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ETHNOMEDICINAL APPROACHES FOR TREATING VARIOUS DISEASE BY IRULAS TRIBALS, KONBANUR VILLAGE, ANAIKATTI HILLS,THE WESTERN GHATS, COIMBATORE DISTRICT

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ABSTRACT

Indigenous traditional Knowledge is an integral part of the culture and history of a local community. It evolves through years of regular experimentation on the day to day life and available resources surrounded by the community. The present paper documented 85 ethno-medicinal plants of Konbanur village, Anaikatti, Coimbatore district, the Western Ghats, Tamil Nadu belonging to 48 families were used by the Irula tribals for various diseases and food. The conventional ethno medicinal plants were mostly used for different inflammation, cough and cold, leucoderma, different skin diseases, ulcers and leprosy. The ethnomedicinal plants are arranged alphabetically followed by botanical name, family, local name and medicinal uses.

Keywords: Psychological Approach, Canadian fiction, Margaret Atwood, nature.

1. INTRODUCTION

India is endowed with a variety of natural resources. All along the West coast the Western Ghats are sprawling. The entire Western Ghats is known for its biodiversity, richness and endemism of different species. India harbours about 15% (3000 – 3500) out of 20,000 medicinal plants of the world. About 90% of these are found growing wild in different climatic regions of the country (Singh, 1997). The tribal and rural populations of India are, to a large extent, dependent on medicinal plants not only to meet their own healthcare needs by self-medication, but also for their livestock. The Western Ghats is richly credited with varied kind of vegetation and unimaginable topographical features. There are about 2,000 plant species that has been found to possess the medicinal value, in all the four systems of indigenous medicine, *viz.,* Ayurveda, Unani, Siddha, and Homeopathy (Hemambara *et al.*, 1996). Irulas are a small tribal community that is part of the Dravidian language group that is spoken in South-Eastern India. They are recognized as a Scheduled Tribe (ST) by the Government of India (Sasi *et al.,* 2011; Ragupathy and Newmaster, 2009). The Irulas are the Dravidian inhabitants and one among the 36 sub-tribal communities in Tamil Nadu that holds the population about 26,000 Irulas living in Tamil Nadu, out of the total population of 558 lakh in the state (Department of Tribal Welfare of Tamil Nadu, Statistic table, July 2006), which is less than 0.5 % of the entire state's population (Census of India, 1991 and 2001). The study area Konbanur village, Anaikatti (11°6'N, 76°45'E). is occupied 250 acres site constitutes a part of the large two square kilometers catchment area. Two hill slopes, northern and southern, also form a part of NBR park. The hills elevate to a height of 80 to 120 meter from the valleys.

2. MATERIALS AND METHODS

The present work is the outcome of intensive field studies undertaken in hamlet inhabited by Irulas community. Explorative field trips were regularly made once in a month of the study area to all habitants to elicit information on medicinal plant used to treat various ailments. Folklore medico botanical investigations were carried out according to the method adopted by Schultes (1960, 1962); Jain (1989) and Martin (1995). Fieldwork is the most significant aspect in this type of study. Extensive field trips were conducted to remote rural settlements. From each village, two or three local herbal healers were interviewed to elicit first hand information in respect of the plant/plant product curing various diseases. The voucher specimen plants collected were identified with the help of Flora of Presidency of Madras by Gamble (1936) and Flora of Tamilnadu and Carnatic by Mathew (1983).

The medicinal plants collected in this way are tabulated. All the collected medicinal plants were arranged their family and genus according to the alphabetical order. The botanical names followed by author citation and synonyms of the plant species, local name of the plant species were also provided. Most of the plants are used as a medicine rest of them served as edible plants.

3. RESULTS AND DISCUSSION

The present study was carried out in the Konbanur village of Anaikatti hills, the Western Ghats, Coimbatore District. Fieldwork is the most significant aspect in this type of study. Extensive field trips were conducted to remote rural settlements. From each village, two or three local herbal healers were interviewed to elicit first hand information in respect of the plant/plant product curing various diseases. In Table 1, data obtained from the field survey are presented. In this study 85 plant species belonging to 48 families have been recorded. Many plant species belonging to families of Solanaceae, Asteraceae and Amarandhaceae are frequently used. The informations collected from this study are in agreement with the previous reports (Pushpangadan and Atal, 1984, Kala, 2005; Jain, 2001; Ayyanar and Ignacimuthu, 2005; Sandhya *et al.*, 2006; Ignacimuthu *et al.,* 2006). For common ailments such as fevers, stomach ache and respiratory disorders, skin diseases, joint pains, hair loss, dysentery, diarrhea, snakebite, jaundice and malaria more number of medication were used. On the other hand, few were used to complicated problems such as heart diseases, kidney disorders skin diseases, cancer and diabetes. The knowledge informants were taken to the field and information on medicinal plants was recorded. The informants were asked to explain therapies of the diseases and to list plants they employ (Table 3-4).In this investigation, there are 20species belonging to 17 families and 18 Genera were reported by the local practice for the treatment of common heart diseases (Table 2). Among them, 17 families represents atleast single species each. Nearly 20 species, they are using for the treatment of common stomach problems which belonging into 12 families and 20 Genera (Table 3) and The Irula communities of the study area selectively used around 15 families with their 19 plant species especially for the treatment of kidney disorders which is belonging into (Table 4) Amarandhaceae, Asclepiadaceae, Cucurbitaceae, Lamiaceae, Fabaceae, Malvaceae, Menispermaceae and Nyctaginaceae etc. For each plant species complete documentation of folklore medicinal information including medicinal property, their vernacular names, family, parts of used, uses and their identified phytochemical compounds was recorded (Coehran and Cornfield, 1951; Martin, 1995).

Table 1. List of Ethnomedicinal plants used by Irula tribalin study area.

S. No	Botanical Name	Family	Vernacular Name	Parts used ,Mode of Preparation, Ethno medicinal uses and some other plants used as ingredients
1	*Abelmoschus esculandus*L.	Malvaceae	Bhendhi	Protect from asthma and diabetes
2	*Abrus precatorius*L.	Fabaceae	Rosary pea, Ratti	Used in stomach pains and diarrhea
3	*Abutilon indicum*Linn.	Malvaceae	Thuthi	Protect from Piles and Pulmonary tuberculosis
4	*Achyranthes aspera*Linn.	Amaranthaceae	Chirchitta	Useful in treatment of Vomiting, Cough, Dysentery
5	*Aconitum heterophyllum*L.	Fabaceae	Athividayam	Extracts used for treating Snakebite, Fever
6	*Acorus calamus*L.	Acoraceae	Vasambu	Rhizome used for cough and fever. Leaf used for Diuretic
7	*Adina cordifolia*(Roxb.)	Rubiaceae	Kadami	Medicine for Stomach-ache, cold cough, fever
8	*Aegle marmelos*(L.) Correa	Rutaceae	Vilvam	Fruits used for Dysentery
9	*Aerva lanata* L.	Amarandhaceae	Kanpulai	Leaf juice cure kidneystone
10	*Ageratum conyzoides*L.	Asteraceae	Chick weed	Treating for stomach pain and antifungal disease
11	*Allium ceba* L.	Liliaceae	Onion	To relieve congestions especially in lungs and bronchial tract.
12	*Allium sativum* L.	Liliaceae	Garlic	To lower blood pressure and cholesterol.
13	*Amarandhus caudatus*L.	Amarandhaceae	Cirukeerai	Avoid diarrhea done by its powder
14	*Amaranthus spinosus* L.	Amaranthaceae	Mullu	Leaf juice used for Diuretic and

				Digestion
15	*Andrographis paniculata* Burm.f.	Acanthaceae	Siriyanangai	Leaf paste mixed with milk internally taken for snake bite
16	*Aristolochia bracteolate* Lam.	Aristolochiaceae	Aaduthinnapaalai	Leaf Paste used externally on the wound of snake bite
17	*Artocarpus heterophyllus*Lam.	Moraceae	Palamaram	Leaf juice used for taken internally for ulcer
18	*Basella alba* L.	Basellaceae	Kodipasalai	Leaves boiled in water and taken internally to cure piles
19	*Boerhaavia diffusa*L.	Nyctaginaceae	Mukkurttaikkoti	Taken for treatment of abdominal pain,tumors
20	*Bryophyllum pinnatum* (Lam.)oken	Crassulaceae	Malaikali	Cure kidney stone and Cough
21	*Burberis vulgaris* Linn.	Berberidaceae	Jaundice barberry	Fruit used to reinforce the heart and liver
22	*Caesalpinia pulcherrima*Linn.	Fabaceae	Peacock Flower	Focusing the diseases like asthma, malaria, kidney stone
23	*Caeselpiniabonduc (L.)* Roxb.	Caesalpinaceae	Kazhichikai	Seed used for Fever. Leaf juice used for diabetics
24	*Camellia sinensis* (L.) Kuntze	Berberidaceae	Tea plant	Tea used for cancer, heart disease, liver disease
25	*Canna indica*L.	Scitaminaceae	Kalvazhai	Root juice are used for diuretic and digestion
26	*Canthiumcoromandelicum*(Burm.f) Alston	Rubiaceae	Bellakarai	Roots and Leaves paste used for Diuretic
27	*Capparissepiaria*L.	Violaceae	Thottichedi	Root and Leaves are pasted with lemon juice and are applied topically to treat swellings.
28	*Cappariszeylanica*L.	Capparaceae	Kevisi	Leaves juice used for Immuno stimulant anti-inflammatory
29	*Carallumabicolour*Ramach, S. Joseph, H. A. John and C. Sofia	Asclepiadaceae	Kattalae	Plant extract used for Weight loss
30	*Caralluma umbellate*Haw.	Asclepiadaceae	Chirukalli	Whole plant roasted for a few minutes and roasted paste applied for indigestion
31	*Carica papaya* Linn.	Caricaceae	Papaya	Cures Abdominal disorders, Amenorrhoea, Atherosclerosis
32	*Cassia occidentalis*(L)	Fabaceae	Ponnavarai	Works as an antibacterial, antifungal, antimalarial
33	*Celosia argentia*L.	Verbenaceae	Kozhikontai	Curing infant fever and Chronic cough
34	*Cissampelospareira*L.	Menispermaceae	Ponmusutai	Treatment of urinary tract
35	*Cocciniagrandis*(L.) J.Viogt	Cucurbitaceae	Koovaikodi	Treating diseases of urinary tract infection, skin diseases, Hypoglycaemic
36	*Coleus aromaticus*benth.	Lamiaceae	Karpuravalli	Working against Anti-tumor and Cholera
37	*Coleus forskohlii* (willd.)Briq	Lamiaceae	Marunthukoorkankizanku	Treating intestinal disorders, asthma
38	*Commiphora mukul* (Jacq.)Eng	Burseraceae	Guggul	oleo-gum-resin used in treatment of nervous diseases, leprosy
39	*Cordiadichotoma*G. Forst.	Boraginaceae	Karadisellai	Seed extract used for Anti-inflammatory
40	*Crataegus oxyacantha* Linn.	Rosaceae	Hawthorn	To reduce cardiac and cerebral damage, when ischemia

41	*Crocus sativus* Linn.	Iridaceae	Saffron	Stamens are used for curing heart disease
42	*Curcuma longa* Linn.	Zingiberaceae	Turmeric	Use in cardiovascular diseaseand gastrointestinal disorders
43	*Cyphomandra betacea* (Cav.) Miers	Solanaceae	Maraththakkali	Fruits used for diuretic, cough and cold
44	*Daturastramonium*L.	Solanaceae	Unmatta	Relieve the diseases urinary retention and ulcer
45	*Digitalis lanata*Linn.	Scrophulariaceae	Wooly foxglove	Used to relive from heart diseases and asthma
46	*Dioscoreaoppositifolia*L.	Dioscoreaceae	Chinese yam	Leaves paste is used as antiseptic for ulcers
47	*Diplocylospalmatus*(L.) Jeffrey	Cucurbitaceae	Sivalingakkodi	Fruits juice used in body pain
48	*Dolichosbiflorus*L.	Fabaceae	Kulattha	Lowering the level of blood sugar
49	*Drynariaquercifolia*(L.) J.Sm.	Polypodiaceae	Mudavattukizhangu	Rhizome juice are taken internally for body pain
50	*Emblica officinalis*Gaertn.	Euphorbiaceae	*Indian gooseberry*	Treatment of jaundice, dyspepsia and cough
51	*Erigeron Canadensis* L.	Asteraceae	Horseweed	Helps for curing Blood clotting andrheumatic complaints
52	*Gloriosasuperba*L.	Liliaceae	Kanvalipoo	Rhizome paste is applied treat wounds.
53	*Glycosmispentaphylla*(Retz.) Dc.	Rutaceae	Melaekulukki	Used for cough, rheumatism, anemia and jaundice.
54	*Gompherna serrate* L.	Amarandhaceae	Arasan con todo	Cures the Kidney problems and live disorders
55	*Guizotiaabyssinica*(L.f.) Cass.	Asteraceae	Malaiellu	Treatment for Stomach ache
56	*Hemidesmusindicus* L.	Asclepiadaceae	Nanari	Refrigerant and for kidney and urinary disorders
57	*Inula racemosa* HOOK. F	Asteraceae	Sunspear	Roots are powerful biological activity.
58	*Jatrophamultifida*L.	Euphorbiaceae	Churakkalli	Protects fromStomach ache, burn
59	*Justiciaadhatoda*L.	Acanthaceae	Aadhatodai	Leaf juice from this plant used for cough, fever and diarrhea
60	*Kalanchoepinnata*L.	Crassulaceae	Ranakalli	Medicine for curing kidney diseases
61	*Lagenariasiceraria* L.	Cucurbitaceae	Surakkai	Treating diseases like Diabetic,Doarrhea and digestive problem
62	*Madhucalongifolia*(Koenig)	Sapotaceae	Iluppai	Medicine for diabetes, Painkiller, Skin diseases
63	*Matricarrecutita*L.	Asteraceae	Chamomile	Cures the digestive problems and acts as an anti-inflammatory, anti-spasmodic.
64	*Momordicacharantia*L.	Cucurbitaceae	Pakkrkai	Cure kidney stone.
65	*Moringa oleifera* L.	Moringaceae	Murungai	Stabilize blood pressure and make strengthen
66	*Nelumbo nucifera* Gaertn	Nymphaeaceae	Indian Lotus	Treatment of diarrhea, tissue inflammation and haemostasis
67	*Pachygoneovata (*Poir.) Diels	Menispermaceae	Perungkaattukodi	Seeds powder used for Snake bites
68	*Pergulariadaemia* (Forsk) Chiv	Asclepiadaceae	Veliparuthi	Treating the diseases like malarial intermittent fevers, toothaches
69	*Phyllandhusniruri* L.	Phyllandhaceae	Keezhanelli	Brain tumor andJaundice
70	*Piper longum* L.	Piperaceae	Long pepper	Therapeutic agent for Alzheimer

				disease, Anti-stress
71	*Psidium guajava* L.	Myrtaceae	Guava	Rich in antioxidant properties
72	*Punica granatum* L.	Puniacaceae	Pomegranate,	Focusing on treatment of diabetics and prevention of cancer, cardiovascular disease
73	*Ricinuscommunis*L.	Euphorbiaceae	Castor	Protect liver damage from certain poisons
74	*Riveahypocrateriformis* Choisy	Convolulaceae	Mustae	Leaves paste used for diarrhea
75	*Scillahyacinthina* (Roth) Macbr.	Liliaceae	Kattuvengayam	Paste made from bulb applied externally for body pain
76	*Scopariadulcis* L.	Scrophuraliaceae	Sarkaraivempu	Cure kidney stone.
77	*Solanumnigrum*L.	Solanaceae	Makoi	Having antiulcer properties cures stomach diseases
78	*Solanumru depannum* Dunal	Solanaceae	Toothuvalai	Leaf juice is taken orally for cough and fever
79	*Strychno spotatorum*L.f.	Loganiaceae	Sillakottai	The whole plants used for Urinary and Kidney
80	*Terminalia arjuna*W. and A.	Combretaceae	White Marudah	Protects the heart, strengthens circulation
81	*Terminalia chebula*Retz.	Combretaceae	Haritaki	Works as anAntioxidant,Antibacterial,
82	*Tribulus terrestis* L.	Zygophyllaceae	Nerunji	Protects the liver and kidney
83	*Withaniasomnifera* Dunal	Solanaceae	Winter cherry	Increases hemoglobin content in the blood
84	*Zingiber officinale* Roscoe.	Zingiberaceae	Ginger	Useful in fighting heart disease, cancer
85	*Zizphus jujube* (L.)	Rhamnaceae	Ber	Increase physical stamina and cures the liver disorders

Table 2. List of medicinal plants used by Irula tribal for the treatment of heart diseases.

S. No	Botanical Name	Common name	Name of the Family	Parts used	Chemical Constituents
1	*Allium ceba* L.	Onion	Liliaceae	Bulb and Leaves	Sulphur compounds(Ajoene, allyl sulfides, and vinyldithiins), quercetinand Allicin(diallyl disulphide oxide)
2	*Allium sativum* L.	Garlic	Liliaceae	Bulb	Sulphur compounds,(Ajoene, allyl sulfides, and vinyldithiins)andAllicin
3	*Burberis vulgaris*Linn.	Jaundice barberry	Berberidaceae	Bark andRoot	Berberine
4	*Camellia sinensis* (L.) Kuntze	Tea plant	Theaceae	Leaves and Leaf buds	Epicatechin (EC),Epigallocatechin (EGC), Epicatechin-3-gallate (ECG), and Epigallocatechin-3-gallate (EGCG)
5	*Coleus forskohlii* (willd.) Briq	Marunthu koorkankizanku	Lamiaceae	Tuberous root	Forskohlin,Arjunic acid
6	*Commiphora mukul* (Jacq.) Eng.	Guggul	Burseraceae	Gum andResin	Guggulsterones,Z–guggulsterone, Guggulipids
7	*Crataegus oxyacantha* Linn.	Hawthorn	Rosaceae	Berries, Leaves andFlowers	Oligomeric proanthocyanidins, Catechin, Quercetin,Epicatechin
8	*Crocus sativus* Linn.	Saffron	Iridaceae	Stigmas	Crocetin, Picrocrocin

9	*Curcuma longa Linn.*	Turmeric	Zingiberaceae	Rhizome	Curcumin(diferuloylmethane) C3
10	*Digitalis lanata* Linn.	Grecin foxglove	Scrophulariaceae	Leaves	Cardiac glycosides
11	*Emblica officinalis* Gaertn.	*Amalaki, amla*	Euphorbiaceae	Fruit	Vitamin C, Gallic acid, Emblicanin A,B
12	*Inularacemosa* HOOK. F	Indian elecampane	Asteraceae	Rootand Rhizome	Alantolactone, isoalantolactone
13	*Nelumbo nucifera* Gaertn	Indian Lotus	Nymphaeaceae	Flowers andRhizome	Quercetin,Luteolin
14	*Piper longum* L.	Long pepper,Thippali	Piperaceae	Fruitand Root	Piperlongumine
15	*Psidium guajava* L.	Guava	Myrtaceae	Fruit andLeaves	Quercetin, Lycopene,vitamin C
16	*Punica granatum* L.	Pomegranate	Puniacaceae	Fruitsand flowers	Hexahydroxydiphenic acid,Gallic acid, quercetin, Punicic acid,
17	*Terminaliaarjuna* W. and A.	Maruthamaram	Combretaceae	Bark	Arjunolic acid,Arjunic acid, Glycosides, Gallic acid, oligomeric proanthocyanidins
18	*Terminaliachebula* Retz.	Haritaki	Combretaceae	Fruit, Bark andseed	Pentacyclictriterpenes, vasicine and vasicinone,Ellagic acid,chebulic acid
19	*Withaniasomnifera* Dunal	Winter cherry, Ashwagandha	Solanaceae	Tuber andRoot	Withaferin A
20	*Zingiberofficinale* Roscoe.	Ginger	Zingiberaceae	Root	Galanolactone

Table 3. List of medicinal plants used by Irula tribal for the treatment of stomach disorders.

S.NO	Botanical name	Name of the family	Common name	Part used	chemical constitution
1	*Abrusprecatorius* L.	Fabaceae	Kuntrymani	Seed	2,3-diphospho-d-glyceric Acid
2	*Achyranthesaspea* Linn.	Amaranthaceae	Chirchitta	whole plant	C-glycosides
3	*Aconitum heterophyllum*L.	Fabaceae	Athividayam	whole plant	Heterophylline,Hetisine
4	*Adina cordifolia* (Roxb.)	Rubiaceae	Kadami	leaf, flower	Rhamnopyranosyl
5	*Ageratum conyzoides*L	Asteraceae	Chick weed	whole plant	Leucoanthocyanins
6	*Caesalpiniapulcherrima* Linn.	Fabaceae	Peacock Flower	Leaf	Terpinene
7	*Carica papaya* Linn.	Caricaceae	Papaya	fruit, seed	cardiac glycosides
8	*Cassia occidentalis*(L)	Fabaceae	Ponnavarai	roots, leaves and seeds	Chrysophanol 1

Table 4. List of medicinal plants used by Irula tribal for the treatment of kidney disorders.

S. No	Botanical Name	Family	Common Name	Parts used	Chemical constitution
1	*Abutilon indicum* Linn.	Malvaceae	Thuthi	Leaf	Ethylacetate, Chloroform, Methanolic, Aphrodisiac,

2	*Aervalanata* L.	Amarandhaceae	Kanpulai	Root, leaf	Laxative, Mucilage β-Sitosterol, α-amyrin,betulin, Hentriacontane, Sitosterylpalmitate, D-glucoside, Glycosides, Rhamnogalactoside
3	*Abelmoschu sesculandus* L.	Malvaceae	Vendai	Fruit	Saponins, Glycosides, linoleic, linolenic ,oleic acid, squalene
4	*Amarandhu scaudatus* L.	Amarandhaceae	Cirukeerai	Root.	β-carotene.Triterpenoids, Saponins, Glycosides, linoleic, linolenic ,oleic acid, squalene
5	*Boerhaavia diffusa* L.	Nyctaginaceae	mukkurttaikkoti	Root	phlobaphenes and ursolic acid
6	*Bryophyllum pinnatum* (Lam.) oken	Crassulaceae	Malaikali	Leaves	β-D-glucopyranoside , nundecanyl, flavanoids ,flavones, falvans, flavanones, isoflavonoids, chalcones,
7	*Coleus aromaticus* Benth.	Lamiaceae	Karpuravalli	Leaves	Oleanolic acid, 2,3-dihydroxyoleanolic acid, Crategolic acid, Ursolic acid, Pomolic acid, ssEuscaphic acid,
8	*Celosia argentia* L.	Verbenaceae	Kozhikontai	Seed, root	, 6-methoxygenkwanin,quercetin, Chrysoeriol, Luteolin, Apigenin, Flavanoneeriodyctol, Flavanol
9	*Cissampelos pareira* L.	Menispermaceae	Ponmusutai.	Leaf,root	7, 12-dimethylbenz(a)anthracene (DMBA),polycyclic aromatic hydrocarbon (PAH), peroxides,
10	*Clerodendrum serratum* L.	Lamiaceae	Thalunarai	Leaf	calcium, magnesium ,uric acid, carbohydrates
11	*Dolichosbiflorus* L.	Fabaceae	Kollu	Root	petroleum ether, Alcohol, Calcium chloride dehydrate, Sodium oxalate, Disodium hydrogen phosphate
12	*Gompherna serrate* L.	Amarandhaceae	Arasan con todo	Whole plant	sulphur, chlorine, potassium, calcium, chromium , manganese, cobalt, Nickel, copper, Zinc
13	*Hemidesmus indicus* L.	Asclepiadaceae	Nanari	Root	4-hydroxy-3-methoxy-cinnamic acid, 4-hydroxybenzoic acid, p-hydroxycinnamic
14	*Lagenaria siceraria**(L.)*	Cucurbitaceae	Surakkai	Fruit	α- and β-amyrins, calcium albumin and alanin transaminase, β-D-glucopyranoside
15	*Moringa oleifera* L.	Moringaceae	Murungai	Root	alkaloids, moriginine, bacteriocide, spirochin, vitamins
16	*Momordica charantia*L.	Cucurbitaceae	Pakarkai.	Leaves	Alkaloid, glycosides, reducing sugar, saponin
17	*Phyllandhus niruri* (L.)	Phyllandhaceae	Keezhanelli	Root	Alkaloid, glycosides, reducing sugar, saponin phosphatase
18	*Scoparia dulcis* (L.)	Scrophuraliaceae	Sarkaraivempu	Root,shoot	Calcium chloride, sodium oxalate, calcium chloride.
19	*Tribulus terrestis* L.	Zygophyllaceae	Nerunji	Whole plant	Peroxide,malondialdehyde,ethanalic,protein,carboxinyl,catalase glutathione, dithiobis, nitrobenzoic acid

The most important aspect of the Irula tribal medicine is that fresh plant material is used for the preparation of medicine. Alternatively, if the fresh plant parts are not available, dried plant materials are used. For this reason several plants served as edible food and alternative remedy to cure a more than single diseases. From this study it is clear that Irula tribal possess innate ability to discern the character of plants and exploit the plant resources to meet their health care needs.

4. CONCLUSION

In the present investigation, a total of 85 species of medicinal plants distributed among 80 genera belonging to 48 families were identified at Konbanur village, Anaikatti hills, the Western Ghats, Coimbatore district**.** In this survey Amarandaceae, Asteraceae and Solanaceae family species served as a food and Asclepiadaceae, Combretaceae, Rhamnaceae and Liliaceae, Euphorbiaceae and etc., families are utilized for various ailments. It is clearly indicates that there is wide usage of local flora by the Irulars community in study area.

This rural area is an important source of traditional medicines. More information may be explored from the peoples residing in the remote villages in this district. The traditional healers are the main source of knowledge on medicinal plants. This knowledge has been transmitted orally from generation to generation; however it seems that it is vanishing from the modern society since younger people are not interested to carry on this tradition. It is also observed that some traditional plants in that area are fast eroding. The conservation efforts are needed by plantation and protection of these plants with maximum participation of local people.

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PHYTOSOCIOLOGICAL OBSERVATIONS ON ECONOMICALLY IMPORTANT PLANTS IN A DRY DECIDUOUS FOREST OF MARUTHAMALAI HILLS, COIMBATORE, TAMIL NADU

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ABSTRACT

The present investigation was carried out in a dry deciduous forest of Maruthamalai hills to know the changes in species composition according to altitude and ecology of economically important plants. A total number of 128 plant species were identified and 112 of them are recognized as economically important. Based on importance value index, the species like *Acacia torta*, *Chloris barbata*, *Eragrostis viscosa*, *Erythroxylon monogynum Pterolobium indicum* and *Zizyphus oenoplia* are ecologically well established plants in the study forest. On the other hand the spices such as *Polygala Jacobi*, *Portulaca guadrifida*, *Ruellia patula*, *Sida rhomboidea*, *Waltheria indica*, *Calotropis gigantean*, *Solanum torvum*, *Acacia leucophloea*, *Acacia nilotica*, *Acacia trotitis*, *Agave Americana*, *Bambusa arundinacea*, *Cassia fistula*, *Chloroxylon swietenia*, *Peltophorum pterocarpum*, *Pithecellobium dulce*, *Pongamia pinnata*, *Prosopis juliflora*, *Samanea saman*, *Thespesia populnea*, *Canavalia mollis*, *Leptadenia reticulata*, *Rivea hypocrateriformis* etc., are considered as ecologically weaker species in the community. Hence priorities must be given to these species so as to protect the genetic stock and species as well.

Keywords: Psychological Observation, Maruthamalai hills, dry deciduous forest.

1. INTRODUCTION

Maruthamalai, the shrine of lord muruga, is situated in the Western Ghats of Coimbatore District, Tamil Nadu. It is also called as Karumalai, Maruthuvamalai and Marundhumalai. In the past 3 yugas of the age of the world, it is well known for its herbal wealth and for the history of Pambatti Siddhar, one of the 18 Siddhars who established the temple at a height of 1175 m above msl. According to Champion and Seth (1968) the vegetation of the Maruthamalai hills comes under the dry deciduous forest. Ramachandran and Nair (1981) documented nearly 66 medicinal plant species in this area. However since last few decades the floristic wealth of Maruthamalai hills is depleted at an alarming rate due to the influence of heavy biotic pressure. In this juncture, the present ecological investigation was aimed to determine the ecological position, the level of establishment and the fitness to the habitat for all component species.

2. MATERIALS AND METHODS

2.1. Study area

The present study was carried out in a dry deciduous forest of Maruthamalai hills, which is situated in the Western Ghats, 15 km away from Coimbatore city. The geographical location of Maruthamalai lies between 76° – 45' and 76°– 55' E longitude and 11° – 0' and 11° – 5' N latitude and forms the western boundary to Coimbatore district. The hill area raises up to 1699 m high, forms scrub jungle up to 700 m with dry rocky soil from the foot hill and evergreen vegetation with grasslands above 700 m height. The trees in this region are small with stunted growth.

2.2. Phytosociological analysis

Phytosociological studies were carried out during the dry month of March, 2011 in a dry deciduous forest of Maruthamalai hills to obtain the quantitative characters such as frequency, density, basal cover and their relative values and importance value index. A one ha plot was established in each of three study plots and it was divided into 20 x 20 m workable units (quadrat). The species and their individuals' occurring in each quadrat were recorded. The basal areas at the point of emergence were measured for all the species. The quantitative characters of the constituent species were calculated as per the following formulae of Cottam and Curtis, (1956).

$$\text{Frequency} = \frac{\text{Number of quadrats in which the species present}}{\text{Total number of quadrats studied}} \times 100$$

$$\text{Density} = \frac{\text{Total number of individuals of the species in all quadrats}}{\text{Total number of quadrats studied}}$$

Since most of the stems are cylindrical, the basal area was calculated by using the formulae:

Basal area = $\pi(r)\ 2$

Where, π = 3.14 and r is the radius of the stem at the point of emergence.

$$\text{Relative Frequency} = \frac{\text{Number of occurence of the species}}{\text{Number of occurence of all species}} \times 100$$

$$\text{Relative density} = \frac{\text{Number of individuals of the species}}{\text{Number of individuals of all species}} \times 100$$

$$\text{Relative dominance} = \frac{\text{Total basal area of the species}}{\text{Total basal area of all species}} \times 100$$

Importance value index (IVI) is the sum of quantities of the relative frequency, relative density and relative dominance expressed per 300.

3. RESULTS AND DISCUSSION

The vegetation of each study plot (1ha) sorted out into four compartments viz., herbs, shrubs, trees and climbers. In all the three studied plots, a total number of 128 species has been enlisted. Off which a high number of 119 species was recorded in the study plot II followed by 117 species in the study plot I and 88 species in the study plot III. Out of 128 species available in three studied plots, 112 species are recognized as medicinally and economically important. The utilization value of the studied plots in a dry deciduous forest of Maruthamalai hills was found to be higher because of the presence of large number of plant species (87.50% of the total flora) as economically important. Paulsamy (2005) also identified a great percentage of economically important species in the floristic list of Nilgiri sholas, adjacent mountain range to the present study area.

The distribution of some of the economically important plants like, *Acacia torta*, *Erythroxylon monogynum*, *Fluggea leucopyrus* and *Zizyphus oenoplia* was even in all three studied plots. It may be explained that the factors like suitability of microhabitat, dispersal mechanism of seeds, germination efficiency, degree of survivability of seedlings and many other intrinsic characters are playing major role for their successful distribution. Many species in three studied plots like *Calotropis gigantean*, *Solanum torvum*, *Acacia nilotica*, *Acacia torta*, *Delonix regia*, *Eucalyptus globules*, *Peltophorum pterocarpum*, *Samanea saman*, *Tectona grandis* etc., have showed restricted distribution. The external factors like topography, soil conditions and biotic disturbances and some intrinsic factors like dispersal mechanism, seed longevity, dormancy period and germination efficiency are some of the environmental variables generally determine the degree of distribution of many plant species (Belsky, 1988).

The density of economically important plants was higher in all three studied plots. The species such as *Acacia torta*, *Eragrostis viscosa*, *Chloris barbata*, *Erythroxylon monogynum*, *Euphorbia hirta*, *Zizyphus oenoplia* etc., were showed high density during the time of sampling. Tansley (2003) stated that in the slopes of mountains where the subtropical and temperate vegetations are available, many local climates are existing which result the variation in the population size of many plant species in the communities. On the other hand, many species like *Calotropis gigantea*, *Solanum torvum*, *Acacia trotitis*, *Agave americana*, *Cassia fistula*, *Eucalyptus globulus*, *Pithecellobium dulce*, *Pongamia pinnata*, *Samanea saman*, *Tectona grandis, Terminalia arjuna, Thespesia populnea, Leptadenia reticulata* etc., were present with low densities in all studied plots may also be due to their poor reproductive potential with less seed output and weaker competitive ability (Chandrasekaran and Swamy, 1995).

Similarly, a high number of economically important plants occupied higher basal area in three studied plots. This may be due to the presence of suitable climate and soil conditions for the growth of such economically important plants in Maruthamalai hills. In addition, the shade provided by the trees also enhancing the growth of these species which naturally being a shade tolerance. Padmavathy (2005) reported in a similar fashion that the forest understories of Nilgiri contained more number of economically important plants with greater density and basal area.

In all the three studied plots of Maruthamalai hills, the ecological picture of economically important plants is highly notable. Among the 112 species of economically important plants, many species like *Acacia torta*, *Chromolaena odorata*, *Euphorbia hirta*, *Erythroxylon monogynum*, *Fluggea leucopyrus*, *Mollugo pentaphylla*, *Pterolobium indicum*, *Tarenna asiatica*, *Zizyphus oenoplia* etc., were determined as well established species on basis of their higher IVI values in comparison to other species. Suitability of habitat, dispersal mechanism of seeds, seed output, reproductive efficiency, longer viability, less demand, rapid regeneration and development of adaptive features according to seasons are accounted to be the reasons for their success in the environmental of present study area (Ramakrishnan, 1991; Paulsamy, 2005).

Table 1. The presence of constituent species in a dry deciduous forest of Maruthamalai hills with their economic importance.

Sl. No.	Species	Family	Parts used	Medicinal/other economic importance	Mode of administration
	HERBS				
1	*Acalypha indica*	Euphorbiaceae	Whole plant	Anti-diabetic activity, Ulcers, bronchitis	Leaf juice, paste, powder
2	*Acanthospermum hispidum*	Asteraceae	Leaves	Cure yellow fever	Leaf juice
3	*Achyranthes aspera*	Amaranthaceae	Whole plant	Antidote, piles, asthma, hydrophobia	leaf paste, root paste
4	*Aerva lanata*	Amaranthaceae	Whole plant	Diuretic, diabetics applied on fresh cuts-burns	Decoction of plant,
5	*Alternanthera pungens*	Amaranthaceae	Whole plant	Diuretic	Decoction of plant
6	*Amaranthus viridis*	Amaranthaceae	Whole plant	Antidote, snakebite, diuretic, inflammations,	Juice, paste
7	*Barleria buxifolia*	Acanthaceae	Leaves, roots	Cough, inflammations	Leaf powder
8	*Barleria prionitis*	Acanthaceae	Leaves, roots	Tooth ache, cough, fever, glandular swelling	Leave juice, root paste
9	*Blepharis mederaspatensis*	Acanthaceae	Entire plant	Venereal diseases	power
10	*Boerhaavia diffusa*	Nyctaginaceae	Whole plant	Asthma, jaundice, antidote, abdominal pain	Leaf juice, paste
11	*Borreria ocymoides*	Rubiaceae	Roots	Tooth warm	Decoction of root
12	*Borreria hispida*	Rubiaceae	Leaves, roots	Tooth warm	Decoction of root
13	*Cassia occidentalis*	Caesalpineaceae	Leaves, roots, fruits	Rheumatism, digestive, diabetes, wheezing, ringworm, saliva secretion, scorpion sting	Decoction of leaves, leaf paste, root power
	Cenchrus ciliaris	Poaceae	-	-	-
14	*Chloris barbata*	Poaceae	-	-	-
15	*Chloris roxburghiana*	Poaceae	-	-	-
16	*Cleome viscosa*	Capparidaceae	Whole plant	Diarrhea, stimulant, cardiac disorders	Leaf juice, powder
17	*Corchorus tridens*	Tiliaceae	-	-	-
18	*Crotalaria verrucosa*	Fabaceae	Leaves	Blood impurities, fever, dyspepsia scabies	Leaf juice, Leaf paste
19	*Croton sparciflorus*	Euphorbiaceae	Seeds	Dyspepsia	powder
20	*Cynodon dactylon*	Poaceae	Whole plant	Diuretic, antidote, stomach trouble	Leaf juice, paste
21	*Desmodium triflorum*	Fabaceae	Whole plant	Cough, antidote, dysentery, diarrhea	Leaf juice, paste
22	*Eragrostis viscosa*	Poaceae	-	-	-
23	*Euphorbia hirta*	Euphorbiaceae	Whole plant	Antidote, asthma, diarrhea, kidney disorders	Plant extract,paste
24	*Evolvulus alsinoides*	Convolvulaceae	Whole plant	Asthma, anthelmintic, bronchitis	Plant juice, power
25	*Gomphrena decumbens*	Amarathaceae	-	-	-
26	*Heteropogon*	Poaceae	Culms of grass	Thatching, stimulant, diuretic, rheumatism	Powder

	contortus				
27	*Hibiscus micranthus*	Malvaceae	Fruits	Febrifuge	Powder
28	*Indigofera enneaphylla*	Fabaceae	Whole plant	Diuretic, anti scorbutic, boiled	plant juice, powder
29	*Indigofera viscosa*	Fabaceae	-	-	-
30	*Justicia tranquebariensis*	Acanthaceae	Leaves	Cooling aspirant, small pox in children	Leaf juice
31	*Leucas aspera*	Lamiaceae	Whole plant	Head ache, cough, cold, chronic rheumatism	Leaf juice, paste
32	*Malvastrum coromandelianum*	Malvaceae	Leaves, flowers	Dysentery, inflamed, scores, antidote,	Decoction of plant
33	*Mariscus cyperinus*	Cyperaceae	-	-	-
34	*Mariscus paniceus*	Cyperaceae	-	-	-
35	*Mollugo pentaphylla*	Aizoaceae	Leaves	Antiseptic, stomachic, ant periodic, earache	Leaf juice
36	*Oldenlandia umbellata*	Rubiaceae	Leaves, roots	Asthma, bronchitis, respiratory tract	Leaf juice, paste
37	*Parthenium hysterophorus*	Asteraceae	Whole plant	Dysentery, scabies, antidote, ulcer, fever	Decoction of root
38	*Pavonia zeylanica*	Malvaceae	roots	Hernia, febrifuge, anthelmintic	Powder
39	*Peristrophe bicalyculata*	Acanthaceae	Whole plant	Eye ailments, bone fracture- sprains	Leaf juice, powder
40	*Perotis indica*	Poaceae	-	-	-
41	*Phyllanthus maderaspatensis*	Euphorbiaceae	Infusion of leaves	Head ache, diuretic, dysentery, jaundice	Leaf juice
42	*Polygala bulbothrix*	Polygalaceae	Leaves, roots	Asthma, chronic, bronchitis, fever	Decoction of root
43	*Polygala jacobi*	Polygalaceae	Roots	Purgative, cold, cough, head ache	Decoction of root
44	*Portulaca guadrifida*	Portulaceae	Leaves	Antiscorbutic, ulcer, gonorrhoea	Decoction of leaves
45	*Rothia indica*	Fabaceae	Leaves, pods	Scarcity	Boiled leaves
46	*Ruellia patula*	Acanthaceae	Whole plant	Psoriasis	Dried powder
47	*Sida acuta*	Malvaceae	Whole plant	Demulcent, diuretic, rheumatism swellings, chest pain, diaphoretic, ulcer, antidote	Leaf juice, root juice, decoction of root, paste
48	*Sida cordata*	Malvaceae	Whole plant	Fever, arthritis, hyper dieresis, diarrhea	Powder
49	*Sida cordifolia*	Malvaceae	Leaves, roots,	Antidote, elephantiasis, dysentery, piles	Plant juice, root powder
50	*Sida rhomboidea*	Malvaceae	Leaves, roots, stem	Rheumatism, emollient, diuretic, febrifuge	powder
51	*Tephrosia purpurea*	Fabaceae	Whole plant	Liver diseases, diarrhea, rheumatism, vomiting, urinary disorders, asthma	Decoction of whole plant, paste, tonic
52	*Tephrosia villosa*	Fabaceae	Leaves, fresh roots	Dropsy, hypoglycemic properties	Paste
53	*Tridax procumbens*	Asteraceae	Leaves	Dysentery, diarrhea, antidote	Paste
54	*Vernonia cinerea*	Asteraceae	Whole plant	Indigestion, piles, malaria, fever,	Leaf juice, paste

55	*Waltheria indica*	Sterculiaceae	Leaves, root	Skin eruption, cleaning wounds, cough	Leaf juice, root powder
56	**SHRUBS** *Acacia torta*	Mimosaceae	Fresh leaves, bark	Menstrual disorders	Decoction of plant
57	*Acalypha fruiticosa*	Euphorbiaceae	Leaves, roots	Antidote, stomachic, gonorrhoea	Leaf juice, powder
58	*Bougainvillaea spectabilis*	Nyctaginaceae	-	-	-
59	*Calotropis gigantea*	Asclepiadaceae	Whole plant	Bite of dog, snake and rat, cough, asthma, healing of wounds and boils, scorpion sting	Powder and paste
60	*Capparis brevispina*	Capparidaceae	Fruits	To reduce body temperature	Decoction of fruit
61	*Capparis roxburghii*	Capparidaceae	-	-	-
62	*Capparis zeylanica*	Capparidaceae	Leaves, roots, bark	Stomachic, fever, body ache, piles	Paste of root bark
63	*Carissa carandas*	Apocynaceae	Fruits, roots	Stomachic, anti scorbutic, digestive	Paste and powder
64	*Carissa spinarum*	Apocynaceae	Whole plant	Purgative, cardiotonic activity	Extract of leaves, tonic
65	*Cassia auriculata*	Caesalpiniaceae	Whole plant	Diabetes, dysentery, tumors, skin, diseases,	Leaf juice, flower powder
66	*Chromolaena odorata*	Asteraceae	Leaves	Antiseptic agent, cure deep cuts and wounds	Leaf juice, leaf paste
67	*Dodonaea viscosa*	Sapindaceae	Aerial part, leaves, roots, bark, seeds	Rheumatism, swellings, cough, backache, sprain, fish poison, wounds and swelling	Boiled leaves, root paste, powder
68	*Erythroxylon monogynum*	Erythroxylaceae	Wood, bark	Fever, dysentery, skin diseases	Ash of the plant
69	*Fluggea leucopyrus*	Euphorbiaceae	Leaves	To destroy worms	Leaf juice
70	*Jatropha glandulifera*	Euphorbiaceae	Roots, fresh bark	Skin diseases, cold, rheumatism, purgative	Paste, oil
71	*Lantana camara*	Verbinaceae	Whole plant	Diaphoretic, dysentery, tumors, piles and rheumatism, fever, ulcers, swellings	Decoction of root, root juice, paste
72	*Phyllanthus reticulates*	Euphorbiaceae	Whole plant	Diuretic, diarrhea, stomachic, burns	Leaf juice, paste
73	*Pterolobium indicum*	Mimosaceae	Dried flower	Fever	Powder
74	*Randia dumetorum*	Rubiaceae	Internal bark, roots, fruits	Dysentery, rheumatism, borne-ache, fever, diaphoretic, asthma ulcers, tumors	Extractions of root and bark, paste
75	*Solanum torvum*	Solanaceae	Leaves, fruits, roots	Digestive, cold, cough, liver diuretic, blood pressure	Decoction of fruit, leaf extract, root paste
76	*Strobilanthes* sp.	Acanthaceae	-	-	-
77	*Tarenna asiatica*	Rubiaceae	Fruits, leaves	skin diseases	Paste
78	*Tecoma stans*	Bignoniaceae	Roots	Diuretic, antidote, vermifuge	Powder and paste
79	*Toddalia asiatica*	Rutaceae	Whole plant	Digestive, stimulant, intermittent fever, cough,, cold, malaria, diarrhea, bronchitis, wounds, ulcers	Leaf juice, paste, flower, juice
80	*Zizyphus oenoplia*	Rhamnaceae	Root bark, fruits	Digestive, antiseptic, healing of wounds	Decoction of root, paste

	TREES				
81	*Acacia leucophloea*	Mimosaceae	Leaves, bark, gum	Stomach ache, fever, anthelmintic, dental caries, oral ulcers, skin diseases, wounds, dysentery, diarrhea	Leaf juice, decoction of bark
82	*Acacia nilotica*	Mimosaceae	Bark, gum	Skin diseases, oral ulcers, liver tonic	Bark paste
83	*Acacia tortitis*	Mimosaceae	-	-	-
84	*Agave americana*	Agavaceae	Leaves, roots, dried, flower stalks	Laxative, diuretic, diaphoretic, antiseptic, dysentery, malaria, other fevers, fish poison	Root juice, paste
85	*Albizzia amara*	Mimosaceae	Leaves, flowers, seeds, gum	Eye diseases, ulcers, swellings, piles, diarrhea, leprosy, leucoderma	Powder
86	*Albizzia lebbeck*	Mimosaceae	Flowers, pods, root gum, stem, seeds	Anti cancer, ophthalmic, wounds, sprains, inflammations, hypoglycemic	Powder
87	*Azadirachta indica*	Meliaceae	All parts	Blood purity, skin diseases, ophthalmic, cough, asthma, ulcers, tumors, liver tonic	Root tonic, bark paste, seeds powder, tonic
88	*Bambusa arundinacea*	Poaceae	Leaves, roots	Diuretic, skin diseases, general debility, nausea, wounds, sprouts	Decoctions of root , leaf bud, paste
89	*Bauhinia variegata*	Caesalpiniaceae	Leaves, flower buds, root bark.	Cough dysentery, tumors, inflammations, diabetes, piles, skin disease	Decoction of root, bark is boiled, paste.
90	*Cassia fistula*	Caesalpiniaceae	Whole plant	Diuretic, dyspepsia, fever, diabetes, skin diseases, ulcers, diuretics, jaundice, cough	Leaf juice, bark powder, root paste
91	*Cassia siamea*	Caesalpiniaceae	Aerial parts , root	Diuretics, to remove intestinal worms	Powder
92	*Chloroxylon swietenia*	Ruutaceae	Leaves, root, bark	Rheumatism, wounds, malaria	leaf Juice, bark decoction
93	*Commiphora berryi*	Bursaraceae	latex	Cracks of feet	Latex
94	*Commiphora caudata*	Bursaraceae	fruits	pickles	Cooked
95	*Delonix regia*	Caesalpiniaceae	Flowers, seeds	Rheumatism, anthelmentic	Powder
96	*Eucalyptus globulus*	Myrtaceae	Leaves , oil	Powerful antiseptic, asthma, diarrhea, vomiting, head ache, cough , cold	leaf oil
97	*Euphorbia antiquorum*	Euphorbiaceae	Roots	Cough, wounds ulcers, rheumatisms	Root juice, powder
98	*Ficus bengalensis*	Moraceae	Whole plant	Diabetes, skin diseases, antidote, tooth ache, cough, ulcers, dysentery, rheumatism	Bark juice, milky juice, extract of aerial root
99	*Ficus tomentosa*	Moraceae	-	-	-
100	*Peltophorum pterocarpum*	Caesalpiniaceae	Barks, seed	Dysentery, muscular pains , sores, anti inflammatory	powder
101	*Pithecellobium dulce*	Mimosaceae	Leaves, bark, seeds	Inflammation of the eyes, blood clotting, dysentery, febrifuge	Extract of seed, powder
102	*Pongamia pinnata*	Fabaceae	Whole plant	Dyspepsia, antiseptic, cough, leprosy, rheumatic pains, foul ulcers cleaning, bleeding	Leaf juice, root paste, decoction of bark and flowers,

				piles, diabetes, fish poison	seeds powder
103	*Prosopis juliflora*	Mimosaceae	mesquite gum	Adulterant, emulsifying agents	As raw
104	*Prosopis spicigera*	Mimosaceae	Barks,leaves,seeds	Dysentery, leprosy, bronchitis, asthma, piles	Paste and powder
105	*Samanea saman*	Mimosaceae	-	-	-
106	*Santalum album*	Santalaceae	Heart wood	Cough, bronchitis, dysentery, jaundice , intermittent fever, skin diseases	Paste of heart wood
107	*Tamarindus indica*	Caesalpiniaceae	Leaves, fruits, roots, seeds	Sore throat, ulcer, wounds, cough, eye disorder, dysentery, disorders, swellings	Leaf paste, seeds powder
108	*Tectona grandis*	Verbinaceae	Whole plant	Antiseptic, diabetes, leprosy, bronchitis, piles, dysentery, urinary troubles, headache	Paste, powder
109	*Terminalia arjuna*	Combretaceae	Twig , leaf, fruit, bark	Body ache, cardio tonic, ear ache, fractures, ulcer, asthma, bronchitis, tumors, dysentery	Leaf juice, paste , bark powder
110	*Thespesia populnea*	Malvaceae	Whole plant	Cough, asthma, diabetes, ulcer, scabies	Fruit juice, decoction of bark
111	*Zizypus rugosa*	Rhamnaceae	Flowers, leaf, bark	Diarrhea, swellings, infection of teeth	Powder
112	*Zizypus trinervia*	Rhamnaceae	leaves	Purify the blood, venereal affections,	Decoction of leaves
113	**CLIMBERS** *Abrus precatorius*	Fabaceae	Leaves, fruits, roots seeds	Cough, cold, colic leucoderma, skin disease, wounds, asthma, ulcers, tonic, jaundice	Leaf juice, root powder seed paste
114	*Canavalia mollis*	Fabaceae	Seeds, leaves	Wound heeling	Paste
115	*Cardiospermum halicacabum*	Sapindaceae	Roots , leaves, seeds	Rheumatism, asthma, diuretic, fever, lumbago naturopathic	Powder
116	*Cissus quadrangularis*	Vitaceae	Whole plant	Bone fracture , asthma , scurvy , wounds digestive , menstrual disorders	Leaf juice, root and stem paste
117	*Clitoria ternatea*	Fabaceae	Leaves, seeds	Diuretic, asthma, ulcers , fever, rheumatism	Leaf juice, root paste
118	*Coccinia indica*	Cucurbitaceae	Whole plant	Sores, scabies skin disease,	Paste and powder
119	*Cocculus hirsutus*	Menspermiaceae	Root and leaves	Chronic rheumatism, inflammation of urinary passages, diabetes, skin disease	Leaf juice leaf paste root paste
120	*Cocculus pendulous*	Menspermiaceae	Leaves	Noise bleeding , anti tumor , anticancer	Extract of leaves
121	*Daemia extensa*	Asclepiadaceae	Whole plant	Cold, Cough, fever, asthma, digestive	Leaf juice, leaf paste
122	*Ipomoea nil*	Convalvulaceae	Seeds	Anti –inflammatory, purgative, skin diseases, dyspepsia, bronchitis,fever	Extract of seeds
123	*Leptadenia reticulata*	Asclepiadaceae	Whole plant	Leprosy, tonic and stimulant	Plant extract
125	*Passiflora foetida*	Passifloraceae	Aerial part, fruits, roots	Anticancer, memory power, asthma, biliousness, hysteria, itches	Decoction of fruit and root, paste, powder
126	*Rivea hypocrateriformis*	Convolvulaceae	Leaves, shoots	Eaten, fragrant	Powder
127	*Sarcostemma intermedium*	Asclepiacaceae	Dried stem, root	Emetic, antidote, hemorrhage	Paste
128	*Tiliacora acuminata*	Menispermaceae	Roots	Antidote	Root juice

Table 2. Importance value index for the ecologically stronger and weaker, economically important plants in a dry deciduous forest of Maruthamalai hills.

Sl. No.	Species	Maruthamalai hills		
		Plot I	Plot II	Plot III
	HERBS			
1	*Acalypha indica*	2.53	2.24	-
2	*Acanthospermum hispidum*	1.68	-	-
3	*Achyranthes aspera*	1.76	1.65	2.00
4	*Aerva lanata*	1.19	-	-
5	*Alternanthera pungens*	1.35	1.21	1.96
6	*Amaranthus viridis*	2.06	1.59	2.30
7	*Barleria buxifolia*	1.71	1.60	-
8	*Barleria prionitis*	-	1.64	-
9	*Blepharis mederaspatensis*	2.09	2.68	2.62
10	*Boerhaavia diffusa*	3.49	1.96	-
11	*Borreria ocymoides*	2.41	3.08	-
12	*Borreria hispida*	-	1.71	-
13	*Cassia occidentalis*	2.32	1.77	-
14	*Cenchrus ciliaris*	3.26	3.40	3.64
15	*Chloris barbata*	9.64	10.19	10.85
16	*Chloris roxburghiana*	-	6.12	-
17	*Cleome viscosa*	1.74	1.76	-
18	*Corchorus tridens*	2.21	3.60	2.92
19	*Crotalaria verrucosa*	1.60	1.79	-
20	*Croton sparciflorus*	1.47	1.04	-
21	*Cynodon dactylon*	3.40	1.72	-
22	*Desmodium triflorum*	3.64	3.88	4.42
23	*Eragrostis viscosa*	10.21	9.82	12.99
24	*Euphorbia hirta*	5.34	5.06	4.53
25	*Evolvulus alsinoides*	3.98	3.73	-
26	*Gomphrena decumbens*	1.30	0.95	1.55
27	*Heteropogon contortus*	4.86	4.72	6.80
28	*Hibiscus micranthus*	4.24	4.46	5.06
29	*Indigofera enneaphylla*	1.23	1.30	1.59
30	*Indigofera viscosa*	1.88	-	-
31	*Justicia tranquebariensis*	-	1.80	2.85
32	*Leucas aspera*	3.24	3.27	2.32
33	*Malvastrum coromandelianum*	1.08	1.34	-
34	*Mariscus cyperinus*	2.02	2.15	-
35	*Mariscus paniceus*	-	-	2.68
36	*Mollugo pentaphylla*	4.91	5.07	3.24
37	*Oldenlandia umbellata*	2.95	3.13	2.68
38	*Parthenium hysterophorus*	1.66	1.11	1.60
39	*Pavonia zeylanica*	2.95	2.59	3.81
40	*Peristrophe bicalyculata*	2.44	2.31	2.95
41	*Perotis indica*	4.61	4.29	5.59
42	*Phyllanthus maderaspatensis*	4.24	4.01	4.33
43	*Polygala bulbothrix*	1.45	1.64	1.99
44	*Polygala jacobi*	0.88	1.22	1.27
45	*Portulaca guadrifida*	0.77	0.81	-
46	*Rothia indica*	1.04	0.93	-
47	*Ruellia patula*	0.86	0.95	-
48	*Sida acuta*	2.15	2.23	2.83
49	*Sida cordata*	2.52	2.37	2.68
50	*Sida cordifolia*	2.03	1.77	-
51	*Sida rhomboidea*	0.67	0.70	-
52	*Tephrosia purpurea*	3.99	3.73	-
53	*Tephrosia villosa*	2.27	2.37	3.13
54	*Tridax procumbens*	4.38	3.94	4.84
55	*Vernonia cinerea*	2.71	2.53	3.45
56	*Waltheria indica*	0.93	0.98	1.22
	SHRUBS			
57	*Acacia torta*	13.52	12.63	15.56
58	*Acalypha fruiticosa*	4.77	4.36	4.96
59	*Bougainvillaea spectabilis*	0.71	0.49	-
60	*Calotropis gigantea*	0.48	0.40	-
61	*Capparis brevispina*	1.56	-	2.02
62	*Capparis roxburghii*	-	1.51	-
63	*Capparis zeylanica*	2.08	2.26	2.93
64	*Carissa carandas*	2.22	2.34	2.60
65	*Carissa spinarum*	2.02	1.83	2.42
66	*Cassia auriculata*	2.61	2.49	-
67	*Chromolaena odorata*	5.40	5.05	6.59
68	*Dodonaea viscosa*	1.92	1.77	2.60
69	*Erythroxylon monogynum*	10.82	10.90	13.66
70	*Fluggea leucopyrus*	8.58	8.00	9.17
71	*Jatropha glandulifera*	2.11	1.92	2.43
72	*Lantana camara*	4.84	4.73	6.08
73	*Phyllanthus reticulates*	3.17	3.12	3.95
74	*Pterolobium indicum*	10.52	9.87	-
75	*Randia dumetorum*	3.44	3.26	5.44
76	*Solanum torvum*	0.34	0.40	0.49
77	*Strobilanthes* sp.	-	5.48	-
78	*Tarenna asiatica*	6.09	-	-
79	*Tecoma stans*	0.76	0.68	0.41
80	*Toddalia asiatica*	4.17	3.88	7.28
81	*Zizyphus oenoplia*	9.97	9.26	15.70
	TREES			
82	*Acacia leucophloea*	0.79	0.74	0.62
83	*Acacia nilotica*	0.81	0.58	-

84	*Acacia tortitis*	0.45	0.51	0.31
85	*Agave americana*	0.50	-	-
86	*Albizzia amara*	3.71	3.60	4.60
87	*Albizzia lebbeck*	1.49	1.42	1.85
88	*Azadirachta indica*	1.81	1.69	0.97
89	*Bambusa arundinacea*	0.93	0.88	0.79
90	*Bauhinia variegata*	1.11	1.10	0.64
91	*Cassia fistula*	0.62	0.70	0.70
92	*Cassia siamea*	1.35	1.39	1.28
93	*Chloroxylon swietenia*	0.90	0.79	2.94
94	*Commiphora berryi*	2.14	1.98	1.25
95	*Commiphora caudata*	2.15	1.96	8.41
96	*Delonix regia*	0.55	0.52	0.49
97	*Eucalyptus globulus*	0.25	0.31	0.31
98	*Euphorbia antiquorum*	3.41	3.66	5.85
99	*Ficus bengalensis*	0.63	0.73	0.44
100	*Ficus tomentosa*	-	0.55	-
101	*Peltophorum pterocarpum*	0.54	0.44	1.67
102	*Pithecellobium dulce*	0.40	0.46	0.30
103	*Pongamia pinnata*	0.78	0.83	0.53
104	*Prosopis juliflora*	0.79	0.66	0.79
105	*Prosopis spicigera*	1.04	0.84	1.18
106	*Samanea saman*	0.53	0.44	0.40
107	*Santalum album*	2.30	1.95	-
108	*Tamarindus indica*	0.83	0.87	0.72
109	*Tectona grandis*	0.25	0.34	0.30
110	*Terminalia arjuna*	0.63	0.70	-
111	*Thespesia populnea*	0.48	-	0.53
112	*Zizypus rugosa*	2.26	2.09	5.72
113	*Zizypus trinervia*	-	2.02	-
	CLIMBERS			
114	*Abrus precatorius*	2.54	2.57	3.88
115	*Canavalia mollis*	0.83	0.85	3.62
116	*Cardiospermum halicacabum*	-	0.67	-
117	*Cissus quadrangularis*	1.65	1.51	2.51
118	*Clitoria ternatea*	3.65	3.40	4.38
119	*Coccinia indica*	2.47	2.38	2.05
120	*Cocculus hirsutus*	2.01	1.85	2.78
121	*Cocculus pendulous*	1.06	1.01	1.43
122	*Daemia extensa*	2.85	2.79	3.12
123	*Ipomoea nil*	2.41	2.32	3.58
124	*Leptadenia reticulata*	0.38	-	-
125	*Passiflora foetida*	1.23	1.38	3.43
126	*Rivea hypocrateriformis*	0.97	0.94	1.31
127	*Sarcostemma intermedium*	2.04	2.33	3.32
128	*Tiliacora acuminata*	-	2.72	-

On the other hand many species such as *Gomphrena* decumbens, *Polygala Jacobi*, *Portulaca guadrifida*, *Ruellia patula*, *Sida rhomboidea*, *Waltheria indica*, *Bougainvillaea spectabilis*, *Calotropis gigantean*, *Solanum torvum*, *Tecoma stans*, *Acacia leucophloea*, *Acacia nilotica*, *Acacia trotitis*, *Agave Americana*, *Bambusa arundinacea*, *Cassia fistula*, *Chloroxylon swietenia*, *Delonix regia*, *Eucalyptus globules*, *Ficus bengalensis*, *Peltophorum pterocarpum*, *Pithecellobium dulce*, *Pongamia pinnata*, *Prosopis juliflora*, *Samanea saman*, *Tamarindus indica*, *Tectona grandis*, *Terminalia arjuna*, *Thespesia populnea*, *Canavalia mollis*, *Leptadenia reticulata Rivea hypocrateriformis* etc., were poorly establishment in the community because of their lower IVI values (less than 1). This may be due to the presence of many intrinsic factors like lower seed output, shorter dormancy, less germination percentage and vigour and poor competitive ability make the species of ecologically weaker category, less available in the communities of shola forests (Padmavathy, 2005).

The floristic composition and ecological studies on various plant species in the study area of Maruthamalai hills indicate that it is an ideal habitat for the growth of many kinds of economically important plants. Further it is known that the population size, density and ecological fitness of the economically important plants in general and medicinal plants in particular are also highly appreciable. Hence the local environment of Maruthamalai is found to suitable for the cultivation of medicinal plants. Therefore it is suggested that the fragile parts of Maruthamalai can be used for the growing of economically and medicinally important plants.

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PHYTOSOCIOLOGICAL ANALYSIS AND FLORISTRIC DIVERSITY OF VANEESWARAM KAVU IN KANNUR DISTRICT, KERALA

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ABSTRACT

Sacred groves act as a treasure house for rare and medicinal plants. Apart from the quantitative analysis quantitative approach to sacred grove gives the potential species and importance of sacred grove, which is the main focuses of this work. There are many sacred grooves are present at Kannur district in which Vaneeswaram Kavu is one of the important one. The flora of sacred groves of has analysed taxonomically and phytosociologically. A total of 64 vascular plants falling under 61 genera and 43 families were documented. About 12 species are reported in the red listed category. In phytosociological studies species like, *Elaeocarpus serratus, Erycibe paniculata* and *Scleria lithosperma* were showing higher IVI. The devastation of species diversity in the study area represent there is an urgent need for regeneration of the species for conservation of biodiversity.

Keywords: Sacred grove, phytosociology, biodiversity, regeneration.

1. INTRODUCTION

The groves are small patches of vegetation types that were traditionally protected and managed by the local communities, through a wide range of management practices (Gadgil and Vartak, 1976). Sacred grooves have existed from time immemorial as patches of densely wooded areas with unique flora and fauna and perennial water sources in their vicinity. These sacred grooves are considered valuable gene pool and the first major effort to recognize and conserve biodiversity. In many parts of India, sacred grooves symbolize surviving examples of climax vegetation and are disappearing with modernization. As an ecosystem, they help in soil and water conservation, preserves the biological wealth, treasure house of rare and endangered animal species abode of many medicinal, endemic, endangered an economically important plants. Sacred grooves are important ecological centres to study the potential vegetation and source to gather indigenous knowledge on local plants, animals, habitat preferences, distribution, life histories and demographic features. Cultural practices and folk beliefs related to sacred groves imply conservation measures of ecosystems and labelled ethno forestry (Pandey 1998).

In Kerala, based on ownership patterns, sacred groves (Kavu in the regional language, Malayalam) can be broadly categorised into three groups namely those managed by individual families, group of families and the statutory bodies for temple management (Devaswom Board). The present study of sacred grove, Vaneeswaram Kavu, Kannur district, Kerala focuses on analyzing floristic composition, medicinal properties and red listed species in the selected area.

2. MATERIALS AND METHODS

2.1. Study area

The study area, Vaneeswaram Kavu (Fig.1) is concentrated in Kannur district, which is located in the northern part of Kerala. The sacred grove is located in Morazha central, which is about 14 kilometers long from Kannur town. The temple lies between 11.987' N latitude and 75.349'E longitude. Here, the climate is very hot and humid with minimum and maximum temperature ranging from 27°C to 31°C. The average annual rainfall is 3614 mm. The study area of sacred grove spread out in one and half acres. Here the worship is "Nagam" (snake).

Fig. 1. Vanneswaram Kavu, Morazha, Kannur district.

2.2. Floristic survey

Field surveys were carried out to know their exact location, extent, presiding deity etc. Whenever any sacred groves visited the neighbouring people and temple worshippers were interviewed to confirm the above facts and also to elicit information about the presence other groves in the vicinity. The extent each grove was ascertained by discussion with local people and latter confirmed with temple records. Plants are identified with the help of Madras Presidency (Gamble, 1915-1936), Flora of Cannanore (Ramachandran and Nair, 1988) and also by using available field keys and taxonomic bulletins. The identification was further confirmed with the help of taxonomic experts in Botany.

2.3. Phytosociological analysis

The minimum quadrat size of 1 x 1 was fixed by the species- area curved method of phytosociological observations. Each time 20 quadrats were laid by the randomized method in each site. The minimum number of quadrat required (ie. 20) was determined as described by Greig – Smith (1974).

The number and type of each species occurring in each quadrat were recorded. For grasses, each tiller was counted as an individual because it is impossible to decide from aerial shoots whether it is separated or connected in the subterranean region, especially in perennial grasses. Different workers have used arbitrary units to represent individual. Armstrong (1907) and Stapledon (1913) have counted the entire individuals as far as possible in the case of erect plants, but in creeping grasses each grasses each rooting units has taken as an individual. Stove and Fryer (1935) have considered an independent root system, as nearly as this could be determined without actually lifting the plant, to be a unit for counting. In the case of creeping plants, any portion of the plant upto 5 cm in length and having functional root was counted as one plant. Only the plants beyond seedling stage (ie. more than 2 cm height in case of monocots and beyond first leaf stage in dicots) were counted. The basal areas at the point of emergence for the constituent species were measured. From the observations, the quantitative characters such as frequency, density, abundance, relative frequency, relative density, relative dominance, importance value index and relative value of importance were calculated (Shukla and Chandel, 1982; Misra, 1980).

Frequency, density and abundance were calculated using the following formulae:

$$\text{Frequency} = \frac{\text{Number of quadrats in which the species present}}{\text{Total number of quadrats studied}} \times 100$$

$$\text{Density} = \frac{\text{Total number of individuals of the species in all quadrats}}{\text{Total number of quadrats studied}}$$

$$\text{Abundance} = \frac{\text{Total number of individuals of the species in all quadrats}}{\text{Number of quadrats of occurrence of the species}}$$

$$\text{Basal area} = \Pi r^2$$

(Π = 3.14 and 'r' is the radius of the stem at the point of emergence.)

Relative frequency, relative density and relative dominance were calculated from the following formulae:

$$\text{Relative Frequency} = \frac{\text{Number of occurence of the species}}{\text{Number of occurence of all species}} \times 100$$

$$\text{Relative density} = \frac{\text{Number of individuals of the species}}{\text{Number of individuals of all species}} \times 100$$

$$\text{Relative dominance} = \frac{\text{Total basal area of the species}}{\text{Total basal area of all species}} \times 100$$

$$\text{IVI} = \text{RD}+\text{RF}+\text{RDo}$$

$$\text{RIVI} = \text{IVI}/3$$

2.4. Ethnobotanical studies and phytosociological analysis

During the field visits, the various uses of plants were gathered. 65 species were recorded by adopting Quadrat method of sampling. The biodiversity induces like frequency, density, abundance, basal cover and important value index and their synthetic attributes like relative frequency, relative density, relative dominance, and relative value of importance were calculated.

3. RESULTS AND DISCUSSION

Among the 64 species available 93 % plant possessed medicinal uses. It indicates the potentiality of two study areas for the inhabitation of medicinal plants. It may be explained that the sacred groves are rich collection of conserved medicinal plants (Vinothkumar *et al.,* 2011). The uses of species for diverse medicinal purposes show the production of different kinds of secondary metabolites with rich varieties of bioactive compounds in the study sites.

There are 12 endangered species in the study area (Table 2). According to Bhagwat (2005) sacred groves are the last home of some endangered species and also are known to represent the only existing climax vegetation communities in Northeastern India.

Table 1. Species composition of Vanneswaram kavu, Morazha, Kannur.

S.No	Species	Quantitative attributes				Synthetic attributes				
		Frequency (%)	Abundance (individuals/m^2)	Density (individuals/m^2)	Basal Cover (mm^2/m)	R.F (%)	R.D (%)	R.Do (%)	IVI	RIVI
1	*Abrus precatorius* L.	25	2.0000	0.5	3.2245	3.0120	1.3495	0.0573	4.4188	1.4729
2	*Abrus pulchllus* Wall.	5	2.0000	0.1	0.6449	0.6024	0.2699	0.0115	0.8838	0.2946
3	*Acacia auriculiformis* A.Cunn. ex Benth	5	1.0000	0.05	20.6369	0.6024	0.1350	0.3665	1.1039	0.3680
4	*Achyranthus aspera* Linn.	5	3.0000	0.15	2.3408	0.6024	0.4049	0.0416	1.0488	0.3496
5	*Acroceras munroanum* (Balansa) Henrard	15	9.3333	1.4	58.9650	1.8072	3.7787	1.0473	6.6332	2.2111
6	*Adenanthera pavonina* L.	10	5.0000	0.5	107.6433	1.2048	1.3495	1.9118	4.4662	1.4887
7	*Adiantum lunulatum* Burm. f.	10	2.0000	0.2	1.0191	1.2048	0.5398	0.0181	1.7627	0.5876
8	*Aglaia elaeagnoidea* (A.Juss.) Benth.	10	1.5000	0.15	1.1943	1.2048	0.4049	0.0212	1.6309	0.5436
9	*Anodendron paniculatum* A. DC.	5	1.0000	0.05	4.6019	0.6024	0.1350	0.0817	0.8191	0.2730
10	*Antidesma menasu* Muell.-Arg.	5	3.0000	0.15	468.2006	0.6024	0.4049	8.3157	9.3229	3.1076
11	*Apocopis mangalorensis* (Hochst. ex Steud.) Henrard	10	16.0000	1.6	5.8904	1.2048	4.3185	0.1046	5.6279	1.8760
12	*Aporosa lindleyana* (Wt.) Bail.	35	2.4286	0.85	87.7070	4.2169	2.2942	1.5578	8.0688	2.6896
13	*Arundinella leptochloa* (Steud.) Hook.f	5	6.0000	0.3	120.4061	0.6024	0.8097	2.1385	3.5506	1.1835
14	*Axonopus compressus* (Sw.) P.Beauv.	5	7.0000	0.35	9.0287	0.6024	0.9447	0.1604	1.7074	0.5691
15	*Calycopteris floribunda*(Roxb.) Lam	15	3.0000	0.45	165.6688	1.8072	1.2146	2.9424	5.9642	1.9881
16	*Carallia brachiata (Lour.) Merr.*	15	2.3333	0.35	56.4291	1.8072	0.9447	1.0022	3.7541	1.2514
17	*Caryota urens* L.	35	3.4286	1.2	238.8535	4.2169	3.2389	4.2423	11.6980	3.8993
18	*Chassalia curviflora* (Wallich)	10	1.0000	0.1	4.5860	1.2048	0.2699	0.0815	1.5562	0.5187
19	*Curculigo orchioides* Gaetrn.	5	3.0000	0.15	13.8057	0.6024	0.4049	0.2452	1.2525	0.4175
20	*Digitaria bicornis* (Lam.) Roem. and Schult.	10	5.0000	0.5	66.9188	1.2048	1.3495	1.1885	3.7429	1.2476
21	*Diploclisia glaucescens* (Blume) Diels.	5	1.0000	0.05	1.9268	0.6024	0.1350	0.0342	0.7716	0.2572
22	*Dendrophthoe falcata* (L.f)	10	1.0000	0.1	1.3455	1.2048	0.2699	0.0239	1.4986	0.4995
23	*Drynaria quercifolia* (Linn.) J. Smith.	5	6.0000	0.3	193.4713	0.6024	0.8097	3.4362	4.8483	1.6161
24	*Elaeocarpus serratus* Linn.	5	2.0000	0.1	1411.1545	0.6024	0.2699	25.0634	25.9357	8.6452
25	*Erycibe paniculata* Roxb.	10	2.0000	0.2	9.1720	1.2048	0.5398	0.1629	1.9075	0.6358
26	*Gomphia serrata* (Gaertn.) Korth.	20	4.5000	0.9	34.6815	2.4096	2.4291	0.6160	5.4548	1.8183
27	*Holigarna arnottiana* J.Hk.	5	1.0000	0.05	74.7174	0.6024	0.1350	1.3270	2.0644	0.6881
28	*Hugonia mystax* Linn.	5	2.0000	0.1	31.6003	0.6024	0.2699	0.5612	1.4336	0.4779
29	*Isachne miliacea* Roth	20	3.7500	0.75	0.5374	2.4096	2.0243	0.0095	4.4435	1.4812

30	*Ischaemum indicum* (Houtt.)	20	20.5000	4.1	1.3057	2.4096	11.0661	0.0232	13.4990	4.4997
31	*Ischaemum timorense* Kunth	10	6.0000	0.6	0.1911	1.2048	1.6194	0.0034	2.8276	0.9425
32	*Ixora brachiata* Roxb.	5	2.0000	0.1	3.1847	0.6024	0.2699	0.0566	0.9289	0.3096
33	*Ixora coccinea* L.	20	2.7500	0.55	19.3113	2.4096	1.4845	0.3430	4.2371	1.4124
34	*Jasminum flexile* Vahl.	10	2.0000	0.2	1.0191	1.2048	0.5398	0.0181	1.7627	0.5876
35	*Jasminum malabaricum* Wight	10	1.0000	0.1	7.1656	1.2048	0.2699	0.1273	1.6020	0.5340
36	*Kyllinga nemoralis* L.	5	5.0000	0.25	25.7962	0.6024	0.6748	0.4582	1.7353	0.5784
37	*Leea indica* (Burm.f.) Merr.	15	2.0000	0.3	27.6115	1.8072	0.8097	0.4904	3.1073	1.0358
38	*Lepidagathis incurve* D.Don	5	2.0000	0.1	4.5860	0.6024	0.2699	0.0815	0.9538	0.3179
39	*Lepisanthes tetraphylla* (Vahl) Radlk	10	1.5000	0.15	4.7771	1.2048	0.4049	0.0848	1.6945	0.5648
40	*Lindsaea ensifolia* Sw.	10	1.0000	0.1	1.1465	1.2048	0.2699	0.0204	1.4951	0.4984
41	*Macaranga peltata* Roxb. Mueller	5	1.0000	0.05	14.3312	0.6024	0.1350	0.2545	0.9919	0.3306
42	*Mangifera indica* L.	5	1.0000	0.05	57.3248	0.6024	0.1350	1.0181	1.7555	0.5852
43	*Melicope lunu-ankenda* (Gaertn.)	10	1.0000	0.1	29.6258	1.2048	0.2699	0.5262	2.0009	0.6670
44	*Memecylon talbotianum* Burm.f.	20	3.2500	0.65	67.0701	2.4096	1.7544	1.1912	5.3552	1.7851
45	*Mimosa pudica* Linn.	10	5.0000	0.5	4.8169	1.2048	1.3495	0.0856	2.6399	0.8800
46	*Olea dioica* Roxb.	30	2.0000	0.6	61.9108	3.6145	1.6194	1.0996	6.3335	2.1112
47	*Piper trioicum* L.	5	4.0000	0.2	1.9268	0.6024	0.5398	0.0342	1.1764	0.3921
48	*Pothos scandens* L.	25	1.8000	0.45	6.0549	3.0120	1.2146	0.1075	4.3342	1.4447
49	*Pseuderanthemum latifolium* (Vahl)B. Hansen	5	2.0000	0.1	4.2118	0.6024	0.2699	0.0748	0.9471	0.3157
50	*Rourea minor* (Gaertn.) Aubl.	20	6.0000	1.2	50.5414	2.4096	3.2389	0.8977	6.5462	2.1821
51	*Rungia pectinata* (L.) Nees.	15	4.0000	0.6	37.4522	1.8072	1.6194	0.6652	4.0918	1.3639
52	*Salacia fruticosa* Wall.	10	2.0000	0.2	20.6369	1.2048	0.5398	0.3665	2.1112	0.7037
53	*Santalum album L.*	10	2.0000	0.2	321.0828	1.2048	0.5398	5.7027	7.4473	2.4824
54	*Sarcostigma kleinii* Wight and Arn.	5	4.0000	0.2	39.8089	0.6024	0.5398	0.7070	1.8493	0.6164
55	*Scleria lithosperma* (L.) Sw.	25	22.0000	5.5	343.3121	3.0120	14.8448	6.0975	23.9544	7.9848
56	*Smilax zeylanica L.*	35	1.7143	0.6	10.7484	4.2169	1.6194	0.1909	6.0272	2.0091
57	*Staurogyne glauca* Kuntze	15	3.3333	0.5	4.8169	1.8072	1.3495	0.0856	3.2423	1.0808
58	*Stemodia verticillata* (Mill.) Hassler	10	1.5000	0.15	7.4642	1.2048	0.4049	0.1326	1.7422	0.5807
59	*Strychnos nux-vomica* L	55	6.8182	3.75	659.5342	6.6265	10.1215	11.7139	28.4619	9.4873
60	*Syzygium caryophyllatum* (L.) Alston	5	2.0000	0.1	101.6640	0.6024	0.2699	1.8056	2.6780	0.8927
61	*Uvaria narum* Wall.	25	5.0000	1.25	238.9530	3.0120	3.3738	4.2440	10.6299	3.5433
62	*Vanda roxburghii* R. Br.	15	9.0000	1.35	189.6019	1.8072	3.6437	3.3675	8.8185	2.9395
63	*Vitex altissima* L.f.	20	2.2500	0.45	63.2006	2.4096	1.2146	1.1225	4.7467	1.5822
64	*Wattakakka volubilis* (L.f.) Stapf	5	2.0000	0.1	1.7914	0.6024	0.2699	0.0318	0.9041	0.3014

R.F-Relative Frequency, R.D- Relative Density, R.Do- Relative Dominance, IVI- Important Value Index, RIVI- Relative Important Value Index.

Table 2. Red listed plants in Vaneeswaram Kavu, Kerala.

Si No	Species	Status
1	*Aglaia elaeagnoidea* (A.Juss.) Benth.	Least concerned
2	*Anodendron paniculatum* A. DC.	Endangered
3	*Arundinella leptochloa (Steud.)* Hook.f	Least Concerned
4	*Curculigo orchioides* Gaetrn.	Endangered
5	*Drynaria quercifolia* (L.) J. Sm.	Endangered
6	*Holigarna arnottiana* J.Hk.	Least concerned
7	*Ixora brachiata* Roxb.	Least concerned
8	*Jasminum malabaricum* Wight	Endangered
9	*Melicope lunu-ankenda* (Gaertn.)	Endangered
10	*Santalum album* L.	Endangered
11	*Staurogyne glauca* Kuntze.	Endangered
12	*Syzygium caryophyllatum* (L.) Alston	Endangered

Out of the 64 species in Vanneswaram Kavu *Strychnos nux-vomica*, *Aporosa lindleyana*, *Caryota urens* and *Smilax zeylanica* shows better frequency value. But *Scleria lithosperma, Ischaemum indicum* and *Apocopis mangalorensis* have distributed abundantly than the other constituent species. Highest density was observed in the species like *Scleria lithosperma, Ischaemum indicum, Strychnos nux-vomica*. Based on the basal cover, *Elaeocarpus serratus* was considered to be the dominant species and secured the basal cover of 1411 mm^2/m. In this site species like *Strychnos nux-vomica, Scleria lithosperma, Elaeocarpus serratus* were registered highest Relative frequency, Relative density and Relative basal cover respectively. Of the various plant species available, *Strychnos nux-vomica* securing higher IVI of 28.46 (Table.1). According to Misra (1980) this may be attributed to their high reproductive capacity, quick dispersal of seeds and wind pollination to produce viable seeds. Their existence is also due to certain taboos, strong and supplemented mystic folklore (Gadgil and Vartak, 1975).

The present study envisages toreveal the potentiality for its richness of biodiversity and ecological status of the sacred grove. It is suggested that the studied sacred groves must be given conservation priority to protect valuable endangered medicinal species. Despite the seasonal changes, the anthropogenic were determined to be most influencing factor to affect the species composition and the quantitative ecological attributes of many sensitive species. Therefore construction activities, over grazing, collection of fire wood, tress passing, dumping of waste and many antisocial elements must be checked so as to protect the species in their habitats. Further, ecosystem- specific management plans must be developed to protect the individual species in these sacred groves. Protection of such activities aid in the regulation of ecological process like energy flow, food chain and food web and cycling of materials which would result in ecological balance and stability of ecosystem.

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QUALITATIVE AND QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS AND *IN VITRO* ANTIOXIDANT ASSAYS IN THE TUBER OF *SOLENA AMPLEXICAULIS* (LAM.) GANDHI. (CUCURBITACEAE)

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ABSTRACT

Most of the traditional medicinal plants in India are not scientifically validated. Scientific evaluation of medicinal property along with traditional knowledge is essential to obtain effective drugs for commercial purpose. *Solena amplexicaulis*belongs to the family, Cucurbitaceae, a traditional medicinal plant species of Tamil Nadu, India is being prescribed to cure various diseases. In this study, the objective was to investigate the qualitative and quantitative determinations of certain phytochemicals and *in vitro* antioxidant capabilities of various alcoholic and aqueous tuber extracts of *S. amplexicaulis*by adapting standard procedures. In all the assays methanolic tuber extract registered significantly high amount of secondary metabolites and also it effectively scavenge the free radicals in a concentration dependent manner than the other extracts. These results were compared with synthetic (BHA and BHT) as well as natural antioxidants (rutin and quercetin). The outcome of the study revealed most valuable information and also supports the continued sustainable use of this species in traditional systems of medicine.

Keywords: *Solena amplexicaulis,* Cucurbitaceae, phytochemical analysis, antioxidant properties.

1. INTRODUCTION

Herbal medicines are in huge demand in the developing countries for primary health care because of their effectiveness, safety and lesser side effects. Now traditional medicine is being reevaluated by extensive research on different plant species and their therapeutic principles. The phytochemical compounds play a significant role in biological functions. There is growing interest in correlating the bioactive components of a medicinal plant with its pharmacological activity. Based on accumulative evidence, in recent decades tremendous interest has considerably increased in finding out the natural substances especially from plants (Dezfuli *et al.,* 2014; Servili *et al.,* 2014). Antioxidant compounds in food plays an important role as a health protecting factor. The main characteristic of an antioxidant is its ability to trap free radicals. These free radicals may oxidize nucleic acids, proteins, lipids and DNA that can initiate degenerative diseases (Carlsen *et al.,* 2010).

Solena amplexicaulis is commonly called as creeping cucumber, belongs to the family, Cucurbitaceae mainly distributed in the dry deciduous forests of southern India (Matthew, 1983; Paulsamy and Karthika, 2014). The traditional healers are prescribing the tubers of this species as astringent, appetizer, carminative, cardiotonic, digestive, diuretic, expectorant, invigorating, purgative, stimulant, sour and thermogenic (Dhananjay, 2006). The whole plant is a potential source of natural antioxidant (Venkateshwaralu *et al.,* 2011; Karthika *et al.,* 2012) and antiinflammatory agent (Arun *et al.,* 2011). It is recognized as CNS active, diuretic, febrifuge and hypothermic (Dhananjay, 2006). Crude leaf juice is used to cure jaundice (Mohammed *et al.,* 2011). Raw unripe fruits are eaten to strengthen the body (Jeyaprakash *et al.,* 2011). The decoction of the root is administered orally to cure stomachache (Abdolbaset *et al.,* 2011). The seeds are used as purgative (Jeyaprakash *et al.,* 2011).

However, no study on antioxidant properties has been available for the tuber of this species. To address this lacuna, an attempt has been made to investigate the qualitative and quantitative phytochemical analysis and certain *in vitro* antioxidant activities of successive extracts (hexane, benzene, chloroform, methanol and water) from the tuber of *S. amplexicaulis.* These antioxidants values were compared with commercially available synthetic as well as natural antioxidants.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH$^{\bullet}$), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS$^{\bullet+}$), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), diosgenin (DE), butylatedhydroxyanisole (BHA), butylated hydroxytoluene (BHT), ferric chloride ($FeCl_3$), ferrous ammonium sulphate ($Fe(NH_4)_2(SO_4)_2.6H_2O$),

Folin-Ciocalteu reagent, gallic acid, polyvinyl polypyrrolidone (PVPP), potassium ferricyanide ($K_3Fe(CN)_6$), potassium persulfate ($K_2S_2O_8$), quercetin, sodium carbonate (Na_2CO_3), sodium nitroprusside ($Na_2[Fe(CN)_5NO]$), trichloroacetic acid (TCA), hydrogen peroxide (H_2O_2), L-ascorbic acid (vitamin C) and β-carotene were purchased from Himedia (Mumbai, India). All other reagents and solvents used were of analytical grade.

2.2. Plant material

The fresh tuber parts of *S. amplexicaulis* were collected from the thorny scrub jungles of Madukkarai, Coimbatore district, Tamil Nadu, India during the month of April, 2014. The authenticity of the plant was confirmed by comparing with the reference specimen (Vide No: CPS 313) preserved at Botanical Survey of India, Southern Circle, Coimbatore. The samples were cleaned, washed with copious amount of water, shade dried and coarsely powdered in a Willy Mill to 60 mesh size (Nippon Electricals, Chennai, India) for extraction.

2.3. Preparation of extracts

About 50g of powdered plant material was extracted (50g/250mL) in a soxhlet extractor for 8 to 10 h, sequentially with the alcoholic solvents *viz.,* hexane, benzene, chloroform and methanol and aqueous. Then the extracts were evaporated to dryness and stored at 4°C in storage vials for experimental use.

2.4. Qualitative estimations

All the extracts were subjected to preliminary phytochemical analysis followed by the methods of Harborne (1998) and Trease and Evans (2002).

2.5. Quantitative estimations

Major non-enzymic antioxidants of the plant extracts were determined by using standard quantitative methods. The alkaloid content was gravimetrically determined by the method of Harborne (1998). The total phenolic and tannin contents were estimated and expressed as mg gallic acid equivalent (GAE)/g extract according to the method described by Siddhuraju and Becker (2003). The total flavonoids content was determined spectrophotometrically using a standard curve rutin as per the method of Zhishen *et al.* (1999) and expressed as mg rutin equivalent (RE)/g extract. Content of ascorbic acid was calculated on the basis of calibration curve of authentic L-ascorbic acid and the results were expressed as mg ascorbic acid equivalent (AAE) /g extract, proposed by Klein and Perry (1982). Total saponin content was determined by the method described by Makkar *et al.* (2007) with some modifications. The values were expressed as mg diosgenin equivalents (DE)/g extract.

2.6. Determination of in vitro antioxidant activity

2.6.1. Reducing power assay

The Fe^{3+} reducing power of the extract was determined according to the method suggested by Oyaizu (1986). The plant extracts (300-700 µg/mL) were mixed with 5.0 mL of 0.2 M phosphate buffer of pH 6.6 and 5.0 mL of 1% $K_3Fe(CN)_6$ and the mixtures were incubated at 50°C for 20 min. The reaction was terminated by adding 5.0 mL of 10% TCA (w/v), and the mixture was centrifuged at 1000 rpm for 10 min. The upper layer of the supernatant (5.0 mL) was mixed with 5.0 mL of distilled water and 1.0 mL of 0.1% (w/v) $FeCl_3$ and the absorbance was read at 700 nm. Rutin, quercetin, BHA and BHT served as the reference materials. Increased absorbance indicates increased reductive capability.

2.6.2. DPPH radical scavenging activity

The hydrogen donating capacity was assessed using the stable DPPH$^{\bullet}$ method (Blois, 1958). Briefly, a solution of 0.1mM DPPH$^{\bullet}$ was prepared using methanol. The samples (50–250 µg/mL) were mixed with 5.0 mL of DPPH$^{\bullet}$ solution. Reaction mixture was shaken, incubated at 27°C for 20 min and the absorbance was measured at 517 nm. Results were compared with the activity of rutin, quercetin, BHA and BHT. Per cent DPPH$^{\bullet}$ discolouration of the samples was calculated using the formula:

DPPH radical scavenging activity (%) = [(Control OD – Sample OD) / Control OD] × 100.

Antioxidant activities of the extracts were expressed as $IC_{50,}$ these values were calculated from the linear regression of the percentage antioxidant activity versus concentration of the extracts. A lower IC_{50} value indicates greater antioxidant activity.

2.6.3. Total antioxidant activity

Total antioxidant activity was performed using an improved ABTS$^{\bullet+}$ method proposed by Siddhuraju and Manian (2007). The ABTS radical cation (ABTS$^{\bullet+}$) was generated by a reaction of 7 mM ABTS$^{\bullet+}$ and 2.45 mM potassium persulphate and the mixture was incubated for 12–16 h at room temperature in dark. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated to obtain an absorbance of 0.700 ± 0.02 at 734 nm. 10 µL/mL of sample was added to 1.0 mL of diluted ABTS$^{\bullet+}$ solution. After 30 min of incubation,

absorbance was read at 734 nm. Trolox was used as a reference material.

2.6.4. Inhibition of β – carotene bleaching

The antioxidant capacity of the extract was evaluated using β–carotene-linoleate model system (Taga *et al.,* 1984).1mg of β – carotene was dissolved in 10 mL of chloroform and mixed with 20 µL of linoleic acid and 200 mg of Tween – 40 emulsifier mixture. Chloroform was completely evaporated using rotary vacuum evaporator at 45°C. 50mL of oxygenated distilled water was added to the flask with vigorous shaking, to form an emulsion. 5mL of emulsion was added to 100 µL of sample from each tube, the zero-time absorbance was measured at 470 nm. Subsequent absorbance readings were recorded at 15 min intervals by keeping the sample tubes in a water bath at 50°C until the colour of the control sample disappeared (about 120 min). A blank, devoid of β – carotene, was prepared for background subtraction. Rutin, quercetin, BHA and BHT were used as standards. β – carotene bleaching activity was calculated as:

$$AA\ (\%) = [1- (A_s^0 - A_s^{120}) / (A_c^0 - A_c^{120})] \times 100$$

Where, A_s^0 - absorbance of sample at 0 min, A_s^{120} - absorbance of sample at 120 min, A_c^0 - absorbance of control at 0 min, and A_c^{120} - absorbance of control at 120 min.

2.6.5. Antihaemolytic activity

Antihaemolytic activity was performed according to the method set forth by Naim *et al.* (1976). The erythrocytes from cow blood were separated by centrifugation (2000 rpm for 10 min) and washed with saline phosphate buffer (pH 7.4) until the supernatant become colourless. The erythrocytes were then diluted with saline phosphate buffer to give 4% (v/v) suspension. 500 µg of extract/mL of saline phosphate buffer were added to 2.0 mL of erythrocytes suspension and made up to 5.0 mL with saline phosphate buffer. This mixture was pre-incubated for 5 min and then 0.5 mL of H_2O_2 solution of appropriate concentration in saline buffer was added. The concentration of H_2O_2 in the reaction mixture was adjusted so as to bring about 90% haemolysis of blood cells after 240 min. After the incubation time, the reaction mixture was centrifuged at 1500 rpm for 10 min and the extent of haemolysis was determined by measurement of the absorbance (at 540 nm) corresponding to haemoglobin liberation. Natural and synthetic standards at the same concentration as sample extract were used for comparison.

The percent haemolysis inhibition was calculated using the formula:

$$\text{Inhibition percentage} = [A_{\text{control}} - A_{\text{sample}} / A_{\text{control}}] \times 100$$

Where, $A_{control}$ - absorbance of control and

A_{sample} – absorbance of sample.

2.7. Statistical analysis

All the values were expressed as mean ± standard deviation (SD) of three determinations and subjected to one-way analysis of variance (ANOVA) followed by post hoc Duncan's multiple range test using SPSS (version 9, SPSS Inc., Chicago, USA). $P < 0.05$ was chosen as the criterion for statistical significance.

3. RESULTS

The study revealed that the percent yield of methanolicextract was higher (9.60%) followed by water extract (4.0%). The other solvents *viz.,* hexane, benzene and chloroform yielded very less quantity of residue only (Table 1).

3.1. Qualitative estimations

The major secondary metabolites present in the extracts were varied across the solvents used (Table 1). The methanolic extract of *S. amplexicaulis* tuber containing more number of secondary metabolites than the other extracts studied. Cardiac glycosides were present in all the alcoholic and aqueous extracts of tuber but resins, steroids, terpenoids and triterpenoids were totally absent in all the extracts. However, the degree of precipitation of phytochemicals varies in all the extracts.

3.2. Quantitative estimations

The quantity of phytochemicals estimated were varied among the extracts tested (Table 2).Among the six components, alkaloids (92.02mg/g dry powder) and saponins(39.4-135.8mg DE/g extract) contents were significantly higher and the tannins(0.01-1.57 mg GAE/g extract) content was very low when compared to the other compounds studied. Further, the degree of precipitation of secondary metabolites varies according to the extractive power of the solvents. Mostly the methanol extract contained high amount of secondary metabolites than the other solvents studied.

3.3. In vitro antioxidant activities

In the reducing power assay, the presence of antioxidants in the samples would result in the reduction of Fe^{3+} to Fe^{2+} by donating an electron which can be monitored by spectrophotometrically at 700nm. The reductive abilities displayed an

apparent linear relationship with concentration. The chloroform extract showed higher reductive capability than the other extracts studied. The activity increases exponentially with the increase in concentration of sample. These values were compared with two natural (rutin and quercetin) and two synthetic (BHA and BHT) antioxidants (Fig. 1a and 1b).

DPPH$^{\bullet}$, a stable organic radical, widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors. It was visually noticeable by a colour change from purple to yellow. It possess concentration dependent scavenging activity. The IC_{50}values of the samples were ranging between 150.91 and 345.75μg/mL and compared with standards as shown in Table 3.

In the evaluation of total antioxidant capacity by measuring ABTS$^{\bullet+}$ method, it is known that all the sample extracts were able to quench ABTS radical more effectively and the values were ranged between 2055.4 and 6226.8 μmol Trolox equivalent/g extract. Among the samples investigated, hexane, methanol and water extracts showed maximum values 6129.0, 6172.8 and 6226.8 μmol Trolox equivalent/g extract respectively. On the other hand, the benzene and chloroform extracts registered markedly low ABTS radical scavenging activity (Table 3).

In β carotene/linoleic acid bleaching assay it is known that all the extracts are having potential to inhibitthe peroxidation of linoleic acid and subsequent bleaching to β carotenein various degrees. Apparently, the most effective extracts were benzene (97.85%) and water (95.41%). Furthermore, these values were comparatively higher than those of natural and synthetic antioxidants tested as shown in Fig. 2.

The protective effect of *S. amplexicaulis* tuber extracts and positive standards against H_2O_2 mediated haemolysis were investigated and presented in Fig. 3. In general, all the sample extracts contributed satisfactory antihaemolytic activity ranged between 34 and 53%. Interestingly these values surpassed the efficiency of synthetic antioxidants, BHA (5%) and BHT (7%).

4. DISCUSSION

Many phytochemicals are now studied extensively for their potential ability of curing diseases. Herbal preparations are effectively and extensively used for their medicinal properties and have become increasingly popular worldwide. Standardization of crude drug is an integral part of establishing its correct identity. Qualitative and quantitative phytochemical screening of *S. amplexicaulis* tuber revealed that alkaloids, cardiac glycosides, flavonoids, glycosides, phenols, saponins, vitamin C and tannins were present in this plant. All the active compounds were excessively present in methanol extract (Table 1). Different solvents have been reported to have different capacity to extract phytoconstituents according to their solubility and polarity and most of the compounds dissolve well in high polar solvents (Karthika *et al.,* 2014).

Table 1. Preliminary phytochemical screening of various extracts of *Solena amplexicaulis* tuber.

Solvent	Yield (%)	Phytoconstituents*										
		A	CG	F	G	P	R	S	St	T	Te	Tr
Hexane	0.60	-	-	-	-	-	-	+++	-	-	-	-
Benzene	0.40	-	-	-	-	-	-	++	-	-	-	-
Chloroform	0.17	-	-	-	-	-	-	++	-	-	-	-
Methanol	9.60	+++	+++	+++	++	+++	-	+	-	+++	-	-
Water	4.00	+	+++	+	+++	-	-	-	-	-	-	-

*A – Alkaloids; CG - Cardiac glycosides; F - Flavonoids; G - Glycosides; P - Phenols; R - Resins; S - Saponins; St – Steroids; T - Tannins; Te - Terpenoids; Tr - Triterpenoids. +++ : highly present; ++ : moderately present; + : low, - : absent.

Table 2. Extractive yield, alkaloids, total phenolics, tannins, total flavonoids, vitamin C and saponinscontents of different solvent extracts of tuber of *Solena amplexicaulis* extracts.

Solvent	Total phenolics (mg GAE/g extract)	Tannins (mg GAE/g extract)	Total flavonoids(mg RE/g extract)	Vitamin C (mg AAE/g extract)	Saponins (mg DE/g extract)
Hexane	0.55 ± 0.02^{a}	0.20 ± 0.03^{b}	Not detected	0.069 ± 0.46^{a}	135.8 ± 0.56^{b}
Benzene	0.21 ± 0.01^{a}	0.02 ± 0.01^{a}	Not detected	0.032 ± 0.25^{a}	121.5 ± 0.27^{b}
Chloroform	0.17 ± 0.01^{a}	0.01 ± 0.02^{a}	Not detected	0.014 ± 0.07^{a}	122.4 ± 0.16^{b}
Methanol	6.32 ± 0.18^{c}	1.57 ± 0.47^{d}	8.94 ± 0.21^{b}	0.666 ± 0.76^{c}	55.6 ± 0.13^{a}
Water	2.51 ± 0.12^{b}	0.79 ± 0.06^{c}	4.59 ± 0.25^{a}	0.293 ± 0.82^{b}	39.4 ± 0.14^{a}

Values are mean ± standard deviation (SD) of three independent experiments.
Values not sharing a common letter in a column are significantly different ($P< 0.05$).

Table 3. DPPH• scavenging and total antioxidant activities of different solvent extracts of *Solena amplexicaulis* tuber.

Solvents/Standards	IC_{50} values (µg / mL) DPPH• scavenging activity	Total antioxidant activity (µmol of TE/g dry weight)
Hexane	345.75±0.14^{e}	6129.01±21.31^{b}
Benzene	264.23±0.25^{d}	2311.93±17.25^{a}
Chloroform	214.12±0.11^{c}	2055.45±15.42^{a}
Methanol	150.91±0.18^{b}	6172.81±29.36^{b}
Water	238.15±0.29^{c}	6226.87±23.51^{c}
Rutin	15.75±0.01^{a}	-
Quercetin	20.71±0.04^{a}	-
BHA	21.41±0.11^{a}	-
BHT	34.74±0.26^{a}	-

Values are mean ± standard deviation (SD) of three independent experiments.
Values not sharing a common letter in a column are significantly different ($P < 0.05$).

The reducing power of a compound may serve as a significant indicator for potential antioxidant activity. Reducing properties are generally associated with the presence of reductones. The presence of reductones in sample extracts might cause the reduction of the Fe^{3+}/ferric cyanide complex to Fe^{2+}/ferrous form which can be monitored by measuring the formation of Perl's Prussian blue with absorbance at 700nm. The study revealed that the methanolic extract of *S. amplexicaulis* tuber due to the presence of reductones might significantly contribute the antioxidant activity (Singhal *et al.,*2011).

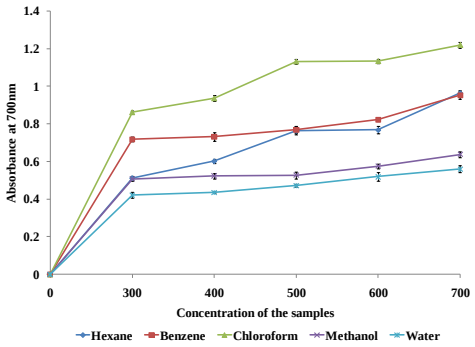

Fig. 1a. Reducing ability of different extracts of *Solena amplexicaulis* tuber. Values are mean ± standard deviation (SD) of three independent experiments.

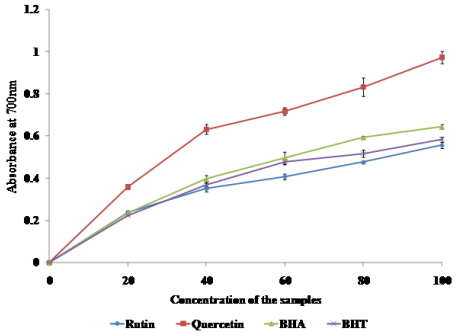

Fig. 1b. Reducing ability of natural and synthetic antioxidants. Values are mean ± standard deviation (SD) of three independent experiments.

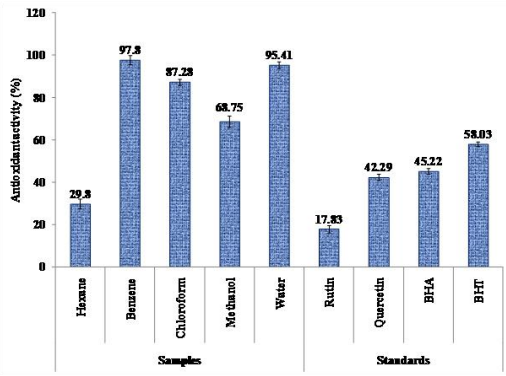

Fig. 2. Lipid peroxidation preventive property of tuber extracts of *Solena amplexicaulis* with certain standardsin β – carotene linoleic acid system. Values are mean ± standard deviation (SD) of three independent experiments.

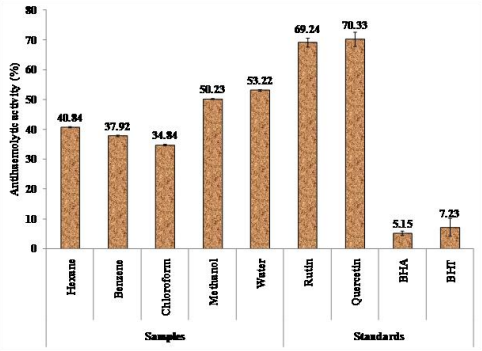

Fig. 3. Antihaemolytic property of tuber extracts of *Solena amplexicaulis* compared with certain standards. Values are mean ± standard deviation (SD) of three independent experiments.

DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable molecule. Antioxidants in the sample on interaction with DPPH•, transfer electron to DPPH• and thus neutralizing its free radical character and convert it to 1-1 diphenyl-2-picryl hydrazine and the degree of discoloration (purple to yellow) indicates the scavenging activity of the drug (Apak *et al.,* 2013).

The results of DPPH• scavenging assay indicate that the methanolic plant extract possess high antioxidant activity (Table 3). The extracts showed a significant correlation with total phenolics, tannins and total flavonoids content (R^2=0.972, R^2=0.952 and R^2=0.953 respectively) and partially correlation with alkaloids and saponins (R^2=0.822 and R^2=0.890 respectively). This high potential of phenolic compounds to scavenge radicals may be explained by their phenolic hydroxyl groups, in addition to synergistic effects of the compounds present in this plant species (Noguer *et al.,* 2014).

$ABTS^{\bullet+}$ (protonated radical) is a blue chromophore produced by the reaction between ABTS and potassium persulphate (Ruby *et al.,* 2009). The characteristic absorbance maxima is at 734nm. The presence of bioactive chemical compounds in the tested extracts that inhibit the potassium persulphate activity may reduce the production of $ABTS^{\bullet+}$. From the results, the aqueous extract of *S. amplexicaulis* tuber possessed the highest $ABTS^{\bullet+}$ scavenging activity. Thus, it might be speculated that the antioxidant activity of the extract may be possibly attributed to the phytochemicals present in it. Further the extract also demonstrated a high correlation with total phenolics and vitamin C content (R^2=0.991 and R^2=0.986 respectively).

In the β carotene bleaching assay, the oxidation of linoleic acid generates peroxyl free radicals due to the abstraction of a hydrogen atom from diallylic methylene groups of linoleic acid. The free radicals well then oxidize the highly unsaturated β carotene. The presence of antioxidants in the sample will minimize the oxidation of β carotene by hydroperoxides. Hydroperoxide formed in this system will be neutralized by the antioxidants from the samples. Thus the degradation rate of β carotene depends on the antioxidant activity of the sample (Chakraborty and Verma, 2010). In the present study all the extracts inhibited peroxidation of linoleic acid and subsequent bleaching to β carotene in various degrees. The extracts showed a significant correlation with total phenolics (R^2=0.999), tannins (R^2=0.993) and vitamin C (R^2=0.988) contents. Therefore, it can be explained that the β carotene bleaching assay of the studied plant extracts may be attributed to the presence of phenolics, tannins and vitamin C in them.

Erythrocytes are considered as major targets for the free radicals which are potent promoters of activated oxygen species. The red blood cells were treated with hydrogen peroxide (H_2O_2) the haemolysis was done. This could be attributed to the oxidizing nature of H_2O_2. In the present study lipid oxidation of cow blood erythrocyte membrane mediated by H_2O_2 induces membrane damage and subsequently haemolysis (Dai *et al.,* 2006). Among the plant samples investigated, benzene and water extracts have higher antihaemolytic activity. Dai *et al.* (2006) also recorded that flavonols and their glycosides are competent antioxidants which are capable of protecting human red blood cells against oxidative haemolysis stimulated by free radical. The extracts demonstrated a high correlation with total phenolics (R^2=0.980), tannins (R^2=0.967), flavonoids (R^2=0.862) and vitamin C (R^2= 0.932) content. The statistical analysis using the Pearson tests indicated a positive linear correlation between the secondary metabolites and antioxidant assays, in agrees with other reports (Ebrahimzadeh *et al.,* 2014; Ghasemi *et al.,* 2014). The analyses were statistically significant ($P<0.05$), showing correlation coefficients greater than 0.748 in this test.

5. CONCLUSION

Based on the active profile exposed through various assays, it can be concluded that major secondary metabolites identified in *S. amplexicaulis*tuber are playing pivotal role in the scavenging of radicals and hence the better antioxidant activity. Hence it is a promising natural source of antioxidant can be used in nutritional or pharmaceutical fields for the prevention of free radical medicated diseases. However, pharmacognostical studies are suggested to confirm the antioxidant ability before going for commercialization.

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ETHNOMEDICINAL PLANTS USED BY THE IRULA TRIBALS OF PALAMALAI HILLS, SOUTHERN WESTERN GHATS OF COIMBATORE, TAMIL NADU, INDIA

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ABSTRACT

The present study is aimed to document the ethnomedicinal plants used for various ailments by the Irula tribes of Palamalai hills, Southern Western Ghats of Coimbatore, Tamil Nadu, India. A total number of 53 plants species belonging to 50 genera and 32 families used by them as herbal medicines to treat several common diseases such as skin diseases, dysentery, cough and cold, cuts and wounds, etc. were documented. Among the plant species Herbs were the primary source of medicine (40%) followed by trees (28%), shrubs (15%) and climbers (17%) and leaves were mostly used (35%) for various illness followed by whole plant (18%), root (13%), stem (7%), bark and rhizome (6%) and seeds and tubers (4%). Most of the medicine prescription by healers is decoction. Therefore, it is suggested to take-up pharmacological and phytochemical studies to evaluate the species to confirm the traditional knowledge of Irulas on medicinal plants.

Keywords: Ethnobotany, Irulas, Palamalai hills, Western Ghats.

1. INTRODUCTION

Ethnobotany word is made from two words ethno and botany and the term was coined by John William Harshberger in the 1896. Ethnobotany is the study of people and study of plants; this is represented good relationship between wild plants (Herbs, Shrubs and Trees) and tribal's. Ethnobotany is the branch of Ethnobiology and complete information about plants and their medicinal uses is given by ethnobotanical studies (Jitin, 2013). India is rich in ethnic diversity and indigenous knowledge that has resulted in exhaustive ethnobotanical studies. There are over 537 different aboriginal groups in India with extensive knowledge of plants (Jain, 1991). Herbal medicine is widely practiced throughout the world from time immemorable. These medicines are safe and environment friendly. The World Health Organization (WHO) defines traditional medicine as practices, knowledge and belief systems which uses minerals, plants and animal based remedies, spiritual therapies and exercises to prevent, treat and maintain well being (WHO, 2003). According to the WHO, about 80% of the population of the world depends on traditional medicine, mostly herbal remedies, for their primary health care needs (Muthu *et al.,* 2006). In the developed countries people are seeking for herbal medicine because of their scarcity side effects compared to the synthetic drugs. According to WHO 70 to 90 percent of world population especially from developing countries, use plant remedies for their health care (Belachew, 1984; Nair and Nathan, 1998). Developing countries like India, Pakistan and China have identified potential usage of medicinal plants, and integrated them in to their overall health care system (Andrew, 1982).

The indigenous traditional knowledge of medicinal plants of various ethnic communities, where it has been transmitted orally for centuries is fast disappearing from the face of the earth due to the advent of modern technology and transformation of traditional culture (Ganesan *et al.,* 2004; Rajadurai *et al.,* 2009). The recent reports have indicated that, 25% of the modern drugs are derived from the extract of medicinal plants. It is estimated that 70% to 80% of the people worldwide rely chiefly on traditional healthcare system and largely on herbal medicines (Robert and John, 1983; Shanley and Luz, 2003; Kaur *et al.,* 2011). Until recently, plants were important sources for the discovery of novel pharmacologically active compounds, with many blockbuster drugs being derived directly or indirectly from plants (Newman and Cragg, 2007; Li, 2010).

Irulars are small tribal community in the part of Dravidian language group which is spoken in south eastern India. They belong to the Negrito (or Negroid) race which is one of the six main ethnic groups that add to the racial mosaic of India (Deepa *et al.,* 2002). The origin of the word "Irula" is not clear. Some surmise that, it is derived from the Tamil word "Irual" implying the dark complexion of the Irula, often being spotted by villagers as distinct silhouettes in the forests and supporting their local name, the Forest People (Fuchs, 1973). They do not practice agriculture and therefore, fully depend on

forest produces and wild animals. Other occupations of the Irulars include intermittent farm labour and the legendary profession of snake charming (Venkatachalapathi *et al.,* 2015; 2016). In recent years some researchers have reported various medicinal plants used by Irula tribals in Anaikatty hills, Siruvani hills and Maruthamalai hills of Coimbatore district (Palanisamy, 1993; Balasubramanian *et al.,* 1997; Nikkitha, 1999; Hamasavalli, 2001; Karthikeyani, 2003; Senthilkumar, 2004; Senthilkumar *et al.,* 2006; Geetha *et al.,* 2007; Paulsamy, 2011; Tamilselvi *et al.,* 2016). Therefore, the aim of the study was, to documentation of traditional knowledge of utilization of medicinal plants used by Irula tribes for various ailments in Palamalai hills of Coimbatore district, Tamil Nadu.

2. MATERIALS AND METHODS

2.1. Study area

The present work was undertaken in the Palamalai hills located in the Coimbatore district of Tamil Nadu, South India. Palamalai is an offshoot of the Eastern Ghats geographically contiguous with the Billigrirangaa hills range as they reach out to merge with the Western Ghats at Nilgiris. It lies at an altitude of 1839 m above mean sea level and an attitude of 1400 m on the Western Ghats (Fig. 1). The ethnobotanical survey was carried out during October 2009 to March 2010 among Irula population residing in this area.

2.2. Data collection

The data on medicinal plants was recorded through interview, discussion and field observation with knowledgeable elder people using standard methods adopted by Jain (1991) and Jain and Goel (1995). Out of 19, 10 were male and 9 female respondents under the age group of 35 to 70 years. The information about plants and their local names, parts of plant used for preparation of drug and mode of administration were documented in the field survey and it was confirmed by cross-checking with respondents and also with the already existing literature.

The collected plant species were identified with help of The Flora of Presidency of Madras (Gamble and Fischer, 1915-1936) and confirmed by comparing authentic specimens in Madras Herbarium (MH) at Botanical Survey of India, Southern circle, Coimbatore and through recent floras and taxonomic revisions. The voucher specimens were deposited at the Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu.

3. RESULTS AND DISCUSSION

The present study revealed the use of 53 species of plants distributed in 50 genera belonging to 32 families which were commonly used by Irula tribal healers of Palamalai hills, southern Western Ghats of Coimbatore district, Tamil Nadu for the treatment of 35 types of ailments. The prominent family was Fabaceae with 6 species, followed by Euphorbiaceae with 4 species, Caesalpiniaceae and Verbenaceae with 3 species each, Acanthaceae, Apocynaceae, Asclepiadaceae, Asteraceae, Lamiaceae, Malvaceae, Poaceae and Rutaceae, Solanaceae contributed with 2 species each and Aizoaceae, Amaranthaceae, Anacardiaceae, Aristolochiaceae, Boraginaceae, Caricaceae, Cleomaceae, Convolvulaceae, Cucurbitaceae, Liliaceae, Meliaceae, Mimosaceae, Myrtaceae, Papaveraceae, Piperaceae, Rhamnaceae, Sapindaceae, Vitaceae and Zygophyllaceae contributed with 1 species each. All the reported species were arranged alphabetically and provided the botanical name of the plant, family, specimen number, local (Tamil) name, life form, part (s) used, ailments treated and mode of administration (Table 1).

Herbs were the primary source of medicine (40%) followed by trees (28%), shrubs (15%) and climbers (17%) (Fig. 2). The frequent use of among the indigenous communities is a result of wealth of herbaceous plants in their environs (Tabuti *et al.,* 2003; Uniyal *et al.,* 2006; Giday *et al.,* 2010) and a Yercaud hills harbours more number herbs as compared to trees, shrubs and climbers (Parthipan *et al.,* 2011). Among the different parts used, the leaves (56%) were most frequently used for the preparation of medicine solely or in combination with other parts. It was followed by bark and whole plant (7% each), roots (6%), fruit and seeds (5% each), stem, flowers and latex (4% each) and bulb (2%) (Fig. 3). Many indigenous communities throughout the world also utilized mostly leaves for the preparation of herbal medicines (Teklehaymanot *et al.,* 2007; Cakilcioglu and Turkoglu, 2010; Gonzalez *et al.,* 2010). The reason why leaves were used mostly is that they are collected very easily than underground parts, flowers, fruits, etc. (Giday *et al.,* 2009).

The preparation and utilization of plant parts were grouped in to five categories (Fig. 4). Of these, most commonly used method preparation was decoction (42%) followed by paste (36%) juice (12%), powder (7%) and raw (3%).

Table 1. List of medicinal plant species used for their health care by Irula tribals of Palamalai hills, Western Ghats of Coimbatore district, Tamil Nadu.

S. No.	Binomial Name	Local name	Family	Parts used	Medicinal uses	Mode of administration
1	**Trees**					
	Aegle marmelos (Linn.) Corr.	Vilvam	Rutaceae	Leaf	Dyspepsia	Decoction
2	*Albizia amara* (Roxb.) B. Boivin.	Arapu	Mimosaceae	Flower and seeds	Piles	Paste
					Diarrhea	Decoction
3	*Azadirachta indica* A. Juss.	Vembu	Meliaceae	Bark	Stomach worms	Decoction
4	*Carica papaya* L.	Pappali	Caricaceae	Latex	Scorpion sting	Paste
					Snake bites	Paste
5	*Emblica officinalis* Gaertn.	Nellikkai	Euphorbiaceae	Fruit	Cold and cough	Decoction
6	*Mangifera indica* L.	Maamaram	Anacardiaceae	Leaf	Cracks	Paste
7	*Pongamia pinnata* (Linn.) Pierre.	Pungamaram	Fabaceae	Leaf	Ulcers	Decoction
					Diabetes	Decoction
8	*Psidium guajava* L.	Koiya	Myrtaceae	Leaf	Dysentery	Juice
9	*Pterocarpus marsupium* Roxb.	Vengai	Fabaceae	Bark	Dysentery	Decoction
10	*Tamarindus indica* L.	Puliyamaram	Caesalpiniaceae	Fruit	Digestive	Raw
11	*Tectona grandis* Linn. f.	Tekkumaram	Verbenaceae	Leaf	Skin diseases	Paste
					Ulcers	Decoction
12	*Vitex negundo* L.	Notchi	Verbenaceae	Flowers	Diarrhea	Decoction
					Cardiac disorders	Decoction
13	*Wrightia arborea* Mabberley.	Karupaalai	Apocynaceae	Bark	Kidney stones	Powder
14	*Wrightia tinctoria* (Roxb.) R. Br.	Veppalai	Apocynaceae	Leaf	Headache	Paste
15	*Ziziphus mauritiana* L.	Ilanthai	Rhamnaceae	Leaf	Wound healing	Paste
16	**Shrub**	Pirammathandu	Papaveraceae	Seed	Cracks at foot	Powder
	Argemone mexicana L.					
17	*Calotropis gigantea* (Linn.) R. Br.	Erukku	Asclepiadaceae	Latex	Wound healing	Paste
18	*Cassia auriculata* L.	Avarai	Caesalpiniaceae	Leaf	Scabies	Paste
					Bone fractures	Paste
19	*Cassia tora* L.	Tagarai	Caesalpiniaceae	Leaf	Leprosy	Decoction
					Ulcers	Decoction
20	*Indigofera tinctoria* L.	Averi	Fabaceae	Root	Snake bites	Decoction
21	*Jatropha curcas* L.	Kattu amanaku	Euphorbiaceae	Stem	Digestion	Juice
22	Lablab purpureus (Linn.) Sweet.	Avarai	Fabaceae	Leaf	Ring worm	Paste
23	*Lantana camara* L.	Unnichedi	Verbenaceae	Leaf	Cuts and wounds	Paste
24	**Herbs**	Thuthi	Malvaceae	Root	Fever	Decoction
	Abutilon indicum (Linn.) Sweet.					

25	*Achyranthes aspera* L.	Naayuruvi	Amaranthaceae	Leaf	Dog bite	Paste
26	*Allium cepa* L.	Vengayam	Liliaceae	Bulb	Boils	Paste
27	*Andrographis echioides* (Linn.) Nees.	Gopuram thangi	Acanthaceae	Leaf	Fever	Juice
28	*Andrographis paniculata* (Burm. f.) Wall. *ex* Nees.	Nilavembu	Acanthaceae	Bark	Fever	Decoction
					Skin diseases	Paste
					Snake bite	Paste
29	*Bambusa arundinacea* (Retz.) Roxb.	Mungil	Poaceae	Leaf	Wound healing	Paste
30	*Cleome gynandra* L.	Veli Keerai	Cleomaceae	Leaf	Ear ache	Juice
31	*Cynodon dactylon* Dress.	Arugampull	Poaceae	Whole plant	Eye disorder	Juice
32	*Datura metel* L.	Oomethai	Solanaceae	Leaf	Respiratory troubles	Juice
33	*Eclipta prostrata* L.	Karasilaganni	Asteraceae	Leaf	Black hair	Paste
					Skin diseases	Paste
					Wound healing	Paste
34	*Euphorbia hirta* L.	Amman pacharisi	Euphorbiaceae	Leaf	Dysentery	Decoction
35	*Evolvulus alsinoides* L.	Vishnukranthi	Convolvulaceae	Leaf	Wound healing	Paste
36	*Mollugo nudicaulis* L.	Parpadakam	Aizoaceae	Leaf	Boils	Paste
37	*Leucas aspera* L.	Tumbai	Lamiaceae	Leaf	Bronchitis	Decoction
38	*Ocimum americanum* L.	Nai thulasi	Lamiacaeae	Leaf	Cold	Decoction
39	*Phyllanthus amarus* Schum. and Thonn.	Kila nelli	Euphorbiaceae	Leaf	Jaundice	Juice
40	*Sida cordifolia* L.	Nilathuthi	Malvaceae	Leaf	Ear ache	Juice
41	*Solanum nigrum* L.	Manathakkali	Solanaceae	Leaf	Mouth sores	Raw
42	*Tribulus terrestris* L.	Nerunjimul	Zygophyllaceae	Fruit	Cough	Juice
43	*Trichodesma indicum* L.	Kavil thumbai	Boraginaceae	Leaf	Diarrhea	Decoction
					Dysentery	Decoction
44	*Tridax procumbens* L.	Kinathupoondu	Asteraceae	Leaf	Wound healing	Paste
45	**Climbers**					
	Abrus precatorius L.	Kundumani	Fabaceae	Root	Cough	Decoction
46	*Aristolochia bracteolata* Lam.	Aaduthinnappalai	Aristolochiaceae	Whole plant	Skin diseases	Decoction
					Snake bites	Decoction
47	*Cardiospermum halicacabum* Linn.	Mudakkathan	Sapindaceae	Whole plant	Swellings	Paste
					Joints pains	Paste
48	*Cissus quadrangularis* L.	Perandai	Vitaceae	Stem	Stomachache	Decoction
49	*Clitoria ternatea* L.	Thuthi	Fabaceae	Whole plant	Fever	Decoction
50	*Coccinia indica* Wight and Arn.	Kovaikai	Cucurbitaceae	Leaf	Diabetes	Powder
51	*Pergularia daemia* (Forsk.) Chiov.	Veliparuthi	Asclepiadaceae	Leaf	Cold	Decoction
					Fever	Decoction
52	*Piper nigrum* L.	Kurumilagu	Piperaceae	Seed	Cough	Powder
					Throat infection	Powder
53	*Toddalia asiatica* L.	Kindu mullu	Rutaceae	Leaf	Stomachache	Decoction

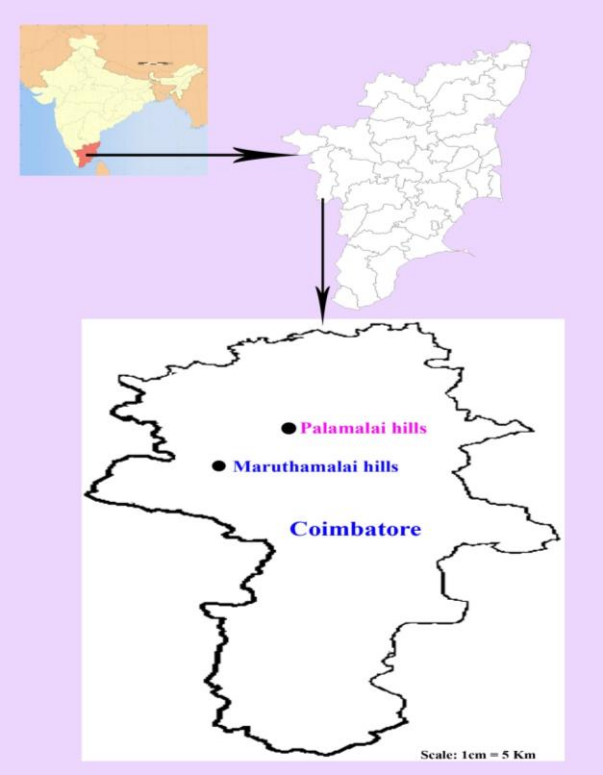

Fig. 1. Location of the study area of Palamalai hills.

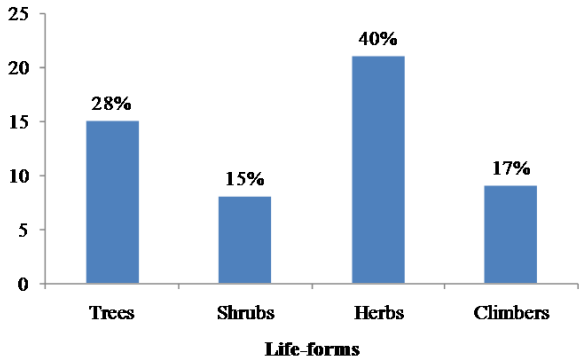

Fig. 2. Per-cent life-forms of medicinal plants used by Irulas in Palamalai hills.

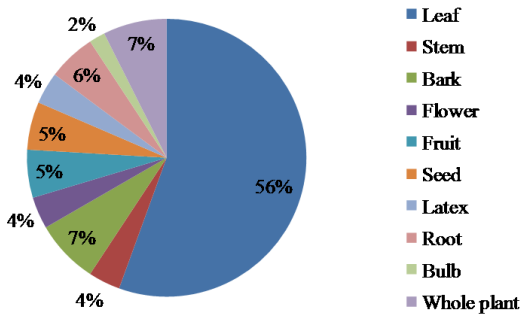

Fig. 3. Per-cent plant parts used for medicine preparation.

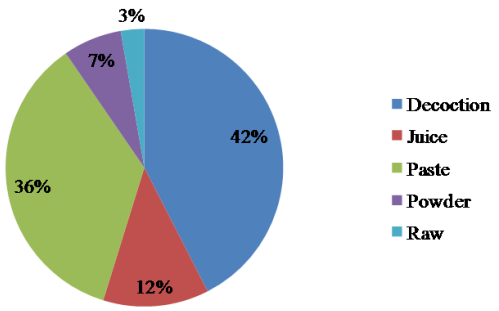

Fig. 4. Per-cent form of medicine preparation by Irula tribe.

Preparation of paste for the treatment of ailments is a common practice among the tribal communities in the world (Giday *et al.,* 2007; Roosita *et al.,* 2008; Giday *et al.,* 2010). The paste was prepared by grinding the fresh or dried plant parts with oil or water. The powder was prepared by grinding of shade dried plant parts. The decoction was obtained by boiling the plant parts in water until the volume of the water reduced to minimum or required amount. The inhalation was done by the burning of plant parts and inhaled the smoke through nose or mouth (Roosita *et al.,* 2008).

The ethnomedicinal studies evidently pointed out that, instead of trying to identify the active compounds and pharmacological actions of plants through massive collection of plants from natural sources, it is better to start investigating the efficacy of the plant based on their use in folk medicine, since most of the commercially proven drugs used in modern medicine were initially tried in crude form in traditional of folk healing practices (Fabricant and Fransworth, 2001).

4. CONCLUSION

All the enumerated plant species are very commonly used for various ailments by the Irula tribes of Palaalai hills, southern Western Ghats of Coimbatore district, Tamil Nadu. A few interesting observations made in the present study are: the use of *Coccinia indica* and *Pongamia pinnata* for diabetes, *Vitex negundo* for cardiac disorders, *Carica papaya* and *Indigofera tinctoria* for poisonous snake bites, *Cardiospermum halicacabum* for joints pains, *Cassia auriculata* for scabies and bone fractures. Although traditional medication is still practiced in this area, it is now fast disappearing due to modern life style. Hence, proper documentation and preservation of traditional skills and technology of medicinal plants is a vital necessity. Further investigations on

pharmacological importance of these plants and their diversity may add new knowledge to the traditional medical and cultural systems.

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PROPAGATION STRATEGIES OF *TRIBULUS TERRESTRIS* L. A PORTENTOUS MEDICINAL PLANT EMPLOYING TISSUE CULTURE TECHNOLOGY

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ABSTRACT

A protocol for micropropagation of *Tribulus terrestris,* an important medicinal herb was established using juvenile explants *viz.,* leaf, node and internode. All the explants were tested for callus induction on Murashige and Skoog's (MS) medium, supplemented with BAP, NAA and 2,4-D. Among the three explants leaf explant responded well (98%) for the callus induction in the MS medium composted with BAP and NAA (4.0 and 0.5 mg/L) followed by the nodal segments (58.75%) in the same medium. Maximum number of shoot induction from the callus of leaf derived explants (91.1%) was perceived on MS medium fortified with BAP 4.0 mg/L and NAA (0.5 mg/L). Moreover, root elongation and profuse rooting percentage (77.19%) were achieved when the well-grown shoots were cultured on MS media supplemented with IAA (2.0 mg/L) for leaf callus derived shoots. The regenerated plantlets were hardened and established at 80% survival rate in hardening media encompassed with red soil, sand and vermicompost in the ratio of 1:1:1 by volume.

Keywords: *Tribulus terrestris,* leaf, node and internodal explants, *in vitro* regeneration.

1. INTRODUCTION

The genus, *Tribulus* comprises about 20 species of creeping shrubs or herbs, of which *Tribulus terrestris* L. (Zygophyllaceae) is the most common. It is also known as 'puncture vine' native of Mediterranean region and that found in warm regions of Europe, Asia, America, Africa and Australia (Frohne, 1999). It is a tap rooted, prostrate, procumbent flowering plant, the stem grows up to 2cm long, leaves are opposite, 4-8 pairs, and spear shaped leaflets, presence of hairs on margin of the leaf. Flowers are yellow. Seeds enclosed in a woody star-shaped structure 5-7mm long and 5-6mm wide (carpels). The leaf parts of *T. terrestris* are used to cure renal problems by the tribals of Al-Rass province, Saudi Arabia (Gamal *et al.,* 2010).Juice of fresh leaves is given to animals in case of colic and chronic cough by the rural farmers and traditional herbal healers from Tikamgarh District of Bundelkhand, Central India (Verma, 2014). The traditional practitioners of Palakkad District prescribed the decoction of dried seeds for diuretic and antilithiatic activity (Smitha *et al.,* 2014). The native communities of Terai forest of Western Nepal were stipulated the entire plant is given orally for the treatment of urinogenital tract infection (Anant Gopal *et al.,* 2012).

Conventionally the plant is propagated through seeds, but it yields very low percentage of germination under natural and laboratory conditions (Raghu, 2005; Raghu and Mohanan, 2006). Due to its high medicinal value and increasing demand, *in vitro* propagation strategies have great importance to reacclimatize the plant in a large-scale is vital in current scenario. Hence, the present study was aimed at to regenerate the plantlets of *T. terrestris* using the juvenile leaf, node and internodal explants and to guard against its overexploitation.

2. MATERIALS AND METHODS

2.1. Collection of the plant materials

Healthy and immature leaf, node and internodal segments of the study species, *Tribulus terrestri* (Fig. 1) were collected foot hills of Maruthamalai, Coimbatore district and used as explants.

2.2. Surface sterilization of the explants

The leaf, node and internodal explants were washed with running tap water and they were cut into small pieces. Apparently, the dust particles on the surface were reduced with a surfactant, tween-20 (5%w/v). After 5 minutes they were rinsed with double distilled water. Likewise, the elimination of fungus on the surface of the explant were done by a fungicide, bavastin (5%w/v) for 5 minutes and rinsed with double distilled water for 3-4 times. Furthermore, screening of bacterial contamination on the explants was carried out with ampicillin (5%w/v) for 5 minutes followed by 3-4 rinses in double distilled water. Finally, removal of toxic chemicals were carried out by dipping the explants

into 0.1% $HgCl_2$ for 5 minutes and then it was rinsed with autoclaved double distilled water, within the Laminar air flow chamber.

2.2. MS media and culture conditions

After surface sterilization the leaf, node and internodal (0.5cm) explants were inoculated on MS media (Murashige and Skoog, 1962) containing 30% sucrose solidified with 8% agar (Tissue culture grade, Hi-Media, India). The pH of the medium was adjusted to 5.6-5.8 with 0.1 N NaOH or 0.1 N HCl prior to the addition of agar. It was autoclaved at 121°C and 15lbs/inch^2 pressure for 15 minutes. The cultures were maintained under white fluorescent light having 2000 lux light intensity. The incubation temperature was adjusted to 25±2°C with 16 hours light and 8 hours dark period in every 24 hours cycle.

2.3. Callus induction

The leaf, node and internodal explants were inoculated on MS medium supplemented with different concentrations and combinations of growth regulators *viz.,* BAP, NAA and 2,4-D. The days required for callus formation, percentage, colour and nature of the callus was perceived.

2.4. Shoot regeneration

For the shoot induction the *in vitro* derived callus was transferred to MS medium containing different concentrations and combinations of growth regulators such as BAP, NAA and 2,4-D. The percentage of explants responding, number of shoots per callus and length of the shoots were recorded after 40 days of culture.

2.5. Root induction and acclimatization

The thrived shoots were shifted to MS medium fortified with various concentrations of IBA, NAA and 2,4-D for root formation. The rooting attributes *viz.,* per cent of shoots responding for rooting, number of roots per shoot and the length of the roots were observed. The rooted plantlets were transferred to the hardening medium containing various combinations of hardening mixtures and the rate of survivability was determined.

2.6. Statistical analysis

In vitro regeneration of the study species were done using thirty explants and were repeated thrice. Data were subjected to statistical analysis (ANOVA) and means of different experiments were compared by using Duncan's Multiple Range Test ($p<0.05$) (Duncan, 1955).

3. RESULTS

The number of days required for callus induction after the inoculation of explants was varied between 12 and 30 for the study species, *T. terrestris* (Table 1). A higher percentage of callus formation (98.97%) in leaf segments were perceived in the MS medium containing the growth regulator, BAP and NAA at 4.0 and 0.5mg/L respectively (Fig. 2, a and b). Next to the leaf explant the nodal explants showed higher degree (58.75%) of callus induction in same concentration. On the other hand, intermodal segments produced very poor response (39.36%) of callus formation in the above said concentration. From the results two well responded explants (leaf and node) were taken for further shoot and root regeneration.

Table 2 shows the shoot induction (91.1%) of leaf derived callus explants of the species, *T. terrestris.* The best shoot induction (91.1%) was noticed in the MS medium fortified with BAP (4.0mg/L) and NAA (0.5mg/L). It also displayed the higher number of shoots (11.56 shoots/callus) and shoot length (6.5cm) (Fig. 2, c). Table 3 and Fig. 3, a and b exhibits the shoot induction from the nodal explants of the study species. The maximum percentage of shoot induction (65.53%) was viewed in the MS medium fortified with BAP (4.0 mg/L) and NAA (0.5 mg/L). The greater number of shoots (12.66 shoots/callus) and shoot length (6.9cm) were also observed in the same medium.

Root induction of leaf derived callus of the species, *T. terrestris* is depicted in Table 4 and Fig.2, d. The MS medium composed with IAA (2.0 mg/L) alone unveiled maximum root proliferation than the other concentration and combinations tried. It also produced higher number of roots (9.37 roots/shoot) and root length (7.3cm). The root induction of the node derived shoot explants of study species is given in Table 5 and Fig. 3, c. The higher percentage (60.67%) of root induction was identified in the MS medium composed with IAA (2.0 mg/L) alone and this concentration also revealed higher number of roots (12.67 roots/shoot) and the root length (7.5 cm).

Hardening experiments were conducted for two explants of the study species, *T. terrestris* by using various hardening media to determine the survivability rate of plantlets (Tables 6 and 7). The survivability rate of leaf callus derived plantlets was significantly higher (80.46%) in the hardening medium comprised by garden soil, sand and vermicompost in the ratio of 1:1:1 by volume followed by 75.24% survivability rate was obtained from decomposed coir waste, perlite and compost in

the ratio of 1:1:1 by volume (75.24%) (Table 6 and Fig. 2, e). On the other hand, it was noticed that the nodal callus derived plantlets registered higher survivability (70.56%) in the hardening medium encompassed by vermiculate, coir waste and forest litter in the of 1:1:1 by volume followed by 66.45% of survivability was seen in decomposed coir waster, perlite and compost in the ratio of 1:1:1 by volume (Table 7 and Fig. 3, d). The study divulged that all hardening media except red soil combined with sand were suitable for maintaining the higher rate of plantlet survivability, above 50%.

Table 1. Effect of different concentrations of growth regulators on per cent callus induction from leaf, node and internode explants of the species, *Tribulus terrestris.*

Growth regulators (mg/L)			Days required for callus formation after inoculation			Callus formation (%)		
			Explants			Explants		
BAP	NAA	2,4-D	Leaf	Node	Internode	Leaf	Node	Internode
0.5	0.5	0.0	23	15	-	65.31±0.82^{i}	45.34±1.63^{h}	11.31±0.14^{i}
1.0	0.5	0.0	23	14	12	70.22±1.63^{g}	51.23±0.82^{d}	19.00±1.63^{g}
1.5	0.5	0.0	27	22	13	72.34±0.82^{f}	49.45±0.82^{e}	28.32±0.82^{ef}
2.0	0.5	0.0	15	23	15	73.44±1.63^{cd}	55.00±0.82^{b}	25.19±1.63^{f}
2.5	0.5	0.0	26	26	14	77.00±1.63^{b}	51.85±0.82^{de}	32.42±1.63^{bc}
3.0	0.5	0.0	28	27	16	75.18±0.82^{cd}	47.45±1.63^{g}	32.17±0.82^{b}
3.5	0.5	0.0	18	15	17	76.56±1.60^{c}	19.00±1.63^{l}	10.00±1.63^{ij}
4.0	0.5	0.0	19	12	-	98.97±0.63^{a}	58.75±1.63^{a}	-
0.5	0.0	0.5	21	13	16	63.76±1.63^{kl}	34.31±0.41^{i}	16.24±2.45^{h}
1.0	0.0	0.5	17	17	18	61.46±1.63^{m}	47.26±1.63^{f}	23.18±0.82^{g}
1.5	0.0	0.5	23	16	17	64.00±0.82^{k}	54.47±0.82^{c}	39.36±0.82^{a}
2.0	0.0	0.5	18	14	13	65.21±1.63^{ij}	31.57±0.33^{j}	9.14±1.63^{jk}
2.5	0.0	0.5	20	15	14	69.64±0.82^{h}	49.89±0.82^{ef}	10.67±0.82^{j}
3.0	0.0	0.5	22	23	17	72.77±1.63^{de}	54.16±1.63^{bc}	28.17±0.24^{de}
3.5	0.0	0.5	23	27	15	76.17±1.63^{bc}	57.00±0.82^{ab}	31.34±0.82^{cd}
4.0	0.0	0.5	25	30	-	73.43±0.82^{b}	24.11±1.63^{k}	-

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

Table 2. Effect of different concentrations of growth regulators on shoot initiation, shoot number and shoot length after the subculturing of leaf derived callus of the species, *Tribulus terrestris.*

Growth regulators (mg/L)			Culture response (%)	No. of shoots/callus	Shoot length (cm)
BAP	NAA	2,4-D			
0.5	0.5	0.0	20.2±0.81^{m}	1.57±0.82^{j}	1.4±0.82^{h}
1.0	0.5	0.0	24.6±0.96^{k}	3.52±1.63^{hi}	2.6±0.82^{g}
1.5	0.5	0.0	34.1±0.99^{j}	5.23±0.82^{g}	3.0±1.63^{ef}
2.0	0.5	0.0	38.4±1.34^{ij}	6.42±1.63^{ef}	3.5±0.82^{de}
2.5	0.5	0.0	50.0±2.23^{h}	5.18±1.63^{fg}	2.6±0.82^{fg}
3.0	0.5	0.0	22.4±0.84^{l}	4.00±0.82^{h}	3.2±1.63^{e}
3.5	0.5	0.0	32.5±1.43^{k}	6.65±0.820^{e}	3.8±1.63^{d}
4.0	0.5	0.0	91.1±1.33^{a}	11.56±1.63^{a}	6.5±0.82^{a}
0.5	0.0	0.5	54.8±1.75^{f}	9.38±2.45^{bc}	5.9±1.63^{ab}
1.0	0.0	0.5	66.2±2.29^{d}	8.76±1.63^{c}	4.2±0.82^{cd}
1.5	0.0	0.5	53.4±1.26^{g}	7.47±1.63^{d}	5.3±163^{b}
2.0	0.0	0.5	58.2±1.22^{ef}	9.98±0.16^{b}	4.7±0.82^{bc}
2.5	0.0	0.5	69.5±1.50^{c}	8.66±0.82^{cd}	4.2±1.63^{c}
3.0	0.0	0.5	77.7±1.49^{b}	6.22±1.63^{f}	3.8±0.19^{d}
3.5	0.0	0.5	58.1±1.00^{e}	4.16±1.63^{gh}	2.7±0.82^{f}
4.0	0.0	0.5	39.4±1.07^{i}	3.18±0.33^{ij}	1.8±0.82^{gh}

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

Table 3. Effect of different concentrations of growth regulators on shoot initiation, shoot number and shoot length after the subculturing of node derived callus of the species, *Tribulus terrestris.*

Growth regulators (mg/L)			Culture response (%)	No. of shoots/callus	Shoot length (cm)
BAP	NAA	2,4-D			
0.5	0.5	0.0	43.24±1.63^{fg}	4.78±0.82^{gh}	1.9±0.82^{j}
1.0	0.5	0.0	51.98±0.82^{d}	5.45±0.8^{g}	2.5±0.82^{h}
1.5	0.5	0.0	55.00±1.63^{c}	6.36±2.45^{fg}	2.8±1.63^{gh}
2.0	0.5	0.0	46.65±1.63^{e}	4.25±1.63^{hi}	4.1±0.82^{d}
2.5	0.5	0.0	34.76±0.82^{i}	2.00±0.82^{i}	3.4±0.82^{f}
3.0	0.5	0.0	28.54±0.82^{l}	7.67±16.3^{de}	1.9±0.82^{jk}
3.5	0.5	0.0	32.34±1.63^{j}	6.97±1.63^{f}	3.2±1.63^{g}
4.0	0.5	0.0	65.53±0.82^{a}	12.66±0.82^{a}	6.9±0.82^{a}
0.5	0.0	0.5	21.58±0.82^{m}	10.73±1.63^{b}	4.1±0.82^{d}
1.0	0.0	0.5	43.34±0.24^{f}	9.86±0.82^{bc}	2.5±0.82^{hi}
1.5	0.0	0.5	38.67±0.82^{h}	9.67±1.63^{cd}	5.5±1.63^{b}
2.0	0.0	0.5	46.14±0.82^{ef}	7.47±0.16^{e}	4.3±1.63^{cd}
2.5	0.0	0.5	41.00±1.63^{g}	9.81±0.82^{c}	4.8±0.16^{c}
3.0	0.0	0.5	57.45±0.82^{bc}	7.15±1.63^{ef}	4.0±0.82^{e}
3.5	0.0	0.5	58.88±0.82^{b}	4.66±0.82^{h}	5.3±0.82^{bc}
4.0	0.0	0.5	29.62±1.63^{k}	8.75±0.24^{d}	3.9±1.63^{ef}

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

Table 4. Effect of different concentrations of growth regulators on rooting percentage, root number and root legth after the subculturing of leaf derived shoot from callus of the species, *Tribulus terrestris.*

Growth regulators (mg/L)			Shoots rooted (%)	No. of roots/shoot	Root length (cm)
IBA	IAA	2,4-D			
0.5	0.5	0.0	42.36±0.82^{kl}	3.35±0.82^{i}	3.1±0.41^{hi}
1.0	0.5	0.0	47.79±1.63^{j}	4.76±0.41^{g}	2.8±0.65^{jk}
1.5	0.5	0.0	53.59±0.82^{gh}	5.48±1.63^{ef}	3.0±0.82^{j}
2.0	0.5	0.0	57.43±0.41^{f}	4.25±0.82^{hi}	4.8±0.65^{k}
2.5	0.5	0.0	61.90±0.16^{e}	6.46±0.82^{cd}	5.4±0.33^{ef}
3.0	0.5	0.0	63.00±0.82^{d}	5.56±1.63^{e}	4.0±0.82^{g}
0.0	0.5	0.0	44.75±0.65^{k}	4.26±0.41^{h}	3.0±0.82^{ij}
0.0	1.0	0.0	53.47±1.63^{gh}	5.38±0.82^{f}	5.8±1.63^{e}
0.0	1.5	0.5	75.38±0.82^{b}	8.76±0.82^{b}	6.5±0.82^{bc}
0.0	2.0	0.5	77.19±1.63^{a}	9.37±1.63^{a}	7.3±0.82^{a}
0.0	2.5	0.5	66.46±0.82^{c}	6.65±0.41^{c}	6.5±0.41^{b}
0.0	3.0	0.5	54.57±0.41^{g}	5.98±0.82^{d}	5.8±0.82^{c}
0.0	0.0	0.5	35.86±1.63^{n}	2.43±1.63^{k}	2.3±1.63^{k}
0.0	0.0	1.0	41.45±0.82^{lm}	3.23±0.82^{ij}	3.3±0.82^{h}
0.0	0.0	1.5	48.34±1.63^{ij}	4.49±1.63^{gh}	4.9±1.63^{f}
0.0	0.0	2.0	49.64±0.82^{i}	5.75±0.41^{d}	5.5±0.41^{cd}
0.0	0.0	2.5	38.28±1.63^{m}	3.00±1.63^{j}	3.0±1.63^{j}
0.0	0.0	3.0	30.17±0.82^{o}	1.37±0.82^{l}	1.7±0.82^{h}

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

Table 5. Effect of different concentrations of growth regulators on rooting percentage, root number and root legth after the subculturing of node derived shoot from callus of the species, *Tribulus terrestris.*

Growth regulators (mg/L)			Shoots rooted (%)	No. of roots/shoot	Root length (cm)
IBA	IAA	2,4-D			
0.5	0.0	0.0	43.24±1.63^{h}	4.78±0.82^{k}	1.9±0.82^{l}
1.0	0.0	0.0	51.98±0.82^{f}	5.45±0.82^{i}	2.5±0.82^{k}
1.5	0.0	0.0	55.00±1.63^{e}	6.36±2.45^{gh}	2.8±1.63^{jk}
2.0	0.0	0.0	46.65±1.63^{g}	4.25±1.63^{kl}	3.1±0.82^{hi}

2.5	0.0	0.0	34.76±0.82^{l}	2.00±0.82^{n}	3.4±0.82^{g}
3.0	0.0	0.0	28.54±0.82^{o}	7.67±1.63^{e}	2.9±0.82^{j}
0.0	0.5	0.0	32.34±1.63^{m}	6.97±1.63^{g}	3.2±1.63^{h}
0.0	1.0	0.0	42.53±0.82^{i}	9.66±0.82^{cd}	5.9±0.82^{c}
0.0	1.5	0.0	21.58±0.82^{p}	10.73±1.63^{b}	6.1±0.82^{bc}
0.0	2.0	0.0	43.54±0.24^{hi}	9.86±0.82^{bc}	6.5±0.82^{b}
0.0	2.5	0.0	60.67±0.82^{a}	12.67±1.63^{a}	7.5±1.63^{a}
0.0	3.0	0.0	46.14±0.82^{gh}	7.47±0.16^{ef}	4.3±1.63^{e}
0.0	0.0	0.5	59.00±1.63^{bc}	9.81±0.82^{c}	4.8±0.16^{cd}
0.0	0.0	1.0	57.45±0.82^{d}	7.15±1.63^{f}	4.0±0.82^{ef}
0.0	0.0	1.5	58.88±0.82^{c}	9.66±0.82^{d}	3.3±0.82^{gh}
0.0	0.0	2.0	29.62±1.63^{l}	8.75±0.24^{de}	3.9±1.63^{f}
0.0	0.0	2.5	41.00±0.82^{ij}	5.16±0.82^{ij}	4.5±1.63^{de}
0.0	0.0	3.0	59.45±0.24^{b}	4.00±0.82^{l}	4.7±033^{d}

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

Table 6. Effect of different composition of hardening medium on survivability rate of leaf callus derived plantlets of the species, *Tribulus terrestris.*

Hardening medium composition (w/v)	No. of plantlets Under hardening	No. of plantlets survived	Survivability (%)
Red soil: sand (1:1)	50	22^{e}	44.31^{e}
Garden soil: sand: vermicompost (1:1:1)	50	42^{a}	80.46^{a}
Decomposed coir waste: perlite: vermicompost (1:1:1)	50	36^{b}	75.24^{b}
Vermicompost : red soil (1:1)	50	30^{e}	65.58^{c}
Red soil: sand: vermicompost (1:1:1)	50	28^{d}	60.11^{d}

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

Table 7. Effect of different composition of hardening medium on survivability rate of node callus derived plantlets of the species, *Tribulus terrestris.*

Hardening medium composition (w/v)	No. of plantlets Under hardening	No. of plantlets survived	Survivability (%)
Red soil: sand (1:1)	50	17^{e}	34.86^{e}
Garden soil: sand: vermicompost (1:1:1)	50	40^{a}	70.56^{a}
Decomposed coir waste: perlite: vermicompost (1:1:1)	50	36^{b}	66.45^{b}
Vermicompost : red soil (1:1)	50	26^{d}	50.25^{d}
Red soil: sand: vermicompost (1:1:1)	50	34^{c}	55.63^{c}

Mean values in columns are followed by different letter (s) are significant to each other at 5% level accordance to DMRT.

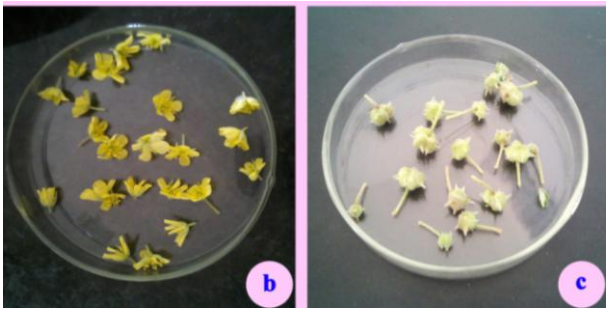

Fig. 1. Habit of the study species, *Tribulus terrestris*. a-Habit; b-Flowers; c-seeds.

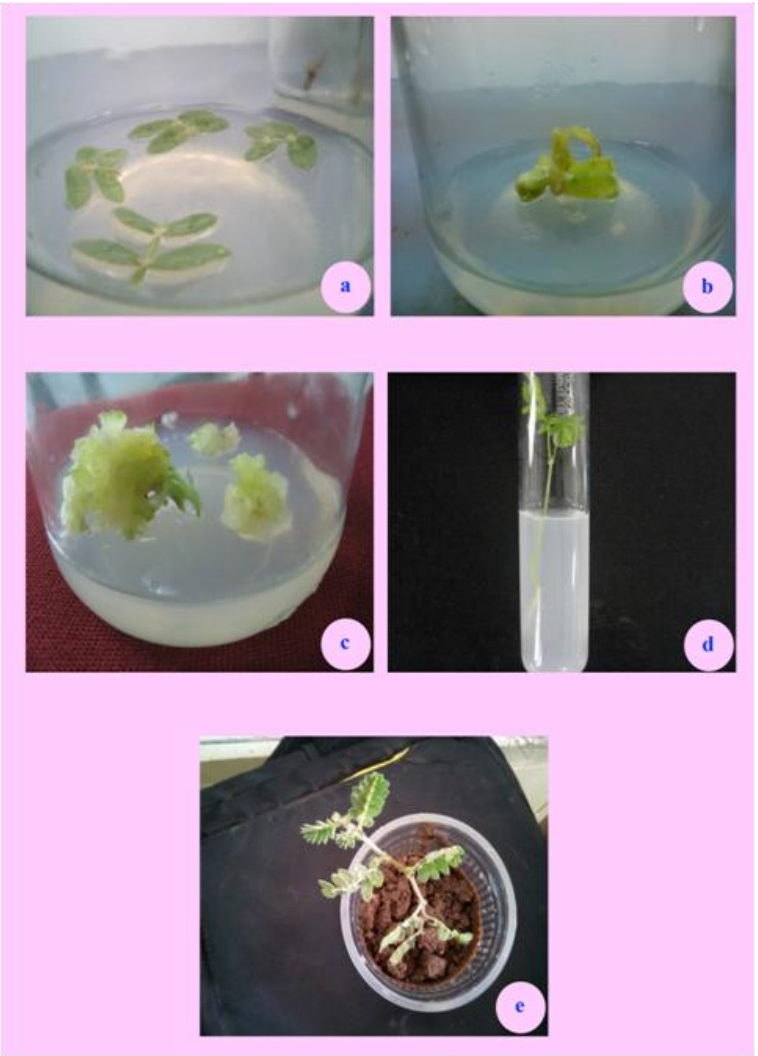

Fig. 2. *In vitro* regeneration of *Tribulus terrestris* using leaf explant.

a - Inocluation of leaf explant on MS medium with BAP and NAA (4mg/L and 0.5mg/L). b - Callus induction of the leaf explant on MS medium with BAP and NAA (4mg/L and 0.5mg/L). c – Shoot induction of *in vitro* derived callus explants in the MS medium with BAP and NAA (4mg/L and 0.5mg/L). d – Root induction of *in vitro* cultured shoots from the leaf derived callus in the MS medium fortified with IAA 2mg/L. e – Acclimatization of *in vitro* plantlets.

Fig. 3. *In vitro* regeneration of *Tribulus terrestris* using nodal explant.

a - Inocluation of nodal explant on MS medium with BAP and NAA (4mg/L and 0.5mg/L).

b - Shoot induction of nodal explants in the MS medium with BAP and NAA (4mg/L and 0.5mg/L). d – Root induction of *in vitro* cultured shoot from the node derived shoot in the MS medium fortified with IAA 2mg/L. e – Acclimatization of *in vitro* plantlets.

4. DISCUSSION

Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture technique has in recent years, become of major industrial importance in the area of plant propagation, disease elimination, plant improvement and productivity of secondary metabolites. Small pieces of tissues (named explants) can be used to produce hundreds and thousands of plants in a continuous process. Endangered, threatened and rare species have successfully, been grown and conserved by micropropagation because of high coefficient of multiplication and small demands on number of initial plants and space.

Due to its high medicinal value and increasing demands, *in vitro* studies have great importance in the propagation of the study species, *Tribulus terrestris.* In the present study, callogenesis and shoot proliferations of the study species were witnessed in MS medium supplemented with higher concentration of cytokinin (BAP 4mg/L) and low concentrations of auxins (NAA 0.5mg/L). In accordance with Singh and Goyal (2016), the best callogenesis and shoot regeneration of *T. terrestris* were also observed in higher concentrations of cytokinin (BAP 2.0 mg/L) and low concentration of auxin (NAA 2.5 mg/L). The study also suggested that the balance of auxin and cytokinin is a decisive morphogenetic factor. The present results exhibited that high concentration of BAP and low concentration of NAA were efficient for the induction of callus and subsequently shoot regeneration. Reports of auxin and cytokinin combinations were supporting the organogenesis differentiation in other species have been well documented by Jamuna and Paulsamy, 2014 (a and b); Jejurker *et al.,*2016).

Rhizogenesis of the study species from the leaf derived callus and nodal explants were noted at MS medium contained with IAA alone 2 mg/L. The result was harmony with previously reported species such as Indrias *et al.,* 2016; Roberto *et al.,* 2016. IAA is generally regarded as the major auxin, universally found in higher plants, that plays a center role in adventitious root formation (Davis and Sankla, 1988).

5. CONCLUSION

In the present study, an efficient regeneration through leaf and nodal explants were achieved by *in vitro* technique. This *in vitro* proliferation method is used for the enhancement the natural stock of plants in wild population. The present protocol will enable the propagation and conservation of *T. terrestris* in an efficient manner.

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CONSERVATION OF SACRED GROVES, CULTURAL CONNECTIONS AND CONTROLLING CLIMATE CHANGE

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ABSTRACT

Sacred groves signify the practice of conserving biodiversity with strong beliefs, customs and taboos and are treasure house of rare and endemic species. Everything within these groves is under the protection of the reigning deity of the grove and the removal of any material, even dead wood or twig is a taboo (Gadgil and Vartak, 1976). Such groves still exist in many parts of the world and represent relict vegetation of the locality, preserved in its original form with minimal disturbance. Preservation of these groves, though on the pretext of religious beliefs, is of importance for conserving germ plasm that is otherwise under threat from human interference (Khiewtan and Ramakrishnan, 1989). Among the sacred groves along the coastal sector centering Pondicherry (90km x 50 km) on the east coast of India, Puthupet (28ha), Senthirankillai (15ha), Thoppaiyankulam (10.5ha), Kotthatai (6.15ha) and Karukkai (5.2ha.) were larger groves, the smaller being Sedrapet, Ramanathapuram and Kumalam, each measuring ca.0.2 ha. Of late, ecologists evince interest in the potential of biodiversity in carbon -'C' sequestration and storage. In some selected groves, the biosequestered atmospheric carbon (C) values ranged from 47.7 to 120.5 Mg ha^{-1}. The quantum of C-storage in a sacred grove, however small it may be, and its implied role in mitigating the climate change, is now confirmed. These groves which have rich, varied and valuable biodiversity conserved in them can also contribute to tackling climate change, which is another most serious environmental problem facing the humankind.

Keywords: Sacred groves, climate change, environmental problem.

1. INTRODUCTION

Nature has always been very vibrant, giving and resilient to a very large extent. One of the critical issues on the national and global agenda is the need to preserve biodiversity for future generations while trying to understand and document the indigenous knowledge of resource management practices. Religion, being a powerful instrument for convincing people, has always been used for meeting the desired objectives of the society

Most religions project the worship of sun, wind, land, trees, plants, and water which is the very base of human survival. Snake worship prevailed in ancient India from time immemorial. Garuda, lion, peacock, crow, cow, mouse, elephant and tiger are part of our cultural ethos emphasizing the conservation of wildlife. Almost the entire living of God Ram and Goddess Sita was very close to nature. The very concept of Sthala vriksha is aimed at arresting the decline of important plant specieds in the wild. The sangam literature depicts that the ancient Tamils evolved every aspect of their life in relation to nature. Some prominent live examples of traditional and cultural forms of biodiversity conservation still exist and are in practice, which include sacred groves, sacred species and sacred landscapes.

The concept of scared groves as instrument of biodiversity conservation was evolved by the ancient people. These have survived through the millennia mainly because of the belief system associated with them. Everything within these groves is under the protection of the reigning deity of the grove and the removal of any material, even dead wood or twig is a taboo (Gadgil and Vartak, 1976). Such groves still exist in many parts of the world and represent relict vegetation of the locality, preserved in its original form with minimal disturbance. Preservation of these groves, though on the pretext of religious beliefs, is of importance for conserving germplasm that is otherwise under threat from human interference (Khiewtan and Ramakrishnan, 1989).

In India, they occur in Western Ghats, Madhya Pradesh, Maharashtra, Meghalaya, Karnataka, etc., and found in variety of habitats from scrub forests of Thar Desert (maintained by Bishnois), to rain forests of Kerala in Western Ghats, Himachal Pradesh in the North and Kerala in the south are specifically known for their large number of sacred groves.

Gadgil and Vartak (1975, 1976) initiated the study in India when they enlisted hundreds of groves on the Western Ghats of Karnataka and Maharashtra

regions. Subsequently, groves were discovered from all over India and several new features were found to be associated with them. Groves in the leeward side of Aravalli regions were sparsely wooded patches dominated by Vanni (*Prosopis cineraria*) trees; those on the North Eastern Himalayan ranges were evergreen or deciduous formation; most of the groves in the plains were mono or multi-species the clusters; some were relicts of past vegetations bearing testimony to the chequered course of ecological history. Nevertheless, despite the divergence on their type and physiognomy, they shared certain common features like having a dense or sparse cover of tress, presence of a village deity in iconic or uniconic form, a routine form worship along with an annual festival, restrictions on the use of natural resources from the groves, taboos and folklores tailored to regulate human behavior and strategic association with a perennial water source.

As more information flowed in, the concept of the sacred groves have also been widened to include any patch of trees, natural or anthropogenic, that is dedicated to a village God and has constrained resource use pattern woven into the tapestry of cultural traditions (Somasheker 1999).

In several regions, the groves are islets of natural vegetation surrounded by either agricultural fields or denuded forest ranges. Interestingly, many of them contained rare and endangered taxa that are seldom found elsewhere. The protection ensured by the cultural traditions and religious sanctions over the millennia, has allowed the plants to grow into tall and robust tress or giant lianas. Hence, they may be a museum of giant specimens, recreation centres and spiritual retreats, refugia of relicts and endemic taxa, laboratories for environmentalists, sanctuaries for birds and fauna, dispensaries of medicinal plants, gene bank of economic species, water-sheds which recharge of aquifers. In fact, certain well preserved groves are even equated to mini-biosphere reserves.

Interestingly some are mono-dominant groves. The choice of species for raising such groves is also socially significant. The geo-climatic considerations coupled to the variety and intensity of utility of the tree for local people have influenced the selection.. Whereas the Bamboo of Sal or teak trees constituted the stands in the hill tracts, Tamarind, Punnai, Ilppai, Panai have been dominating the rural landscapes in the plains.

The UNESCO–WWF sponsored nationwide survey led by Prof. Ramarakrishnan of Jawaharlal Nehru University, New Delhi has documented the existence of groves from all parts of India (Ramakrishnan *et al.,* 1998) Still, Tamil Nadu's contribution to this compilation is meagre and minimal. It is not that the Tamil-speaking areas were bereft of the groves, as the recent initiatives have proved (Amirthalingam, 2014).

For Tamil Nadu – Pondicherry region, Meher – Homji (1986) from the French Institute, Pondicherry first reported a patch of tropical Dry Evergreen Forest (TDEF) dedicated to Lord Manjiny and Airyanar which was protected a strong religious belief system and cultural traditions. Occupying over 20 ha, it harboured 104 plant species belonging to 44 Angionsperm families. It is a relict patch of erstwhile tropical dry evergreen vegetation (Champion and Seth, 1968).

Amirthalingam (1998) C.P. Ramasawmy iyer foundation of Chennai (CPRF) has undertaken the stupendous task of survey and restoration of Tamilnadu groves and enlisted 82 groves with appreciable floristic value from the Districts of Pudhukottai and Tiruchirappalli. Subsequently, CPRF has surveyed and resurrected several denuded groves and successfully restored several them to their original status in Tamilnadu and Andhra Pradesh (Nanditha and Javanthi, 1997; Amirthalingam, 1998). Nenmeli grove near Chengalpattu is a classic example of restoration wherein the stake-holders displayed a keen interest.

Parthasarathy's group from Pondicherry University studied the phytosociology of three groves in Pondicherry – Cuddalore region and declared these are essentially TDFF patches viz. Thirumanikkuzhi, Puthupet, Kulandhaikuppam. (Parthasarathy and Karthikeyan, 1997; Parathasarathy and Sethi, 1997). King (1997) found a grove at Suriyampet, near Cuddalore which could also be considered a relict of TDFF. Swamy *et al.* (1998) from Madurai Kamaraj University, enumerated scores of groves in Madurai, Sivangangai and Kanyakumari districts and noted the aboundance of *Artocartpus integribolius* and *A. heterophyllus* in the less – disturbed groves. Some of them were > 10 ha in extent.

The coastal sector covering Pondicherry, Cuddalore and Villupuram districts has been the focus of research by our group. Stretching over 350 Sq. Km, this sector has 180 groves, of which 50 had rich biodiversity values (Kadamban, 1998; Pravenkumar, 1999; Ramanujam *et al.,* 2002; Krishnan, 2004) After Puthupet, Senthirankillai and Thuthipattu measuring >10.5 ha each were larger groves; the smallest was Ranganavaram, Ramanathapuram and Kumalam measuring ca.0.2 ha each.

A major outcome from these diverse initiatives is that there are hundred of groves with biodiversity potential in Tamilnadu especially in Pondicherry-Villupuram-Cuddalore-Pudhukottai sector. They may be a museum of giant specimens, recreation centres and spiritual retreats, refugia of relicts and endemic taxa, laboratories for environmentalists, sanctuaries for birds and fauna, dispensaries of medicinal plants, gene bank of economic species and water-sheds which recharge the aquifers. In fact, certain well preserved groves are even equated to mini-biosphere reserves.

However, there are alarming signs of their degradation mainly due to defective management policies. Chandran and Hughes (1997) reviewing the status of sacred groves attributed the ascendance of religious/cultural considerations over conservation ethos, spread and establishment of monotheistic religions, influence of western culture and modernism which shifted the primacy from biodiversity to temple construction and worship pattern and conversion of forests to agriculture or plantations. It is imperative that the conservation strategy is reoriented to revive them since they contain significant biodiversity and botanical values.

The concept of sacred grove was not a component of protected Area Network (PAN). Nevertheless, their biodiversity potential is immense and needs to be preserved on a priority basis. The earth summit of 1992 that adopted the "Convention of Biodiversity" (CBD) provided the necessary impetus to the sacred grove initiatives. The Forest Department of Govt. of Puducherry has proposed to declare them as 'Community Reserves" under Wildlife Protection Act of 1972. Realising the value of the rich flora and fauna a contained within the groves, Alternatively, they can be declared as "Biodiversity Heritage Sites" (BHS) under the Biodiversity Act 2002. The National Biodiversity Authority has framed rules facilating their new status as BHS.

2. TASKS REMAINING

Nevertheless, certain grey areas remain. Their role in nutrient enrichment, soil preservation and moderation of microclimate is yet to be substantiated in different climatic zones. Though most are associated with water bodies, the water-shed management value is unclear. More important is the livelihood support documented in hill regions but not in the plains. As they have more shrubs and trees, their capacity to absorb atmospheric Carbon and thereby reducing the C-impact on climate change is almost untouched. Ecologists have started evincing interest in the potential functional relationship between biodiversity and carbon-'C' sequestration and storage (Kirby and Potvin, 2007). Forests sequester and store more C than any other territorial ecosystem and are an important 'brake' on climate change (Gibbs *et al.,* 2007).

In this background, an attempt was made to assess the potential of such groves in sequestering atmospheric carbon which is the major contributing factor to global warming. While the preservation of groves will conserve local biodiversity, it will indirectly mitigate the climate change through enhanced carbon capture. That is a major aspect of clean development mechanism (CDM).

To fill this void, the woody plant diversity, phytosociology and conservation of seven coastal and three interior groves in Cuddalore district of Tamil Nadu was taken up by Praveenkumar (2011) recently. The ten groves are: Aiyanar grove at Sedapalayam Pudhur (SP), Kasambu Nayagi grove at Chinnakumatti (CK), Chetty Veerappaswamy Grove at Kothattai (KT), Ponni Amman grove at Indiranagar (PK), Pacahaivazhi Amman grove at Palvalthunnan (PT), Muni Aiyanar and Nallanayagi grove at Senthirankillai (SK), Aiyanar grove at Anayankuppam (BM), Kurumbaiyanar grove at Karukkai (KK), Aiyanar grove at Elavathadi (EL) and Muthu Muneeswaran grove at Thoppayankulam (TP).

Together they occupy 38.11 ha. While SP (10.06 ha), KT (5.32ha) and SK (3.04 ha) are larger groves exceeding 10 acres, EL (1.2 ha), BM (0.7 ha) are smaller. The species richness ranges from 13 (BM) to 37 (SK). A total of 80 Woody species, including 62 species of trees and 18 species of lianas have been enumerated from the ten groves. They are distributed in 64 genera belonging to 39 families. Of the constituent species, evergreens dominated the coastal groves. In KK deciduous elements take over (394 individuals belonging to 10 species). Family–wise, Rubiaceae with six genera is the most represented family followed by Rutaceae with five genera, Euphorbiaceae and Fabaceae-Faboideae with three and Fabaceae-Caesalpinioideae with two genera.

The above ground biomass (AGB) has been calculated by Praveenkumr to understand the growth status of forest stand (Murali *et al.,* 2005). The above ground biomass (AGB) values of the ten sites ranged between 130 Mg ha to 219.7, averaging 163.6. As the AGB is an extrapolation of BA, the tree architecture and height contribute to the estimations immensely. These values were also used for calculating the carbon stock values, as adopted by Gairola *et al.* (2011). As recommended by IPCC, 0.47

of the AGB is taken as carbon percentage since broad leaved species and deciduous species constituted the vegetation in the study sites; it is 0.26 for root system (Mc Groddy *et al.,* 2004) It is represented as total carbon density TCD (Mg C ha^{-1}).

3. MODERATION OF MICROCLIMATE

The air temperature was invariably less by 1-3° C inside the groves compared to the open surroundings. In TP, SK, PT and SP, where the canopy was thick and continuous, it was cooler by 2-3° C but differed by just 1° C in the dry tracts of BM and PK. The temperature inside the grove despite the nature of the canopy always remained lower than outside. While it is tempting to downplay the impact on microclimate as shade effect, the cool and comfort provided by the trees is really enjoyable. This is in line with the observations of Jaryan *et al.* (2009) who for the first time assessed the microclimate in Shivbari grove in Himachal Pradesh.

4. CARBON STOCKING

The total carbon sequestered by the sacred grove sites, taken as a 0.47 fraction for aerial parts and 0.26 fraction of the root system as recommended by IPCC (Mc Groddy *et al.,* 2004), Wherever the proportion of crooked stems and stunted trees was high or the shrub layer is dense, the biomass values have decreased correspondingly. The carbon stock values varied between 47.7 to 120.5 Mg $ha^{-1)}$. Only well grown forests of Garhwal Himalaya have a the total carbon (TCD) stock of 178.4 Mg ha^{-1} (Gairola *et al.,* 2011).

Table 1. Carbon stocking of the 10 sacred groves in Cuddalore district of Tamilnadu.

S. no	Grove	SP	CK	KT	PK	PT	SK	BM	KK	EL	TP
1	Biomass (AGB) (Mg ha^{-1})	101.6	157.9	146.9	135.1	205.3	219.7	256.5	196.8	170.2	172.7
2	Carbon stock- ACD (Mg C ha^{-1})	47.7	74.2	69.1	63.5	96.3	103.3	120.5	92.5	79.8	81.2
3	Carbon stock- BCD (Mg C ha^{-1})	26.4	40.9	38.0	35.1	53.4	55.6	66.7	51.2	44.2	44.9
4	Total Carbon stock- TCD (Mg C ha^{-1})	74.1	116.7	107.1	98.6	149.7	158.9	187.2	143.7	124.0	126.9

(Calculated On The Basis Of Above Ground Biomass Values as recommended by inter governmental panel on climate change - IPCC) (Mg = Metagram = 10-15)

The maximum biomass and carbon have been stocked in SK, PT, BM and KK, and minimum in SP. While the high density of stems has contributed to the higher biomass values in SK, fewer but tall and robust trees did it for PT and KK. Though the stem density may be low at EL, the trees are voluminous. In other groves too, the stocked carbon levels are appreciable since the biosequestered atmospheric carbon (C) is uniformly 47% of the biomass. Despite smaller size of groves and varying ABG values, the contribution of sacred groves to mitigation of global warming is certainly remarkable.

Name of the Grove	Village	Area(ha.)
Veera Aiyanarappan Thirukkoil	Sedapalayam-Pudur	8.2 – low tree cover
Kasambunayaki (Informal)	Chinnakumatti	2.16 - dense and tall trees
Chettiveerappa Swami Kovil(Informal)	Kothattai	6.2– low tree cover
Pachavazhaiamman Kovil	Puduchathiram	2.17 –sparse tall trees
Ponniyamman Kovil	Senthirankillai	1.2- dense and tall trees
Muniyanar Kovil Thoppu	Senthirankillai	3.5- dense and tall trees
Aiyanar Kovil	Anainkuppam - B.Mutlur	1.1 – sparse tree cover
Kurumbu Aiyanar Kovil	Karukai	5.1 sparse, tall trees
Aiyanar Kovil	Elavathadi	1.2- sparse tall trees
Munieeswaran Kovil	Thopayankulam	3.8- dense and tall stands

Accordingly the short statured strands in SP, PK, and KT scored the lowest values compared to the dense and tall stands of SK, PT and TP.

- Any how, these values tally with the 90.25 to 173.1 range from the coastal sites and 73 to 138.73 for inland sites
- It also compares favourably with the total Biomass density (TBD) 135.6 Mg ha^{-1} for an Indian forest estimated in 1993 and AGB range of 171.9 to 380.3 Mg ha^{-1} reestimated in 2011(Gairola *et al.* 2011).

Considering that these values are only approximations, the quantum of C-storage in sacred groves, however small it may be, and its implied role in mitigating the climate change, is certainly significant.

5. CONCLUSIONS

In conclusion, the carbon capturing potential of the selected sacred groves among the agricultural/urban societies. It confirms that these groves which have the rich, varied and valuable biodiversity conserved in them can also contribute to tackling Climate change, which is another most serious environmental problem we facing the human kind.

It now demands a paradigm shift in the attitude of the public as well as the administrators to acknowledge the tangible and intangible benefits from the groves. With no economic dependence, not even incentives, disinterest of the farming community cannot be faulted upon either. But given their social status and educational background of the elders and the adverse impacts of rationality, urbanization and modernism on the young and middle classes, it seems a distant dream to integrate them into mainstream conservation measures. It is imperative to strengthen the people – grove interface by imprinting the values of ecosystem services in them. Nothing could be more alluring than the earning carbon credits through the Clean Development Mechanism (CDM).

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IN VITRO REGENERATION AND MASS PROPAGATION OF *AZIMA TETRACANTHA* [LAM.] FROM THE LEAF EXPLANTS THROUGH CALLUS CULTURE

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ABSTRACT

In the present study the protocol for callus induction and regeneration in *Azima tetracantha* has been developed in culture medium. The young apical leaf explants were used for callus induction on MS medium containing BAP and NAA at 1.0 and 0.4mgl^{-1} respectively showed maximum callus induction (73%). The amount of callus responded for shoot formation (74%) was obtained in the MS medium containing BAP (1.5 mgl^{-1}) and NAA (0.3mgl^{-1}).The elongated shoots were rooted on half strength medium supplemented with IBA (1.5 mgl^{-1}) and Kn (0.4 mgl^{-1}) for shoots rooted. Regenerated plantlets were successfully acclimatized and hardened off inside the culture and then transferred to green house with better survival rate.

Keywords: *Azima tetracantha*, MS medium, Growth hormones, Acclimatization.

1. INTRODUCTION

India is richly endowed with a wide variety of plants having medicinal value. These plants are widely used by all sections of the society whether directly as folk medicines or indirectly as pharmaceutical preparation of modern medicine (Uniyal, 2003). In India, thousands of medicinal plant species are known to have medicinal value to cure specific ailments. However, it is estimated that about 15,000 species of medicinal plants are globally threatened; the causes include loss of habitat, over harvesting and pollution. Biotechnological tools are important for the multiplication and the genetic enhancement of the medicinal plants by adopting techniques such as *in vitro* regeneration and genetic transformation. Microprobagation method is specifically applicable to species in which clonal propagation is needed (Gamborg and Phillips, 1995). Among the important medicinal plant species, one of the species *Azima tetracantha* belongs to the family Salvadoraceae. It is a potent diuretic to treat rheumatism, dropsy, dyspepsia, chronic diarrhorea; it is used as stimulant tonic after child birth. *A. tetracantha* is used to treat cough, phthisis, asthma, small pox and diarrhorea. Rheumatism has been cured by its leaves, root and root bark. Leaves are used as stimulant, expectorant and antispasmodic. It is also used in cough and asthma. Bark is used as antiperiodic, astringent and expectorant. In western India, juice of the leaves is applied as ear drops against earache and crushed leaves are placed on painful teeth. In India and Sri Lanka the root, root bark and leaves were administered with food as a remedy for rheumatism, dropsy, dyspepsia, chronic diarrhea and is considered as stimulant tonic and given to pubertal women immediately after confinement. Locally, the traditional healers from Tirunelveli district of Tamilnadu use the root bark (paste with buttermilk) as potent remedy for jaundice. This paper describes the first results of experiments carried out to induce organogenesis in tissue culture of *Azima tetracantha* under influence of different combinations of growth regulators.

2. MATERIALS AND METHODS

Leaf segments from young and healthy branches of *A. tetracantha* were used as explants. They were collected from pot cultured individuals maintained in a mist chamber. For surface sterilization, the collected immature leaves were washed with tap water twice and then treated with 5 % tween–20 solutions for 5 min followed by rinsing in tap water. To eliminate fungal contamination, explants were further treated with 5 % antibiotics (Amphicillin and Rifampicin) for 30 min followed by 3 rinses in sterile double distilled water. Further, surface sterilization was carried out by dipping the explants in 0.1% $HgCl_2$ for 3 min followed by 3-4 rinses in sterile double distilled water.

2.1. Media and culture condition

Murashige and Skoog (MS) (1962), medium containing 3 % sucrose solidified with 1 % agar (tissue culture grade, Himedia, India) was used. The pH of the medium was adjusted to 5.6-5.8 prior to the addition of agar before autoclaving at 121° C for 15 min. All the culture bottles were kept in culture chamber at 25± 2° C under 16/8 hr (light/dark) photoperiod with a light intensity of 2000 lux supplied by cool white fluorescent tubes and with 60-65% relative humidity.

2.2. Callus induction medium

The explants were transferred to culture bottles containing 25 ml MS medium supplemented with different concentrations and combinations of BAP and NAA for callus induction.

2.3. Shoot induction medium

MS medium containing different concentrations and combinations of BAP (0.3, 0.6, 0.9, 1.2, 1.5 and 1.8 mg/l) and NAA at 0.3 mg/l was used for shooting attributes.

2.4. Rooting of elongated shoots and acclimatization

After proper shoot induction, the plantlets were carefully removed from the medium and washed with sterilize double distilled water properly, so as to avoid any trace of medium on roots. *In vitro* regenerated shoots (5-6 cm long) were excised and transferred onto the rooting media containing half strength MS medium supplemented with IBA and Kn for rooting. After proper root formation, these rooted plantlets were transferred to hardening medium composed by garden soil, sand and vermicompost in different proportion and maintained in greenhouse condition to know the survivability rate.

2.5. Statistical Analysis

All the experiment was done atleast twice using triplicate. The data was statistically processed and means were compared using Duncan's Multiple Range Test (P<0.05).

3. RESULTS AND DISCUSSION

The Calli formation was observed in leaf explants after 26 days. The best response of callus (73%) was observed in the MS medium supplemented with cytokinin BAP (1.0 mgl^{-1}) and auxin, NAA (0.4 mgl^{-1}) (Table 1). A similar result was shown by Thambiraj and Paulsamy (2012). Further studies were carried out for shoot regeneration capacity of the callus. Shoots were initiated from the callus obtained leaf explants. The best result of shooting (74%) was observed on the MS medium fortified with BAP (1.5 mgl^{-1}) and NAA (0.3 mgl^{-1}). The maximum number of multiple shoots 9.06 shoots/callus and shoot length (6.8 cm) were produced in the same concentrations and combinations of growth regulators (Table 2). The superiority of BAP over the other cytokinins on shoot bud production and prolifehration of shoots has been reported for several medicinal and aromatic plant species by Jebakumar and Jayabalan, 2000; Hussain and Anis, 2006; Raja *et al.*, 2008; Faisal and Anis, 2003 and Chakradhar and Pullaih, 2014.

Table 1. Effect of growth regulators on callus induction from leaf explants of the species, *Azima tetracantha.*

Growth regulators (mg/l)				Days required for callus formation after inoculation	Callus formation (%)
BAP	**2, 4-D**	**NAA**	**IAA**	**Leaf Explant**	**Leaf Explant**
0.2	0.0	0.4	0.0	16	$39.21^{g} \pm 0.82$
0.4	0.0	0.4	0.0	19	$48.02^{h} \pm 1.63$
0.6	0.0	0.4	0.0	24	$60.34^{j} \pm 0.82$
0.8	0.0	0.4	0.0	25	$67.14^{l} \pm 1.63$
1.0	0.0	0.4	0.0	26	$73.23^{i} \pm 1.63$
1.2	0.0	0.4	0.0	25	$65.18^{i} \pm 0.82$
0.0	0.3	0.3	0.0	18	$25.56^{a} \pm 1.63$
0.0	0.6	0.3	0.0	20	$33.76^{b} \pm 0.82$
0.0	0.9	0.3	0.0	21	$39.17^{c} \pm 1.63$
0.0	1.2	0.3	0.0	26	$44.46^{f} \pm 1.63$
0.0	1.5	0.3	0.0	25	$55.00^{h} \pm 0.82$
0.5	0.0	0.0	0.2	18	$25.21^{b} \pm 1.63$
1.0	0.0	0.0	0.4	20	$31.38^{d} \pm 1.63$
1.5	0.0	0.0	0.6	23	$58.45^{g} \pm 1.63$
2.0	0.0	0.0	0.8	24	$69.64^{h} \pm 0.82$
2.5	0.0	0.0	1.0	26	$70.32^{i} \pm 1.63$
3.0	0.0	0.0	1.2	26	$54.17^{i} \pm 1.63$
0.0	0.3	0.0	0.3	15	$15.89^{a} \pm 0.82$
0.0	0.6	0.0	0.3	17	$23.43^{b} \pm 0.82$

0.0	0.9	0.0	0.3	18	41.00^{d} ± 0.82
0.0	1.2	0.0	0.3	20	46.00^{e} ± 1.63
0.0	1.5	0.0	0.3	19	47.00^{e} ± 1.63

Means in columns followed by different letter (s) are significant to each other at 5% level according to DMRT.

Table 2. Effect of different concentrations of growth regulators on shoot initiation, shoot number and shoot length after the subculturing of leaf derived callus of the species, *Azima tetracantha.*

Growth regulators (mg/l)					Culture response (%)	No. of shoots/callus	Shoot length (cm)
BAP	NAA	Kn	IBA	GA_3			
0.5	0.0	0.0	0.3	0.0	24.02^{a} ± 0.82	2.47^{abc} ± 0.82	1.4^{a} ± 0.82
1.0	0.0	0.0	0.3	0.0	32.17^{fg} ± 1.63	3.12^{ab} ± 1.63	2.6^{abc} ± 0.82
1.5	0.0	0.0	0.3	0.0	49.10^{h} ± 0.82	4.23^{bcd} ± 0.82	3.0^{abc} ± 1.63
2.0	0.0	0.0	0.3	0.0	45.78^{c} ± 1.63	6.42^{def} ± 1.63	3.5^{abc} ± 0.82
2.5	0.0	0.0	0.3	0.0	43.24^{de} ± 0.82	5.18^{cde} ± 1.61	4.6^{abc} ± 0.82
3.0	0.0	0.0	0.3	0.0	42.27^{cd} ± 1.63	4.00^{bcd} ± 0.82	3.2^{abc} ± 1.63
0.3	0.3	0.0	0.0	0.0	38.48^{h} ± 1.63	3.65^{cde} ± 0.82	3.8^{abc} ± 1.63
0.6	0.3	0.0	0.0	0.0	47.00^{i} ± 0.82	4.49^{efg} ± 1.63	4.4^{abc} ± 0.82
0.9	0.3	0.0	0.0	0.0	60.43^{j} ± 1.63	5.38^{def} ± 2.45	5.6^{bc} ± 1.63
1.2	0.3	0.0	0.0	0.0	65.67^{i} ± 1.63	7.47^{hi} ± 1.63	5.8^{bc} ± 1.63
1.5	0.3	0.0	0.0	0.0	74.42^{k} ± 0.82	9.06^{i} ± 1.21	6.8^{c} ± 0.82
1.8	0.3	0.0	0.0	0.0	58.46^{h} ± 1.63	7.98^{ghi} ± 0.82	4.7^{abc} ± 0.82
0.5	0.0	0.2	0.0	0.0	32.38^{g} ± 0.82	4.66^{fgh} ± 0.82	3.4^{abc} ± 1.63
1.0	0.0	0.2	0.0	0.0	39.00^{de} ± 0.82	4.22^{def} ± 1.63	3.9^{abc} ± 0.82
1.5	0.0	0.2	0.0	0.0	48.67^{h} ± 1.63	5.16^{abc} ± 1.63	4.7^{abc} ± 0.82
2.0	0.0	0.2	0.0	0.0	52.89^{i} ± 0.82	3.08^{ab} ± 0.12	4.8^{a} ± 0.82
2.5	0.0	0.2	0.0	0.0	55.55^{j} ± 0.82	2.67^{a} ± 0.62	5.0^{abc} ± 1.63
3.0	0.0	0.2	0.0	0.0	59.26^{h} ± 1.63	3.47^{bcd} ± 0.31	3.4^{abc} ± 0.82
0.5	0.0	0.0	0.0	0.3	33.12^{i} ± 0.82	4.65^{cde} ± 1.63	3.6^{abc} ± 1.63
1.0	0.0	0.0	0.0	0.3	39.66^{j} ± 0.82	5.86^{abc} ± 1.63	4.2^{abc} ± 1.63
1.5	0.0	0.0	0.0	0.3	44.37^{g} ± 0.82	6.46^{a} ± 0.82	4.0^{abc} ± 0.82
2.0	0.0	0.0	0.0	0.3	55.47^{ef} ± 1.63	5.14^{ab} ± 0.82	3.8^{abc} ± 0.82
2.5	0.0	0.0	0.0	0.3	67.76^{cd} ±1.63	4.56^{bcd} ± 1.63	3.1^{abc} ± 1.63
3.0	0.0	0.0	0.0	0.3	42.00^{b} ± 0.82	4.98^{def} ± 0.82	2.7^{abc} ± 0.82

Means in columns followed by different letter (s) are significant to each other at 5% level according to DMRT.

Table 3. Effect of different concentrations of growth regulators on rooting percentage, root number and root length after subculturing the leaf derived callus of the species, *Azima tetracantha.*

Growth regulators (mg/l)			Shoots rooted (%)	No. of roots/shoot	Root length (cm)
IBA	IAA	Kn			
0.5	0.2	0.0	36.16^{b} ± 0.21	3.75^{abc} ± 0.16	2.9^{a-d} ± 0.40
1.0	0.2	0.0	38.79^{d} ± 1.63	3.96^{abc} ± 0.41	3.1^{ab} ± 0.65
1.5	0.2	0.0	42.09^{e} ± 0.34	4.18^{bcd} ± 1.23	3.5^{abc} ± 0.81
2.0	0.2	0.0	46.43^{g} ± 0.41	4.25^{abc} ± 0.82	4.3^{c-f} ± 0.21
2.5	0.2	0.0	50.14^{h} ± 1.63	5.26^{cde} ± 0.12	4.9^{ef} ± 0.33
3.0	0.2	0.0	44.10^{d} ± 0.17	4.56^{bcd} ± 1.61	3.8^{b-e} ± 0.42
0.3	0.0	0.4	42.11^{c} ± 0.65	3.76^{abc} ± 0.41	3.0^{abc} ± 0.12
0.6	0.0	0.4	52.45^{e} ± 1.63	4.38^{bcd} ± 0.17	3.8^{abc} ± 1.61
0.9	0.0	0.4	57.07^{fg} ± 0.41	5.38^{a} ± 0.82	4.2^{b-e} ± 0.19

1.2	0.0	0.4	$67.08^{i} \pm 0.82$	$6.76^{cde} \pm 0.34$	$5.2^{a} \pm 0.82$
1.5	0.0	0.4	$72.46^{i} \pm 0.19$	$10.24^{de} \pm 0.41$	$6.6^{def} \pm 0.12$
1.8	0.0	0.4	$62.38^{j} \pm 1.61$	$8.21^{e} \pm 1.21$	$5.1^{f} \pm 0.04$
0.0	0.3	0.0	$32.16^{a} \pm 1.24$	$2.13^{ab} \pm 1.63$	$1.5^{abc} \pm 1.61$
0.0	0.3	0.0	$34.45^{b} \pm 0.82$	$3.03^{ab} \pm 0.71$	$2.2^{b-f} \pm 0.16$
0.0	0.3	0.0	$39.34^{d} \pm 1.63$	$4.00^{abc} \pm 1.60$	$3.1^{abc} \pm 0.82$
0.0	0.3	0.0	$42.04^{d} \pm 0.27$	$4.75^{bcd} \pm 0.41$	$3.4^{a-d} \pm 0.33$
0.0	0.3	0.0	$40.28^{e} \pm 1.17$	$4.00^{cde} \pm 1.23$	$2.5^{ab} \pm 0.41$
0.0	0.3	0.0	$38.17^{ef} \pm 0.82$	$3.67^{abc} \pm 0.82$	$2.2^{a-d} \pm 0.16$

Means in columns followed by different letter (s) are significant to each other at 5% level according to DMRT.

Table 4. Effect of different composition of hardening medium on survivability rate of leaf callus derived plantlets of the species, *Azima tetracantha.*

Hardening medium composition (V/V)	No. of plantlets under hardening	No. of plantlets survived	Survivability (%)
Red soil + sand (1:1)	50	24	$42^{a} \pm 1.23$
Garden soil + sand + vermicompost (1:1:1)	50	43	$75^{e} \pm 0.42$
Decomposed coir waste + perlite + compost (1:1:1)	50	37	$70^{d} \pm 0.41$
Vermicompost + soil (1:1)	50	31	$68^{c} \pm 1.17$
Red soil + sand + vermicompost (1:1:1)	50	28	$57^{b} \pm 0.82$

Means in column followed by different letter (s) are significant to each other at 5% level according to DMRT.

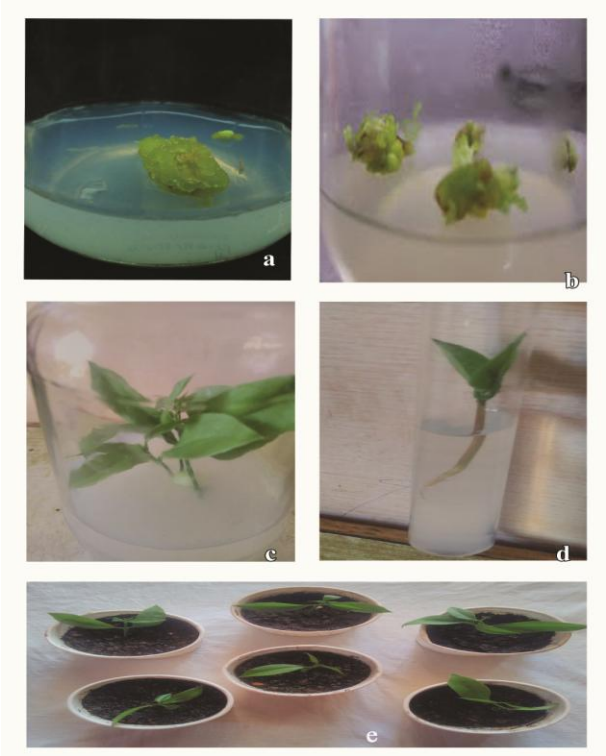

Fig. 1. *In vitro* regeneration through leaf explant *Azima tetracantha.*

a-b : Effective callusing in MS medium supplemented with BAP and NAA at 1.0 and 0.4mg/L respectively. c: Successful shooting by subculturing of leaf derived callus in the MS medium with BAP and NAA at 1.5 and 0.3mg/L respectively. d :High amount of rooting during the subculturing of shoots in the MS medium fortified with IBA and Kn at 1.5 and 0.4mg/L respectively. e: Under hardening in the mist chamber.

Induction of rooting is an important step for *in vitro* plant propagation. Excised shoots were inoculated on MS medium with IBA and Kn for proper root development. The rooting responses were summarized in Table 3. Maximum rooting (72%), number of roots (10.24 roots/ shoot) and root length (6.6 cm) was observed on the MS medium supplemented with IBA and Kn at 1.5 and 0.4 mgl^{-1} respectively (Table 3). These findings are in agreement with those reported by Sreekumar *et al.,* 2000; Martin, 2002; Raghuramulu *et al.*, 2002 and Chakradhar and Pullaih, 2014.

After the development of roots, the plantlets were taken out from the culture bottles and washed with sterilized distilled water to remove adhering agar medium, so that the chance of contamination could be stopped. Then these juvenile plantlets were transferred to the hardening medium containing garden soil, sand and vermicompost (1:1:1 ratio by volume) where the leaf callus derived plantlets survivability rate was higher 75% (Table 4). Admixture of all these three components may offer condusive environment by providing proper nutrients, adequate aeration and required minerals respectively to the plantlets. From the above study, it is concluded that multiple shoot and root cultures of *Azima tetracantha* were established from leaf explants on MS medium supplemented with combination of hormones. This protocol has potential for large-scale micropropagation and

application in molecular plant breeding research programs.

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STUDY OF ANTIMICROBIAL ACTIVITY OF THE FOLKLORE MEDICINAL PLANT, *ACALYPHA FRUTICOSA* FORSSK.

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ABSTRACT

The purpose of the study is to examine the antimicrobial efficacy of root extracts of the folklore medicinal plant species, *Acalypha fruticosa* Forssk by using three alcoholic solvents *viz;* petroleum ether, ethyl acetate and methanol were tested against ten human pathogenic bacteria *viz*., *Pseudomonas aeruginosa, P. stutzeri, Escherichia coli, Micrococcus* sp., *Lactobacillus* sp*., Servatia* sp., *Moraxetta* sp., *Bacillus subtilis*, *B. thuriengensis*, and *Klebsiella pneumoniae* and ten human pathogenic fungi *viz., Aspergillus niger, A. flavus, A.baumannii, Fusarium oxysporum, F. solani, Mucor rouxii, Alternaria alternata, Candida albicans, Cladosporium* sp. and *Rhizopus* sp*.* for assessing the antimicrobial properties by adapting disc diffusion method. The results of the study revealed that all extracts showed varied degree of antimicrobial activity against the tested pathogens. However, the methanol extracts exhibited higher inhibition zone (21.83 mm) against the bacterium, *Bacillus subtilis* and ethyl acetate extracts showed higher inhibiotion zone (24.83 mm) against the fungus, *Rhizopus* sp*.* Results concluded that this spices contain high amount of secondary metabolites due to these metabolites they have high antimicrobial activity and it can be used as good bio- preservater and it can also use for medicinal purpose.

Keywords: *Acalypha fruticosa*, Antimicrobial activity, Disc diffusion, Microorganisms.

1. INTRODUCTION

Plants have been a valuable source of natural products for a long period of time to maintain human health, especially with more intensive studies in the last decade for natural therapies (Gislene *et al.,* 2000). In many parts of the world, the extracts and essential oil of medicinal plants are used in folk medicine for their antimicrobial and antiviral properties (Hassawi and Kharma, 2006), that have been used. During the last two decades, the pharmaceutical industry has made massive investments on pharmacological, clinical and chemical researches all over the world in an effort to discover still more potent plant drugs; in fact, a few new drug plants have successfully passed the tests of commercial screening. Many of the plants used today were known to the people of ancient culture throughout the world for their preservative and medicinal powers (Zaika, 1975). However several plants are used in India in the form of crude extracts. A lot of plants with medicinal value used in the Indian traditional medicine have not yet been characterized for their active principles. Hence, one such plant of great medicinal importance is *Acalypha fruticosa* which are found to have diverse photochemical compounds of medicinal properties. This study plant species, *Acalypha fruticosa* belongs to the family, Euphorbiaceae is one such folklore plant used in traditional system of medicine in Coimbatore district of Tamil Nadu, India. The species is mainly distributed in tropical regions of India, Arabia, peninsular Burma and Africa. In India it is abundantly present in the foot hills of the Western Ghats (Matthew, 1995). Locally in Western districts of Tamil Nadu, the plant is administered to treat the diseases like jaundice, fever, and even as an antidote. The root is used for gonorrhea. Leaves and roots are used in siddha system of medicine for the treatment of skin diseases (Pullaiah, 2006). The plant is also used to cure cough, cold and headache. The boiled roots are used to cure cerebral malaria (Sahoo, 2001). A root decoction is drunken to treat convulsions, fever, colds and swellings of the scrotum and to treat whooping cough. Root decoction is taken to snake bites, fever and ulcer of venereal origin. However, no published works are available for the antimicrobial property of root of this plant. Hence in the present study, an attempt has been made to focus the plant in this angle and hence to assess its therapeutic potency.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh root parts were collected from the population of *A. fruticosa* present in the Maruthamalai Hills of Coimbatore District and washed under running tap water, air dried and then homogenized to fine powder and stored in air tight bottles.

2.2. Preparation of extracts

250g air-dried root powder was subjected to 250ml of methanol in soxhlet extraction for 8 hours (50-85˚C). The extracts were concentrated to dryness in a flask evaporator under reduced pressure and controlled temperature (50-60˚C) to yield crude residue, which was then stored in refrigerator. To obtain petroleum ether and ethyl acetate extracts, the same method as used to obtain methanol extract was adopted.

2.3. Media used

Freshly prepared Nutrient Agar medium and PDA medium were used for the culture of bacteria and fungi respectively.

2.4. Microorganisms

In vitro antimicrobial activity was examined for the chemical extracts of root part of the study plant, against ten bacterial species which include the gram positive strains *viz*., *Micrococcus* sp., *Lactobacillus* sp., *Bacillus subtilis*, *B. thuriengensis* and gram negative strains *viz., Pseudomonas aeruginosa, P. stutzeri, Escherichia coli, Klebsiella pneumoniae, Servatia* sp. and *Moraxetta* sp. and fungal species *viz., Aspergillus niger, A. flavus, A. baumannii*, *Fusarium oxysporum, F. solani, Mucor rouxii, Alternaria alternata, Candida albicans, Cladosporium* sp. and *Rhizopus* sp*.* All these microorganisms were obtained from the Department of Microbiology, Tamil Nadu Agricultural University, Coimbatore. All the microorganisms were maintained at 4˚C on nutrient agar slants (for bacteria) and PDA slants (for fungi) until further use.

2.5. Antimicrobial assay

The alcoholic extracts were tested for their effect against the growth of pathogenic bacteria and fungi by disc diffusion method (Bauer *et al.*, 1966). Both the organisms, bacteria and fungi tested were inoculated into nutrient agar and PDA media respectively. After an incubation period of 24 hrs at a temperature of 35˚C, three or four colonies isolated from these media were inoculated into 4ml of nutrient broth and incubated for 2 hrs at 35˚C. The cultures were adjusted with sterile saline solution to obtain turbidity. Petri dishes containing Muller-Hinton agar medium and PDA medium were streaked with these microbial suspensions of bacteria and fungi respectively. Disks of 6mm diameter were impregnated with the extracts of petroleum ether, methanol and ethyl acetate separately. Tetracycline is used as positive control. After equilibrium at 4˚C, the plates were incubated overnight at 37˚ C and the diameter of any resulting zones of inhibition was measured. Each experiment was repeated at least three times.

3. RESULTS AND DISCUSSION

The antibacterial activity of the all the alcoholic root extracts of the study species, *Acalypha fruticosa* generally ethyl acetate extracts showed inhibitory activity against all the bacterial growth and methanol extracts showed inhibitory activity against *Bacillus subtilis, B. thuringiensis, Micrococcus* sp., *Lactobacillus* sp., *Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa* and *P. stutzeri* except *Serratia* sp. and *Moraxetta* sp. However, petroleum ether extracts has comparatively less activity against most of the tested pathogens (Table 1). It is explained that the different phytochemicals like alkaloids, flavonoids, glycosides, saponins, terpenoids etc., which have extracted by different solvents may be responsible for their antibacterial effects (Anandakumar *et al.*, 2009). Further, the methanol extract has determined to have highest inhibitory activity (21.83 mm diameter inhibitory zone) against the bacterium, *Bacillus subtilis* (gram positive) and (20.63 mm diameter inhibitory zone) against the bacterium, *Bacillus thuringiensis* followed by the ethyl acetate extract against the bacterium, *Bacillus subtilis* (gram positive) (16.13 mm diameter inhibitory zone). It indicates the presence of effective active principle compounds in the ethyl acetate and methanol extracts of root part of *A. fruticosa* to suppress both gram negative and gram positive bacteria. It has been observed further that the methanol extracts showed significant inhibitory activity against the colonial growth of *Bacillus subtilis*, *B. thuringiensis, Klebsiella pneumonia* when compare with the commercially available antibiotic, tetracycline. This fact shows the higher therapeutic potential of methanol and ethyl acetate extracts of the study species. The petroleum ether extract has comparatively less activity against most of the tested pathogens. It may be attributed to the presence of respective active compounds with insufficient quantities in this crude extract (Taylor *et al.*, 2001).

The antifungal activity of various alcoholic root extracts of the study species, *Acalypha fruticosa* against the ten studied fungal species is given in Table 2. The results of the study report that the ethyl acetate extract has the highest inhibitory activity (24.83 mm diameter inhibitory zone) against the fungus, *Rhizopus* sp. and the methanol extract has the higher inhibitory activity (15.73 mm diameter inhibitory zone) against the fungus, *Fusarium solani*. The petroleum ether extract has comparatively less activity against most of the tested pathogens.

Table 1. Antibacterial activity of certain alcoholic root extracts of the species, *Acalypha fruticosa*.

Plant extract	Diameter of zone inhibition (mm)									
	Gram positive bacteria				Gram negative bacteria					
	Bacillus subtilis	***B. thuringiensis***	***Micrococcus* sp.**	***Lactobacillus* sp.**	***Klebsiella pneumoniae***	***Escherichia coli***	***Pseudomonas stutzeri***	***P. aeruginosa***	***Serratia* sp.**	***Moraxetta* sp.**
Standard *	30.83± 0.80	30.73± 0.67	20.67± 0.59	23.63± 0.60	12.13± 0.71	25.67± 0.61	13.73± 0.67	21.73± 0.70	14.23± 0.49	20.83± 0.80
Petroleum ether	9.67± 0.75	-	-	-	8.13± 0.71	-	-	7.32± 0.49	-	-
Ethyl acetate	16.13± 0.38	15.16± 0.38	9.73± 0.67	10.73 ± 0.67	8.77 ± 0.71	8.73± 0.75	9.77± 0.75	11.73± 0.67	7.77± 0.71	8.77± 0.71
Methanol	21.83± 0.60	20.63± 0.60	8.06± 0.31	8.63± 0.60	10.16± 0.47	9.67± 0.58	9.76± 0.86	7.67± 0.61	-	-

*Tetracycline

Table 2. Antifungal activity of certain alcoholic root extracts of the species, *Acalypha fruticosa*.

Plant extract	**Diameter of zone inhibition (mm)**									
	Aspergillus niger	***A. flavus***	***A. baumanii***	***Fusarium oxysporum***	***F. solani***	***Mucor rouxii***	***Alternaria alternata***	***Candida albicans***	***Cladosporium* sp.**	***Rhizopus* sp.**
Standard *	45.77± 0.61	39.87± 0.85	41.73± 0.70	44.53±0.68	23.73± 0.75	40.76±0.80	42.83±0.80	12.73± 0.70	44.73± 0.67	33.73± 0.70
Petroleum ether	9.76± 0.58	8.83± 0.80	8.76± 0.80	7.67± 0.49	8.76± 0.80	7.87 ±0.85	-	-	9.43± 0.65	7.83±0.80
Ethyl acetate	20.67± 0.53	13.87±0.90	14.83± 0.91	10.83±0.85	10.76± 0.65	14.73± 0.70	10.76± 0.75	-	13.76± 0.80	24.83± 0.90
Methanol	12.77± 0.58	8.73± 0.75	9.77± 0.80	-	15.73± 0.75	9.77± 0.75	8.77± 0.80	7.83± 0.91	8.76± 0.70	10.73± 0.70

*Tetracycline

This fact indicats the existence of strong antifungal activity of root part of the study species, *A. fruticosa* and hence its effective healing property against the infectious diseases. The variation in antifungal activity across the extracts studied may be due to the polarity of the solvents used. Significantly higher inhibitory activity of ethyl acetate extract is nearly to the commercially available antibiotic tetracycline against the fungus, *Rhizopus* sp. observed shows the superior healingness of root part of *A. fruticosa.* Proper isolation and purification of active compounds by using ethyl acetate solvent would ensure the therapeutic value of this folklore medicinal plant when it will be used commercially.

The overall study on antimicrobial activity reports that the study species contains adequate variety of active compounds to reduce or check the growth of microbial colonies. It confirms the therapeutic value and hence the traditional usage of the root part of the study species, *A. fruticosa* against various ailments. Further, the alcoholic extracts of root part of this plant in general and ethyl acetate and methanol extracts in particular are suggested for the therapy of infectious diseases caused by pathogens and further studies are recommended to purify the active compounds for the formulation of new drugs, while go for commercialization.

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IN VITRO- RAPID MULTIPLICATION OF AN IMPORTANT MEDICINAL PLANT *TURNERA SUBULATA* L.

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ABSTRACT

A highly stable, efficient and cost-effective, protocol for direct and indirect regeneration of a medicinal plant *Turnera subulata*. was established through the nodal culture using Murashige and Skoog's (MS) nutrient medium supplemented with various concentrations and combinations of growth hormones. The highest shoot proliferation (86%) was observed on the MS medium enriched with BAP and IAA at 2.22 and 0.90μm/l respectively. Effective shoot multiplication and elongation was observed on the same combination of growth regulators. The regenerated shoots was successfully rooted on MS medium supplemented with IBA alone at 2.46μm/l. After sequential hardening the survival rate of the plantlets was determined to be 93.8% under green house condition.

Keywords: Plant tissue culture, medicinal plant, *Turnera subulata*.

1. INTRODUCTION

Medicinal plants are the traditional sources for chemicals used as pharmaceutical products, food colors, fragrances and flavors. Plants high medicinal value are oldest friend for humans the backbone of folk medicine (Farnsworth, 1994). Almost 80% of the world population's primary health care need are fulfilled by plant based medicines. More than 25 % of the drugs are plants based phyto compounds and 87% of human cancer therapy treated depend on plant based products (Shaikh *et al.*, 2011).

Plant tissue culture plays a major role in the development ofthe commercial production of pathogen-free plants (Fay, 1992), and helps in the germplasm conservation of rare and endangered species (Campbell *et al.*, 2003). The biotechnology applications like tissue culture plays an important role in the propagation of selected genotypes (Barrett, 2002). The plant raised through seeds, are highly heterozygous and shows greater variations in growth and yield when it is released as a commercial crop. Likewise, the majority of the plants are not responsive through vegetative propagation like cutting and grafting, which limits the multiplication of desired cultivars. Plants verities propagated through vegetative means contain plant pathogens like bacteria, fungi and viruses which may affect the appearanceandquality of selected items. In recent years, tissue culture has emerged as a promising technique to obtain genetically pure elite populations under *in vitro* conditions rather than having indifferent populations.

Turnera subulata belongs to the family Turneraceae which is one of the 28 angiosperm family showing heterostyly (Urban, 1883). *Turnera*is one of the most important genera of the family turneraceae which comprises more than 100 species, grouped into nine series (Barrett, 1998). Which are largely distributed in the tropical and subtropical region of Asia and Africa (Lorenzi, 2008). *T. subulata* is a polymorphic polyploid complex of perennial weeds commonly called 'Butter cup'. This variety is a dense, compact shrub that has dark green foliage and light yellow flower. *Turnera*is adopted as an ornamental plant, being used as the foundation, border, mass planting and ground cover (Gracioso *et al.*, 2002). *Turnera* is also used as a tea for the treatment of disease related mainly to gastric dysfunction, research has produced data indicating that the plant extract has a significant antiulcerogenic effect (Kumar *et al.*, 2005). The present study reports an efficient protocol through *in-vitro* culture by using nodal explant to increase their population to meet the needs.

2. MATERIALS AND METHODS

Study species were collected in and around Maruthamalai hills,Coimbatore. Healthy, young and disease free portion of the branches was selected and used as explants. Healthy nodal explants were selected and washed thoroughly under running tap water for 15 min to wash off the dust, dirt's and microbes present on the surface. The explants were cut (1-2 cm) separately and they were washed with a detergent solution (Tween 20) for 10 min. After, they were thoroughly washed under running tap water until the traces of detergent solution were removed. Further steps of surface sterilization were carried out under aseptic conditions in the laminar air flow chamber. The shoots were then subjected to 70% ethanol treatment for 30 seconds and again washed with sterilized double distilled water at least three to

four times, follows with mercuric chloride (0.12%w/v $HgCl_2$) solution for 3 min and rinsed four to five times with sterilized double distilled water. Sterilized explants were inoculated on MS medium for callus and shoot induction.

2.1. Shoot initiation and multiplication

For shoot induction, the nodal explants were cultured on MS medium supplemented with plant growth regulators like BAP and IAA in combinations for rapid shoot organogenesis. Twenty explants were used for each culture. The percent of explants responding for shoot formation were recorded after 35 days. In the subsequent subcultures, the multiple shoots from the nodal explants were carried out at the regular interval of 15-20 days.

2.2. Rooting of in vitro produced shoots

Shoots with 5-6 cm height was separated and individual shoots were transferred for rooting to MS medium containing different concentration of Kn, IAA and IBA. The cultures were incubated under 16 h photoperiod for 15-20 days until the micro shoots developed the roots. Then the rooting frequency was measured.

2.3. Acclimatization of plantlets

The well-developed plantlets were removed from the culture bottles and washed with tap water to remove traces of agar and dipped in fungicide for a few minutes. Then the plantlets were planted on to net pot contains different type of potting media and survivability rate were determinate after 20 days of step-wise hardening processes. Hardened plants were transferred to a pot containing mixture of decomposed coir waste, garden soil and vermiculite (1:1:1 ratio). The pots were watered at two days interval under shade house condition. After 60 days, the frequency of survival was calculated.

3. RESULTS AND DISCUSSION

The morph genic response of nodal explants was observed on MS medium containing different concentration and combination of BAP and IAA. MS medium without growth regulators (control) induced no shoots. However, the multiplication rate, shoot number was higher in culture supplemented with growth regulators. The percentage of response varied with varying concentrations of growth regulators used. All the concentrations of BAP and IAA facilitated shoot bud differentiation. Swelling of dormant axillary bud took place within 8 days of inoculation and then differentiation into multiple shoots occurred after 35-40 days. The highest amount (86.50%) of culture response was observed on MS medium supplemented with BAP and IAA at 2.22 and 0.90μm /l respectively.

Table 1. Effect of different concentration of growth hormones added to the MS medium on shoot proliferation from nodel explants of *Turnera subulata.*

Growth Regulators (μm /l)		%of culture response
BAP	IAA	
2.22	0.90	86.50±0.81
4.44	0.90	83.33±0.53
6.66	0.90	75.33±0.51
8.88	0.90	56.66±0.42
10.10	0.90	40.66±0.61
12.32	0.90	11.83±0.46
2.22	1.8	72.16±0.04
4.44	1.8	70.50±0.54
6.66	1.8	55.66±0.51
8.88	1.8	41.33±0.75
10.10	1.8	29.16±0.42
12.32	1.8	18.33±0.46
2.22	2.7	57.16±1.04
4.44	2.7	45.16±0.56
6.66	2.7	32.50±0.39
8.88	2.7	30.83±0.63
10.10	2.7	21.50±0.75
12.32	2.7	11.16±0.81

Table 2. Effect of different concentration of growth regulators on per cent of shoot multiplication, shoot number and shoot length after subculturing of *in vitro* derived shoots of *Turnera subulata.*

Growth Regulators (μm /l)		Percent explants with multiple shoots	No. of shoots/ explant	Shoot length (cm)
BAP	IAA			
2.22	0.90	84.33±1.36	4.33±1.03	4.83±0.75
4.44	0.90	82.00±0.89	2.83±0.75	4.33±1.03
6.66	0.90	71.83±0.75	2.16±0.75	3.33±0.81
8.88	0.90	51.66±0.42	1.66±0.33	2.50±0.54
10.10	0.90	41.55±0.54	3.66±0.81	1.50±0.54
12.32	0.90	21.33±0.33	2.33±0.51	0.66±0.51

2.22	1.8	62.00±0.63	3.33±0.51	3.00±0.63
4.44	1.8	61.66±0.51	3.00±0.63	2.66±0.81
6.66	1.8	51.50±0.54	3.16±0.30	2.33±0.51
8.88	1.8	41.50±0.54	2.66±1.03	2.00±0.81
10.10	1.8	21.33±0.51	2.33±0.33	1.50±0.54
12.32	1.8	11.16±0.40	2.00±0.44	0.91±0.04
2.22	2.7	47.83±0.75	2.00±0.57	2.83±0.75
4.44	2.7	41.66±0.81	2.00±0.63	2.50±0.54
6.66	2.7	31.66±0.49	1.50±0.54	1.83±0.75
8.88	2.7	28.33±0.33	1.33±0.51	1.50±0.54
10.10	2.7	21.33±0.51	1.00±0.63	1.51±0.03
12.32	2.7	17.00±0.57	0.85±0.71	0.83±0.75

Table 3. Effect of different concentration of growth regulators on rooting percentage, root number and root length after subculturing of shoots of *Turnera subulata.*

Growth Regulators (μm /l)			% Shoots rooted	Number of roots/shoot	Root length (cm)
Kn	IAA	IBA			
5.35	-	-	70.33±0.33	4.16±0.47	3.85±0.69
10.74	-	-	63.83±0.04	3.26±0.04	3.66±0.51
15.05	-	-	51.66±0.30	2.50±0.42	2.85±0.69
20.40	-	-	24.16±0.63	2.00±0.36	3.00±0.63
25.75	-	-	50.16±0.42	1.16±0.13	1.16±0.75
-	2.86	-	46.53±0.75	3.66±0.51	2.85±0. 75
-	5.72	-	34.82±1.03	2.33±0.61	2.00±0.63
-	8.58	-	23.62±0.81	2.00±0.63	2.00±0.63
-	11.44	-	21.43±0.03	1.16±0.75	1.00±0.89
-	14.30	-	13.16±0.63	0.80±0.83	0.85±0.69
-	-	2.46	87.34±0.52	6.00±.63	5.66±0. 81
-	-	4.92	84.83±0.81	5.5±0.54	4.83±0.75
-	-	6.38	44.66±0.75	4.33±0.51	3.26±0.04
-	-	8.84	24.16±0.32	2.83±0.75	2.42±053
-	-	10.30	10.16±0.63	1.50±0.83	1.53±0.02

Table 4. Effect of different composition of hardening medium on survivability of plantlets of *Turnera subulata.*

Hardening medium composition(v/v)	No. of plants under hardening	No. of plants survived	Percentage of survivability
Garden soil	50	28	55.83
Vermiculite	50	30	60.83
Decomposed coir waste	50	40	80.50
Decomposed coir waste: garden soil: vermiculite(1:1:1)	50	47	93.83

Follows that 83.33% of culture response was observed on MS medium containing BAP and IAA at 4.44 and 0.90 μm /l respectively. The remaining concentration BAP and IAA produced satisfactory results. Further, the increasing concentration of IAA in MS medium registered decreasing culture response. The result reported that, the nodal explant of the study species induced shoots at low concentration of growth regulators in the MS medium (Table 1). A similar kind of result was reported for the species, *Bupleurum distichophyllum* (Karuppusamy and Pullaih, 2007).

The *in-vitro* produced shoots was subcultured in MS medium enriched with different

concentration of BAP and IAA for shoot multiplication. The highest (84.33%) shoot multiplication was observed on the medium containing, BAP and IAA at 2.22 and 0.90μm /l respectively. Whereas the highest number (4.33shoots) of shoot/ explant and high shoot length (4.83cm) was observed in the MS medium enriched with BAP and IAA at 2.22 and 0.90μm /l respectively (Table 2). However, the MS medium containing high concentration of growth regulator registered low percentage shoot multiplication. The varying response of explant for shoot multiplication depends on endogenus levels of growth hormones. The synergistic effect of auxin and cytokinin has been demonstrated in several medicinal plants *viz*., *Buplerum fruticosum* (Fraternale *et al.*, 2002) and *Rotula aquatic* (Martin, 2003). In accordance with these reports, the present investigation also exemplifies the positive role of cytokinin in combination with auxin respects to induction of multiple shoots. *In vitro* produced shoots were transferred to MS medium supplemented with Kn, IAA and IBA for root induction. Root formation from the basal cut end of the shoots was observed seven days after the transfer of shoots to the rooting medium without callus formation. The highest percentage (87.34%) of rooting was achieved in MS medium containing IBA alone at 46µM/l. The highest number (5.66 roots) of roots per shoot and increased length (81cm) of root were recorded after 20 days of culture (Table 3). Similar kind of results was reported in medicinal plants like *Disporum leschenaultianum* (Senthilkumar *et al.*, 2009).The well-developed healthy *in-vitro* rooted plantlets were washed thoroughly and planted in different hardening substrate (Table 4). Among the four different substrate used, hardening medium composed of decomposed coir waste, garden soil and vermiculite in the ratio of (1:1:1)was observed with higher survival percentage (93.83%).

The simple, reproducible protocol raised in the present investigation could be used for mass multiplication of the plantlets of the *T. subulata.*

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ANTHOCYANIN, THE NATURAL COLORANT AND ITS IMPLICATIONS IN HEALTH AND FOOD INDUSTRY: A SEARCH

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ABSTRACT

Anthocyanins are unique plant pigments since they are critical for most of the red, purple and blue pigmentation of flowers, fruits and vegetables. Meanwhile, they are reactive in nature, anthocyanins degrade easily, or react with other compounds such as reactive metals such as iron, aluminum, and tin in the media, to form colorless or brown colored by products. Anthocyanins are glycosides of anthocyanidins (aglycones) and sugars. Anthocyanidins are almost always glycosylated in the 3-position, though glycosylation in other positions and in more than one position at a time is also encountered. Furthermore, the sugar moiety may be acylated with aliphatic or aromatic acids. Anthocyanidins are less in number but anthocyanins show much diversity offered by glycosylation and acylation. 635 anthocyanins were identified in nature, featuring six common aglycones and various types of glycosylations and acylations. Reports suggest that dietary consumption of anthocyanins is good for health. Based upon many cell-line studies, animal models, and human clinical trials, it has been suggested that anthocyanins possess anti-inflammatory and anti-carcinogenic activity, cardiovascular disease prevention, obesity control, and diabetes alleviation properties, all of which are more or less associated with their potent antioxidant property. Evidence suggests that absorption of anthocyanins occurs in the stomach and small intestine. Epithelial tissue uptake seems to be highly efficient, yet transportation into circulation, tissue distribution, and urine excretion are very limited. The bioactivity of bioavailable anthocyanins should be a focus of future research regarding their putative health-promoting effects.

Keywords: Anthocyanin, pigments, colorant, dietary, economical, therapeutic roles.

1. INTRODUCTION

Anthocyanins are flavonoid group of phytochemicals common in teas, honey, wines, fruits, vegetables, nuts, olive oil, cocoa, and cereals. The flavonoids, perhaps the most important single group of phenolics in foods, comprise a group of over 4000 C_{15} aromatic plant compounds with multiple substitution types. The primary members in this group include the anthocyanins like cyanidin, pelargonidin, petunidin, the flavonols (quercetin, kaempferol), flavones (luteolin, apigenin), flavanones (myricetin, naringin, hesperetin, naringenin), flavan-3-ols (catechin, epicatechin, gallocatechin), and, although sometimes classified separately, the isoflavones (genistein, daidzein). They are commonly referred as bioflavonoids due to their multifaceted roles in human health maintenance and anthocyanins in food are typically ingested as components of complex mixtures of flavonoid components (Skibola and Smith, 2000).

The anthocyanins are anthocyanidins with sugar group(s) mostly 3-glucosides of the anthocyanidins. The anthocyanins are subdivided into the sugar free anthocyanidin aglycones and the anthocyanin glycosides. Anthocyanin pigments are assembled from two different streams of chemical raw materials in the cell: both starting from the C2 unit acetate (or acetic acid) derived from photosynthesis, one stream involves the shikimic acid pathway to produce the amino acid phenylalanine. The other stream (the acetic acid pathway) produces 3 molecules of malonyl-Coenzyme A, a C3 unit. These streams meet and are coupled together by the enzyme chalcone synthase (CHS), which forms an intermediate chalcone via a polyketide folding mechanism that is commonly found in plants. The chalcone is subsequently isomerized by the enzyme chalcone isomerase (CHI) to the prototype pigment naringenin, which is subsequently oxidized by enzymes like flavonoid hydroxylase and coupled to sugar molecules by enzymes like UDP-O-glucosyl transferase to yield the final anthocyanins. More than five enzymes are thus required to synthesize these pigments, each working in concert (Raghvendra *et al.*, 2011). The most common combination of side groups and their names are displayed in Table 1.

Anthocyanidin	R_1	R_2	R_3	R_4	R_5	R_6	R_7	main colour
Apigeninidin	-H	-OH	-H	-H	-OH	-H	-OH	orange
Aurantinidin	-H	-OH	-H	-OH	-OH	-OH	-OH	orange
Capensinidin	$-OCH_3$	-OH	$-OCH_3$	-OH	$-OCH_3$	-H	-OH	bluish-red
Cyanidin	-OH	-OH	-H	-OH	-OH	-H	-OH	magenta
Delphinidin	-OH	-OH	-OH	-OH	-OH	-H	-OH	purple, blue
Europinidin	$-OCH_3$	-OH	-OH	-OH	$-OCH_3$	-H	-OH	bluish red
Hirsutidin	$-OCH_3$	-OH	$-OCH_3$	-OH	-OH	-H	$-OCH_3$	bluish-red
Luteolinidin	-OH	-OH	-H	-H	-OH	-H	-OH	orange
Pelargonidin	-H	-OH	-H	-OH	-OH	-H	-OH	orange, salmon
Malvidin	$-OCH_3$	-OH	$-OCH_3$	-OH	-OH	-H	-OH	purple
Peonidin	$-OCH_3$	-OH	-H	-OH	-OH	-H	-OH	magenta
Petunidin	-OH	-OH	$-OCH_3$	-OH	-OH	-H	-OH	purple
Pulchellidin	-OH	-OH	-OH	-OH	$-OCH_3$	-H	-OH	bluish-red
Rosinidin	$-OCH_3$	-OH	-H	-OH	-OH	-H	$-OCH_3$	red
Triacetidin	-OH	-OH	-OH	-H	-OH	-H	-OH	red

2. DIVERSITY OF MAJOR PIGMENTS

2.1. Pigments in general

Pigments are chemical compounds that absorb light in the wavelength range of the visible region. Produced color is due to a molecule-specific structure (chromophore); this structure captures the energy and the excitation of an electron from an external orbital to a higher orbital is produced; the non absorbed energy is reflected and/or refracted to be captured by the eye, and generated neural impulses are transmitted to the brain where they could be interpreted as a color.

2.2. Classification

2.2.1. By their origin

Pigments can be classified by their origin as natural, synthetic, or inorganic. Natural pigments are produced by living organisms such as plants, animals, fungi, and microorganisms. Synthetic pigments are obtained from laboratories. Natural and synthetic pigments are organic compounds. Inorganic pigments can be found in nature or reproduced by synthesis.

2.2.2. By the chemical structure of the chromophore

Pigments can be classified by taking into account the chromophore chemical structure as:

Chromophores with conjugated systems: carotenoids, anthocyanins, betalains, caramel, synthetic pigments, and lakes.

Metal-coordinated porphyrins: myoglobin, chlorophyll, and their derivatives.

2.2.3. By the structural characteristics of the natural pigments

Tetrapyrrole derivatives: chlorophylls and heme colors.

Isoprenoid derivatives: carotenoids and iridoids. N-heterocyclic compounds different from tetrapyrroles: purines, pterins, flavins, phenazines, phenoxazines, and betalains.

Benzopyran derivatives (oxygenated heterocyclic compounds): anthocyanins and other flavonoid pigments.

Quinones: benzoquinone, naphthoquinone, anthraquinone.

Melanins

2.2.4. As food additives

By considering the pigments as food additives, their classification by the FDA is

Certifiable: These are manmade and subdivided as synthetic pigments and lakes.

Exempt from certification: This group includes pigments derived from natural sources such as vegetables, minerals, or animals, and manmade counterparts of natural derivatives.

The colorants that occur naturally in food plants have been the source of the traditional colorants of raw as well as the processed food. However, they can also be obtained from microorganisms and animals, but few of them are available in sufficient quantities for commercial use as food colorant. Although, biocolorants are structurally much diversified and from a variety of sources, the three most important are: tetrapyrrols, tetraterpenoids, and flavonoids. The main pigments and their potential natural sources are discussed below.

Pigment	Coloring principle from plant origin
Purple	Anthocyanins - Red Cabbage
Blue	Anthocyanins - Red Cabbage
Turquoise	Anthocyanins - Red Cabbage
Red	Beet Red
Yellow	Curcuma (Turmeric)
Orange	Annatto - Bixin
Green	Cu-Chlorophyllin
Light Green	Red Cabbage and Curcuma
Dark Brown (charcoal)	Caramel Color
Medium Brown (slight red tint)	Caramel Color
Light Brown (slight yellow tint)	Caramel Color

Conjugated systems for electron bond chemistry that causes these molecules to have pigment. On the basis of this pigments may be classified as

1. **Heme/porphyrin-based**: chlorophyll, bilirubin, hemocyanin, hemo globin, myoglobin

2. **Light-emitting:** luciferin
3. **Carotenoids:**

a. Hematochromes (algal pigments, mixes of carotenoids and their derivates)

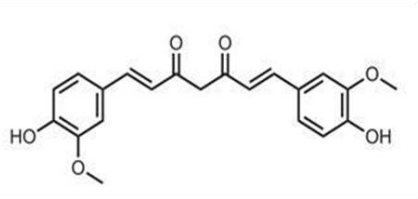
b. Carotenes: alpha and beta carotene, lycopene, rhodopsin

c. Xanthophylls: canthaxanthin, zeaxanthin, lutein

4. **Proteinaceous:** phytochrome, phycobiliproteins
5. **Polyene enolates:** a class of red pigments unique to parrots
6. **Other:** melanin, urochrome, flavonoids

Carotenoids

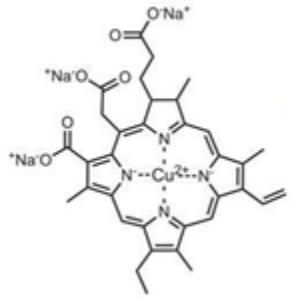
Carotenoids are familiar as food ingredient such as beta-carotene, apocarotenal, lycopene, annatto, paprika and lutein. They can deliver shades from weak yellow to a reddish colour, and anything in between.



They all decay by oxidation, losing their colour, so incorporating antioxidant ingredients is the key to stability and a good shelf-life in the warehouse, during processing and over time on the supermarket shelf. This is commonly done with ascorbic acid, ascorbyl palmitate or tocopherol. Temperature, pH, air and light are also important. Food production often includes heat treatment to control bacteria, which can affect the colour. Orange-red annatto is derived from the seeds of the *Bixa orellana*. The main coloured component is the oil-soluble carotenoid bixin- a carboxylic acid group at one end of the conjugated chain, and a methyl ester at the other. Norbixin is the de-esterified diacid, which is water soluble. It's in widespread use in dairy products such as cheddar, colby and red leicester cheeses, where it has been used for centuries to impart a characteristic orange colour.

2.4. Canthaxanthin

Canthaxanthin, meanwhile produces a bright deep red colour and when people taking canthaxanthin capsules as a sun-tanning aid developed reversible deposits of canthaxanthin crystals in their retinas.



Anthocyanin

The largest group of water-soluble pigments is the anthocyanins, whose colour tends to change with pH. They're basically indicators. Anthocyanin can be anywhere from red to a purple to blueish at neutral pH, and if pH increased further it will go green or brown, and ultimately colourless. This pH sensitivity makes food applications a real challenge. Synthetic colours remain a constant shade regardless of pH. Anthocyanins are also often light sensitive. In contrast to the carotenoids, which need ascorbic acid to stabilise them, they will be destroyed by ascorbic acid.

Curcumin

Another water-soluble pigment, curcumin, is extracted from turmeric. Its vibrant lemon-yellow colouration fades very rapidly in beverages as it is not light stable. Yellow sweets are commonly coloured with curcumin, and it performs brilliantly in confectionery, with a fantastic shelf-life and maintaining its vibrancy. But in a beverage or anywhere else with an excess of free water, it will fade very rapidly (Kulkarni *et al.*,2012).

Carmine

One natural pigment that many food manufacturers are moving away from is carmine, which, as it is derived from the cochineal beetle, is not vegetarian, kosher or halal. Carmine is a very stable red, and while anthocyanins are a successful replacement in beverages. Here, the colour of choice is often one derived from beetroot, which contains the indole-based pigment betanin. Beetroot used for commercial colour production are selectively bred to contain more betanin.

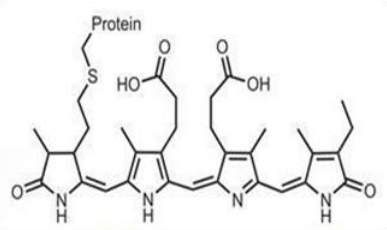
Copper chlorophyllin

Copper chlorophyllin has a vibrant bluish green colour. Green can be achieved using chlorophyll and copper chlorophyllin, a more stable derivative of chlorophyll with a more vibrant blue-green shade, and a more lime-green shade is possible

when mixed with curcumin. It's not acid stable (Alison and Paul, 2000).

Phycocyanin

Blue is difficult to achieve with natural colours, and the only way is spirulina blue from spirulina blue–green algae. 'The colouring portion is phycocyanin, which is a pigment–protein complex, Its proteinaceous nature means it's limited to pH neutral applications. It's not a pure chromophore, and often contains a small amount of a reddish phycorubin component that can lend brown undertones when used with yellow to make a green. Phycocyanin, a pigment protein complex, is concentrated from algae.



GLOBAL VALUE

Biocolourant lost their appeal with the synthetic colors arrived on the scene, as they provide less consistency, heat stability and color range than their chemical alternatives. The global market for natural carotenes has reduced after the introduction of synthetic colour. Moreover, biocolourant are more costly and unstable in nature. The leading markets for natural colours are the UK, Germany, France, Italy and Spain. Currently, there is also a flourishing market in China, India and South Korea. The demand for natural colours are increasing regularly because of the following factors such as

- Health-promoting features of food with natural colours
- Biocolourant has been the public priority
- Low lipid content preferred for improved food formulations, replacing high fats or other synthetic food additives
- Demand of consumer for organic food
- Variety and international preference of natural food colour and flavours.

The market for natural food colours is estimated to increase by approximately 12% annually. Fig. 1 and 2 display the global scenario about the usage of biocolourant. Many of the raw materials for colours and flavours require growing conditions which are more favourable in countries outside Europe. So they imports natural colours and flavours estimated to € 2,765 million or 789 thousand tonnes. Developing countries like India and china may play a major role in supplying natural colours either in processed forms or as raw materials to the markets, due to their favourable climatic and production conditions coupled with the rise in their middle income family. In essence, the message to consumers that "Natural is Better" is gaining popularity day-by-day. Although, natural colors are on the rise but they are unlikely to be a total replacement for synthetic dyes because the area of land required for production of natural colorants yielding plants increasing due to inadequate strategies and horticultural practices on this crops. It was estimated that to provide sufficient vegetable dyes to dye cotton alone, about 462 million ha would be needed, i.e., 31% of the world's current agricultural land, which appear unlikely. Thus, natural dyes is likely to occupy a small niche market, unless technology of horticultural practices and pigments extraction is redefined and standardization on modern scientific lines (Glover and Pierce,1993).

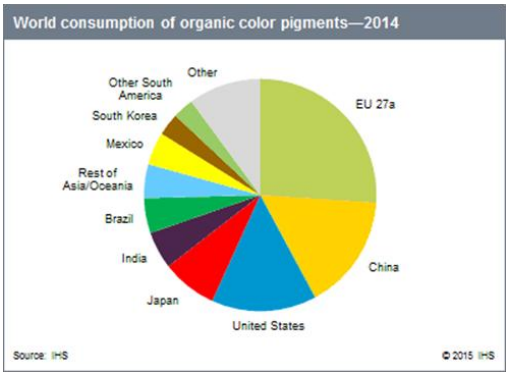


Fig. 1. World consumption of organic colour pigments

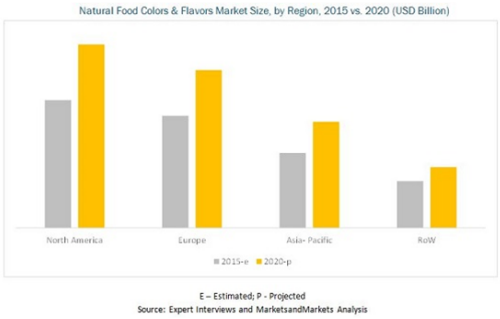


Fig. 2 Natural colour usage in different continents

COLOUR CHOICE

Commonly single natural coloring agents may not give the desired effect, the background color and of neighboring colored substances make a large impact in the color outlook. Product concepts, requiring blue or green, limit the choice from certified colors only. Bluish purple can be achieved

with carmine, but it does not create a true blue. Annatto or turmeric tends to represent a cheese color or have an eggy tone compared with the bright color produced with the

FD and C-yellow. Now-a-days, fluorescent colors are also getting importance in food industry as consumers favor foods to glow under conditions. Turmeric is highly fluorescent, thus it is commonly used in food (Martin *et al.*, 2007).

3. APPLICATIONS IN FOOD AND PHARMACEUTICAL INDUSTRY

3.1. Food preservatives

Generally, natural biocolorants possess antagonistic activity against certain bacteria, viruses and fungi for protecting the food from microbial spoilage. (Chattopadhyay *et al.*, 2008) reported that dyes were active against protozoans such as *Leishmania brasiliensis*, and insects like *Calliphora erythrrocephala.* Carotenoids are also known to act as sun screen for maintaining the quality of food by protecting them from intense light. Norton (1997) reported that corn carotenoids inhibit the synthesis of aflatoxin by *Aspergillus flavus* (90%) and by most of the *A. parasiticus* (30%) strains.

3.2. Quality control markers

Generally for maintenance of good manufacturing practices, level of anthocyanin is used as an indicator to evaluate the quality of colored food (Boyles and Wrolstad, 1993). Anthocyanin profiles have been used to determine the quality of fruit jams. Adulteration of blackberry jam with strawberries can also be detected efficiently by the analysis of pelargonidin and cyanidin-3-glucoside content (Garcia-Viguera *et al.*, 1997).

3.3. Nutritional supplements

Natural colours includes phytochemicals produced by plant cells, which are known as the vegetal active principles. These are sources for obtaining biologically active drug substances and many other natural compounds used in various industries such as food, pharmaceuticals, cosmetics, with important commercial value (Filimon, 2010). Carotenoids are also used as vitamin supplements, since β-carotene is the precursor of vitamin A. In under developed countries, the diet is primarily of rice, there is every possibility of inadequate supply of vitamin A, which leads to night blindness and in extreme cases to xerophthalmia. Riboflavin is another example of natural food grade biocolorant which is an essential vitamin source and available in milk and in several leafy vegetables, meat, and fish (Counsell *et al.*, 1979 and Nagaraj *et al.*, 2000). Yellow β- xanthins, are potential as food colorant and also may be used as a means of introducing essential dietary amino acids into foodstuffs.

3.4. Medicinal

Plant dyes play remarkable roles in human health as they contain biologically active chemicals, which possess a number of pharmacological features like strong antioxidant, antimutagenic, anti-inflammatory and antiartheritic effect (Hari *et al.*, 1998; Saleem *et al.*, 2004). Carotenoids also act as biological antioxidants, protecting cells and tissues from the damaging effects of free radicals and singlet oxygen and also as a good source of anti-tumor agent (Zeb and Mehmood, 2004). Lycopene is particularly effective at quenching the destructive potential of singlet oxygen. Lutein, zeaxanthin and xanthophylls are believed to function as protective antioxidants in the mascular region of the human retina (Landrum *et al.*, 1997). These compounds also act against aging, mascular degeneration, and senile cataracts. Betacyanin also contain antioxidant and radical scavenging properties. Since betanin exerts a good bioavailability, red beet products may provide protection against certain stress related disorders. It has been established that flavonoids present in different plant products show good antioxidant activity, sometimes better than the commercially available antioxidants. Allomelanins (free of proteins) from plants are found to suppress growth of tumorigenic cells of mammals. Grape seed extract is the primary commercial source of a group of powerful antioxidants known as oligomeric proanthocyanidins (OPCs), also generically called pycnogenol, a class of flavonoids. Canthaxanthin also shows antioxidant property. Astaxanthin is another naturally occurring xanthophyll with potent antioxidant properties. Other health benefits of biocolorants include enhancement of immune system function, protection from sunburn, and inhibition of the development of certain types of cancers (Bendich, 1989; Mathews-Roth, 1990). Lycopene prevents oxidation of low-density lipoprotein (LDL) cholesterol and reduces the risk of developing atherosclerosis and coronary heart disease. Epidemiological studies revealed that there is a positive correlation between the consumption of chlorophylls and decreased risk of colon and other cancers.

4. MODERN TECHNOLOGY VS NATURAL DYES

Even though there are many natural dye yielding plants only a few are reported and explored for the same. Therefore, modern technology could be a solution for biopharming coloring compounds which are difficult to synthesize by traditional

methods of extraction. Biotechnology and cell line culture are alternatives for this process. Biotechnological production of such colorants, plants and microorganisms are more suitable due to understanding of proper cultural techniques and processing.

4.1. Microbial cell culture for biocolorants production

Bradyrhizobium sp. strain are known to produce canthaxanthin (4, 4'-diketo-β- carotene) and the carotenoid gene cluster was fully sequenced (Asker and Ohta, 1999). This keto-carotenoid was also found in *Halobacterium.* Culture of *Flavobacterium* sp. (Shepherd *et al.*, 1976) in nutrient medium containing glucose or sucrose, sulphur-containing amino acids such as methionine, cystine or cysteine, pyridoxine and bivalent metal ions was able to produce zeaxanthin. *Haematococcus lacustris* is commercially used for the production of astaxanthin using bioreactor. Besides, echineone and canthaxanthin are also identified in *Haematococcus* cultures. *In vivo* and *in vitro* studies have shown that high astaxanthin production required high level of oxygen (aerobic conditions) and high C/N ratio but cell growth requires low C/N ratio (Yuan *et al.*, 1997; Yamane *et al.*, 1997; Chumpolkulwong *et al.*, 1997a). Also, it is suggested that the addition of ethanol during the second stage enhanced the production of astaxanthin 2.2 times whereas compactin resistant mutants of *H. pluvialis* (compactin inhibits HMGR that strongly blocks cholesterol formation) showed 2 times enhanced yield (Chumpolkulwong *et al.*, 1997b). *Dunaliella bardawil* and *Dunaliella salina* produce β-carotene as their main carotenoid (Phillips, 1995). *Blakeslea trispora* is known to produce β-carotene. The cell growth and β-carotene production are enhanced in medium containing surfactants such as Span or Triton, except Triton X-100 (Kim *et al.*, 1997). *Phycomyces blakesleeanus* is known for β-carotene production (Ootaki *et al.*, 1996). In *Blakeslea trispora*, sexual stimulation of carotene biosynthesis remains essential to increase yield significantly (Mehta *et al.*, 1997). Several strains of *Monascus* are also being exploited for commercial production of red and/or yellow pigments. The red yeast, *Xanthophyllomyces dendrorhous* synthesizes astaxanthin and zeaxanthin as its main carotenoids. Commercial production of carotenoids using microorganism has been achieved in case of astaxanthin, by red yeast fermentation. *Rhodotorula* including species *R. glutinis, R. gracilis, R. rubra,* and *R. graminis* synthesize carotenoids (Sakaki *et al.*, 2000; Simova *et al.*, 2004; Tinoi *et al.*, 2005).

The Czech Republic's Ascolor Biotech is awarded patents of compounds from new fungal strains that produce a red colorant which can be applied in the food and cosmetic industries. The strain *Penicillium oxalicum* var. armeniaca CCM, obtained from soil, produces a chromophore of the anthraquinone type.

4.2. Plant cell culture for biocolorants production

Cells culture is the most common practice for production of plant pigments, as culture ensures uniform quality and continuous production of pigments. *Vitis vinifera*, *Aralia cordata*, *Aralia cordata*, *Fragaria anansa, Perilla frutescens*, *Daucus carota*, *Crocus sativus, Bixa Orellana* and *Beta vulgaris* produce via cell culture produce anthocyanin, crocin, carotenoid, bixin and norbixin betalain, betacyanin, betaxanthins (portulaxanthin-II and vulgaxanthin-I), muscaauri-VII, dopaxanthin, and indicaxanthin and other similar compounds (Rymbai *et al.*, 2011).

5. SIDE EFFECTS OF BIOCOLOURANT

Natural pigments are the most important precursor of several nutrients (β- carotene is the precursor of vitamin A, as well as many other carotenoids) and they have always been present in the diet of man. Food allergy and intolerance among human beings has increased in recent years, efforts to identify foods and food constituents that may cause reactions have also increased. Thus a variety of foods and food constituents have been identified which cause allergies. The consensus adopted by the Codex Alimentarius Commission of the WHO (1998) experts for investigating food colourants, they consider eight foods or food groups to be the major causes of food allergy. Natural color additives are justifiably not included among the foods and food groups identified by the Codex. Lucas and Taylor (1998) critically evaluated of the available information and demonstrated that reactions to natural color additives are rare. Studies of turmeric and carotenoid pigments administered in mixtures with other food colourings failed to definitely identify reactions to either color additive and also found no reports of sensitivities to grape skin extract or grape color extract and hence concluded that the ingestion of natural color additives presents a very low risk of provoking adverse reactions.

6. IMPORTANCE

The use of natural colours may show benefits over synthetic colours**.** Natural dyes are less toxic, less polluting, less health hazardous, non-carcinogenic and non-poisonous and prevent chronic diseases such as prostrate cancer. In addition to this,

they are harmonizing colours, gentle, soft and subtle, and create a restful effect. Most of them are water-soluble (anthocyanins), which facilitates their incorporation into aqueous food systems. These qualities make natural food colorants attractive. Above all, they are environment friendly and can be recycled after use. Thus, they attribute to food-both for aesthetic value and for quality judgement and also they tend to yield potential positive health effects, as they possess potent antioxidant and improve visual acuity properties. Anthocyanins also possess antineoplastic, radiation-protective, vasotonic, vasoprotective, anti-inflammtory, chemo- and hepatoprotective potentialities.

7. PITFALLS OF PIGMENTS

Natural colours in spite of having many benefits, natural dyes have some limitations as well. Tedious extraction procedures of colouring component from the raw material, low colour value and longer time make the cost of dyeing with natural dyes considerably higher than synthetic dyes. Some of the natural dyes are fugitive and need a mordant for enhancement of their fastness properties while some of the metallic mordents are hazardous. Besides, there are problems like difficulty in the collection of plants, lack of standardization, lack of availability of precise technical knowledge of extracting and dyeing technique and species availability. The use of these colorants in food products may also face some problems due to their instability during processing due to their sensitivity to temperature, oxygen, light and pH. They can also be decolourised or degraded during storage. Anthocyanin degradation and brown pigment formation cause color loss in food products. Curcumin is very prone to photobleaching and beetroot color has low heat stability. However, stability of biocolourant can be maintained by adding dextrins additives extracted from tart cherries or maltodextrin extracted from Roselle as a stabilizer. It has been demonstrated that increased glycosidic substitution, and in particular, acylation of sugar residues with cinnamic acids and reduced water activity will enhance stability and anthocyanin pigments in dried forms can exhibit high stability.

8. CONCLUSION

The review highlights the need to tap existing indigenous knowledge and understanding of plants to promote art education and technical skills development so that traditional values can be incorporated in the schools and colleges curricula towards the creative information particularly in the vocational subjects. This exercise also offers opportunity for common man about the nature of the plants, their characteristics, local and botanic names, medicinal value, dye-yielding quality, and their uses across the country. Since the dye extraction and application project involves visits to nature reserves and the indigenous textiles production centers where plant dyes are traditionally used on a large scale, the students will be knowledgeable in using dyes extracted from plant source for use as colourants for food and textiles to sustain life and generate employable skills. This will also offer opportunity for people to learn skills in tie-dye, batik, printing, dyeing of yarns for macramé, and crocheting at little or no cost and only buy synthetic dyes and food colourants for examination purposes since practically does not recognize the use of natural dyes and pigments for this purpose.

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CULTIVATION AND ECONOMICAL PERSPECTIVES OF *GRACILLARIA*: MARINE SEAWEED

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ABSTRACT

For decades, seaweed has been of biological, industrial, and pharmaceutical importance. Because of their nutraceutical potential, seaweed has been used as a food throughout Asia. Traditional Chinese medicine used aqueous hot extracts of certain seaweeds in the treatment of cancer. Further, the Japanese and Chinese cultures have used seaweeds to treat goiter and other glandular problems since 300 BC. The Romans used seaweeds in the treatment of wounds, burns, and rashes. The Celts noted that ordinary seaweed contracted as it dried and then expanded with moisture. In Scotland during the 18th century, physicians used dried seaweed stem to successfully drain abdominal wall abscesses. They also inserted seaweed into the cervix in an attempt to treat dysmenorrhea. Many reports outline the use of seaweed to induce abortion. Seaweed was employed intravaginally for ripening of the cervix and was used rectally for strictures. In this juncture, culture and therapeutic potential of *Gracilaria* was reviewed. *Gracilaria* is a genus of red algae notable for its economic importance as an agarophyte, as well as its use as a food for humans and various species of shellfish. Various species within the genus are cultivated among Asia, South America, Africa and Oceania. Gracilaria is used as a food in Japanese, Hawaiian, and Filipino cuisine. In Japanese cuisine, it is called ogonori or ogo. In thePhilippines, it is called gulaman and used to make gelatin. In Jamaica, it is known as Irish moss. The moisture content is 12% and protein is 8%. The species are used by local people as salad, preparation of various curries and industrially many by products are synthesized from this sea weed. Medicinally as microbicidal, antiinflammatory, antimetastatic and immuno modulatory potential.

Keywords: *Gracilaria*, seaweeds, culture, medicinal, nutraceutical.

1. INTRODUCTION

Gracilaria species are common warm water seaweeds. There are more than one hundred species in the world, some of which have very important economic value. *Gracilaria* is used as food and in the preparation of food products.

In China, *Gracilaria* species were used as food and as binding material in the preparation of lime for painting walls. In course of time it was popularized in several Asiatic countries, until its content of agar were discovered by the Japanese and the Western countries. A diversity of cultivation methods has been developed in different places, which is also consistent with the great variety of species of gracilarioids and their great range of temperature and latitudinal tolerance (Hansik and Ryther; 1984).

In the late fifties to early sixties, commercial cultivation was carried out in the Guangdong Province of China by inserting branches of the seaweed into splits of bamboo sticks about 15 cm. long, which were then planted in littoral farms. Later, cultivation of *Gracilaria* was carried out by scattering juvenile thallus fragments (5-6 cm. long) in littoral farms sheltered from wave action. Thalli in the fish ponds were covered with old fishing nets to avoid drifting. The method was initiated in Taiwan and by the late seventies, the *Gracilaria* ponds occupied over 200 hectares in area with production reaching over 6800 tonnes and worth almost US$ 4.7 million. Experiments on growing *Gracilaria* in ponds or lagoons were later done in Brazil, Italy, Sri Lanka, Malaysia, Philippines and Japan. The method has proved to be successful in Taiwan province of China, China, Vietnam, Thailand and Indonesia. Two cultivation methods of *Gracilaria* were developed in the 1980s. In St. Lucia, Lesser Antilles, *Gracilaria* was successfully cultivated on lines suspended near the surface by floating bamboo. These were later replaced by plastic bottles. A system of suspended cultivation with ropes and buoys was later developed in Namibia, while experiments with suspended ropes have been made also in Cuba, South Africa, India, the Philippines and New Zealand. At present, the most successful cultivation system with ropes and significant economic returns is being

developed in China with *Gracilariopsis lemanaeformis* (Santelices and Doty; 1989).

During the 1980s, a different cultivation method was developed in Chile. Because the thallus can survive burial in sandy mud for month, thallus fragments can be pushed in the mud with a fork or held down with sand-filled polyethylene tubing until the plants are established and growing. Harvesting is by hand, taking care not to remove the underground thalli. This method is the basis of the commercial production of *Gracilaria chilensis* and is confined to sheltered intertidal and shallow subtidal muddy sandy areas. Experimental tank cultivation of *Gracilaria* also has proved to be technically feasible, first in Florida for energy and later in Sweden, to improve agar quality and quantity (Ohno and Critchely; 1993).

2. MAJOR PRODUCTS

The FAO statistics for several species of *Gracilaria* are combined within the map shown below. Data are separated for what FAO has called *Gracilaria* spp. and warty *Gracilaria*. The last taxon is supposed to correspond to *G. verrucosa*, (now *G. gracilis*) being produced in China, Taiwan Province of China and other places of Asia and amounting to some 1.5 million tonnes. *Gracilaria* spp. includes all other species, being produced in Africa (Namibia and South Africa), America (Argentina, Brazil, Chile and Peru), Asia (Indonesia, Korea Philippines and Viet Nam). Not all of them, have productions registered in the national statistics. By 2011, the total production of *Gracilaria* spp. amounted to 700 000 tonnes (Bird and Porse; 1984).

2.1. Biology of Gracilaria

The typical habitats of these species are sheltered environments such as bays, estuaries, or river mouths. They grow on intertidal or subtidal rocky, sandy or sandy-muddy substrates, or on rocky outcrops associated with sandy beaches. They can be intertidal or sub tidal, down to 20 m depth, attached to small stones, partially covered by sand or anchored in sand. Often in areas with good water circulation. The fertilization of the egg in the carpogonium of the female gametophyte by the spermatium results in the production of the zygote, which in turn develops in the macroscopic carposporophyte within the fertile structure called cystocarp in the female gametophyte.

The carpospores (2n) produced by the carposporothyte develop into the tetrasprophyte. Meiosis takes place in the tetrasporangia, resulting in the production of tetraspores (n) which in turn develop into the gametophytes. The gametophyte and the sporophyte are the large phases in the life cycle of this seaweed. These seaweeds are characterized by their high vegetative regenerative capacity, a characteristic used by farmers to propagate the crop.

3. CULTIVATION

Given the diversity of cultivation methods used in the production of *Gracilaria* spp., the four most important are described below (Shang; 1976).

3.1. Pond Farming

Supplies of seed stocks are sourced from the wild, from crops obtained in other ponds or from nursery-reared cuttings. Ponds are generally located in areas not exposed to strong prevailing winds, near sources of freshwater and seawater. Usually pond depth is kept at 30-40 cm and water is exchanged every 2-3 days. Salinity is adjusted by freshwater additions while expected seasonal temperature regimes can be modified by pond depth (deeper ponds in warmer areas or seasons). Mineral, especially nitrate and phosphates can be added for seaweed growth. Stocking densities are 5-6 tonnes /ha. After 30-35 days of growth in summer and 40-45 days in winter, harvesting is undertaken removing one third to half of the total biomass. Annual production figures of about 34 tonnes / ha have been reported for this production system.

3.2. Rope Farming

Two methods of out-planting using ropes are utilized to grow *Gracilaria*. One starts from vegetative materials that are tied or inserted within a rope. The supplies of seed stocks also may be sourced from the wild, from crops obtained in previous cultivation or from nursery-reared cuttings. The second method involves reproductive materials which are used as a source of spores which are left to settle on the surface of the ropes. The ropes or lines used can be monofilament, nylon or other suitable line. Durability of the line in seawater should be tested before large quantities of line are purchased. The rope can be opened to insert the *Gracilaria* fragments or they could be tied at regular intervals to the main rope using pieces of raffia or tape. Once the *Gracilaria* is attached, ropes are then suspended, stretched between stakes buried in the sediment or supported at different levels by buoys or rafts. Light intensity and transparency of the water column are important factors that may limit survival and growth of the species under cultivation. Field conditions are site and species specific and should be tested previously to large-scale investment on farming. Spores may be set onto lines, allowing them to settle on the lines after laboratory induction of spore

release or lying ropes as substratum for spores in natural populations of fertile *Gracilaria*. Rope farming results in different levels of production, depending on climatic conditions and the species being cultivated. In Ceylon, up to 3.5 kg per m-1 could be obtained per crop while in China, productions up to 2 tonnes per ha-1 (dry) are reported.

3.3. Bottom stocking

The simplest method is to transfer rocks bearing the thalli to places where an increase in density is wanted. If the species is already growing on a site, this labor-intensive method is often successful. A more complex procedure is to remove thalli or major branches and put them in places where they are wanted. Two further common approaches are to tie the thalli to rocks, or to secure them to rocks by means of rubber bands. Another method used on non-consolidated bottoms is to push the proximal ends of whole thalli or major branches into the planting site bottom. This is feasible and is used in Chile with some promise. It is especially useful for intertidal mud flats where farmers can easily push bundles of thalli into the sand or mud during low tides. However, materials planted in substrate may tear loose during harvesting or during periods of much water movement. This problem has been common in the shallow intertidal flats of southern Chile. In addition planting and harvesting are high labor-consuming activities which can be economic only in areas with low labor costs (Zemekewhite, 2004).

A more elaborate system has been designed for subtidal beds on sandy bottom. *Gracilaria* is anchored using soft polyethylene tubes 1 m long, 0.1 mm thick and about 4 cm in diameter, which are completely filled with dry sand (ca. 2.5 kg) and knotted at both ends. Keeping them wet and cool, apical and middle portions of thalli about 40 cm long, collected from natural beds, are secured to the sand-filled polyethylene tubes with rubber bands. Five to six strands of the *Gracilaria* (about 90 g) are tied along one side of each anchoring unit. These units are then placed on a boat, transported to the planting area, dropped from the boat and arranged underwater by divers. Normally they are set in parallel rows, about 1 m apart and positioned perpendicular to the coastline. When setting up the rows, precautions are taken to rest the centers of the bundles directly on the substratum, where they are held in place by the weight of the sand in the tubes. Experimental evaluation of the polyethylene tube method has suggested potential yields of about 21 tonnes (dry) per ha for a 6 months growing period (Fredericq, 1989).

3.4. Tank Farming

Growing *Gracilaria* in tanks permits control over the whole production process. Also the method has the promise of achieving more sophisticated ends, e.g., the processing of polluted water to obtain some specific material (e.g. agarose) in addition to clean water. This method is particularly attractive in high-labor-cost areas, where capital returns are the principal benefits. Also, it provides the greatest productivity per unit area, much greater than any other type of farming (some 127 tonnes per ha per year). Efficiency of the system is dependent upon the input of various types of energy (compressed air for bubbling, carbon dioxide and pumping of water). Influent water should be filtered or held in sedimentation tank to reduce the problem of siltation in the tank. The tank cross-section may be v- or u-shaped and constructed from any number of convenient materials. Aeration systems are provided using air compressor and perforated PVC pipes.

For several reasons not completely understood, tank yields have been found to be directly proportional to seawater exchange rates of between 1 and 30 culture volumes/day. Carbon dioxide addition and pH adjustments can substitute for up to ten water exchanges per day. Under low water flow, *Gracilaria* growth may be limited not only by mineral nutrient availability but also by CO2 limitations. Accepting this, high production of *Gracilaria* in a land-based pond or raceway culture system would be carbon limited; the solution would be to add large volumes of water or large amounts of CO2 gas to the culture. In addition, there must be sufficient aeration to maintain the seaweed in suspension and to rotate it. Periodic aeration (15 min per hour, for a total of 7 h a day) proved to be as efficient as continuous aeration. Increases in duration of aeration up to 24 h per day were accompanied by increases in productivity but at such cost that little economic gain occurred after about 12 h; 11 h daily appeared to be the most economical.

Contrary to common expectations, continuous mineral nutrient additions to the tanks resulted in a reduction in the growth of *Gracilaria* due to enhanced growth of epiphytes (Kain;2003). However, two biological findings suggested an optimum nutrient management program for *Gracilaria*. First, it was found that epiphytes such an *Enteromorpha* take up nitrogen mainly in response to light, while *Gracilaria* takes up nitrogen at night as well as by day. Secondly, seaweeds have the capacity to store mineral nutrients when external supplies are available and then draw upon these reserves when external concentrations are low. Therefore, if

fertilizing is done only at night, and at 3 to 6 day intervals, such green algae as *Enteromorpha* may not become a pest. Nutrient supply to cultures has been successfully provided by pulse feeding of various concentrations at suitable frequencies.

The *Gracilaria* crop should be harvested periodically to maintain a high level of productivity. If the standing crop became too thick, self-shading may result. The harvesting methods vary depending on the size of the tanks and harvesting frequency. It may be adequate to use hand nets. Larger operations may require continual removal by mechanical scoops. Tanks could be drained into a net but efficiency might be compromised by the time needed to refill the tank.

Although the sustained yield of *Gracilaria* cultivated in tanks is among the highest for any seaweed tested, this production system for biomass is economical for a few types of production only (e.g. *Gracilaria* as fresh food). The method of cultivation employed is very energy intensive because it requires large amounts of flowing seawater and aeration. In addition, in many developed nations the requirement of large acreages of coastal land for land-based tank or raceway systems seems to be economically prohibitive. The FAO reported statistics for this group of seaweeds separate the warty *Gracilaria* from the other species of *Gracilaria* (*Gracilaria* spp.). The warty *Gracilaria* is supposed to correspond to *Gracilaria verrucosa*. This is an invalid name and the species now is recognized as *Gracilaria gracilis*. Since *G. verrucosa* was a common name in the past, many of the classical reports might be referring to different species (Patra and Muthunaman *et al.*, 2013).

Landing of the two types of *Gracilaria* distinguished have steadily increased over the last decade. By 2011, about 2 257 919 tonnes of *Gracilaria* were reported. The totality of what is recognized as warty *Gracilaria* (1 518 455 tonnes) is produced by cultivation mainly in China (1 513 590 tonnes) and Taiwan province of China (some 4 865 tonnes). In the case of *Gracilaria* spp., 94.2 percent of the production (some 697 240 tonnes) is by cultivation while the remaining (42 224 tonnes) are gathered from wild stocks. The most productive countries in America are Chile, Perú and Argentina. In Asia, there is productive cultivation of *Gracilaria* is Indonesia, Vietnam, the Philippines and Korea while in Africa only Namibia reports *Gracilaria* production.

The principal product of *Gracilaria* is agar. Industrial applications are dominated by three quality grades, a) sugar reactive agar, b) standard agar and c) food-grade agar. In the sugar reactive agar, the gels are stronger as a function of sugar concentration. It is obtained largely from *Gracilariopsis lemaneiformis*, at present the most important species under cultivation in China. Standard agar is recognized because the gel has the temperature, consistency and structure for microbiological purposes. It is produced largely by other seaweed groups such as *Gelidium, Pterocladia* or *Pterocladiella*. The food grade agar designates any kind of agar not meeting the requirements for sugar-reactive or bacteriological agar. It is extracted from a wide variety of *Gracilaria* species (Kumar *et al*; 2013).

Market demands for species of *Gracilaria* to produce agar have increased markedly in a decade (1999-2009). Table 2 summarizes several important market parameters. Global agar production increased from 7 500 to 9 600 tonnes, with sale prices of USD 17/kg increasing, on average, to USD 18/kg. The world agar sale value increased, therefore, from USD 128 million in 1999 to USD173 million in 2009. In 1999, about 63 percent of the total agar production was produced by *Gracilaria*. In 2009, the relative importance of *Gracilaria* had increased to 80 percent of the total agar production. These data indicate that although the industrial agar growth has been modest, it has been enough to generate the cash flows necessary to support the overheads needed for regulatory reform and capital investment needed to improve plants and equipment. Selling price increases have generally been adequate to offset seaweed, energy and chemical costs. In addition, the cultivation of *Gracilaria* has provided enough raw materials to support expansion. In fact, *Gracilaria* has grown in importance for extracting agar between 1999 and 2009 while *Gelidium* is declining in importance (Luhan; 1996).

Unknown quantities of *Gracilaria* are used in several places (e.g. Japan, China, Hawaii, St. Lucia) in the fresh vegetable market for human consumption. There, *Gracilaria* prices can be high (e.g. 5-7 USD /kg) although the volumes consumed are relatively low (e.g. a few tonnes per year).

The use of *Gracilaria* as marine invertebrate feed has developed in Asia, especially in the fish pond system of Taiwan Province of China, leading to development of polycultures of *Gracilaria* and abalone which uses *Gracilaria* as the sole source of food of these gastropods. In the past the emphasis was to produce *Gracilaria* biomass for agar production. Now it is more profitable for the pond operators to supply their *Gracilaria* as fresh food to abalone farmers (Bixler and Porse; 2011).

4. OTHER USES

Intensive research over the last decade is showing several potential economic uses for different species of *Gracilaria* and *Gracilariopsis*. For sake of clarity, the findings have been grouped in four main uses. However, it is clear that with adequate technology several of these uses could be accomplished reutilizing the same material.

4.1. Paper making

During the agar extraction process from *Gracilaria* or *Gracilariopsis*, considerable amounts of solid residues are produced as extraction wastes. The potential for using agar extraction residues as raw materials for pulping and paper making has been explored in China (Pei *et al.*, 2013) finding that the extraction wastes indeed could be utilized for papermaking (e.g. as a fiber source, as a functional filter, etc.). The higher contents of algal materials in the hand sheets samples resulted in lower permeability and stronger antimicrobial effects than the common paper. Algal material, when used as a partial substitute for wood pulp, resulted in improved paper density, waterproofness, grease proofness, and antimicrobial effects, indicating its potential use in the food packaging industry.

4.2. Biofuel

The large carbohydrate contents of same species of *Gracilaria* (about 45 percent of dry weight; Amanullah *et al.*, 2013) and which is normally used as agar or food, could have an alternative use for ethanol production. Amanullah *et al.* (2013) recently demonstrated such possibility using field cultivated *Gracilaria edulis* and fermenting its polysaccharides to ethanol, using *Saccharomyces cerevisiae* in the fermentation process. In a related study Kumar *et al.* (2013) developed an efficient strategy for agar extraction using the resultant pulp for bioethanol production. Thus, they suggested an integrated biorefinery process as the kind of activity most likely to obtain maximum economic returns from *Gracilaria*. Furthermore, they noticed that after ethanol production the leftover residues still contained good amounts of organic matter and useful minerals, and eventually could be used as biofertilizer.

4.3. Multi-products source

A diversity of studies on chemical composition of species of *Gracilaria* and on the effects of some of its many bioactive compounds are revealing a large variety of compounds and effects (Francavilla *et al.*, 2013; Patra and Muthunaman, 2013; Tabarsa *et al.*, 2012) to the point of suggesting some of the species of this genus could be characterized as a multiproduct source for biotechnological, nutraceutical and pharmaceutical applications even though more investigations are required for separating, purifying and characterizing many of these compounds.

4.4. Bioremediation capacity

The capacity of seaweeds to remove inorganic nutrients from the water media has been recognized for many decades. In present day integrated multitrophic aquaculture, the species of seaweeds are viewed (Chopin *et al.*, 2001) as renewable biological nutrients scrubbers that take up nutrients. Various species of *Gracilaria* have been evaluated in their removal capacity of nutrients produced from invertebrate or fish farms. For example, Troell *et al.*, (1997) concluded that a suspended culture of 1 ha, at a stocking density of 1 kg wet weight m2, removes 5 percent of the dissolved inorganic phosphorus released from a 227-tonnes mixed salmon farm. Thus different species of *Gracilaria* are being considered as good candidates or the establishment and exploitation of multithrophic cultivation systems.

5. STATUS AND TRENDS

Several fronts of active research are focusing on *Gracilaria* spp. For example, there are renewed efforts to characterize species and strains of productive taxa, using molecular analysis. The final goal is to maintain culture collections of the most productive species, strains and cultivars perfectly identified as to trace their distribution and genetic changes over time and space.

Closely related to the above goal is the present concern to learn on the genetic diversity of cultured stocks. Several studies are showing that cultured populations exhibit reduced genetic diversity which seemingly results in production decline and increased frequency of outbreaks of pests and pathogens. Studies are oriented to analyze genetic diversity of cultured stocks as well as to obtain genetic improvement.

The effect of various environmental factors on growth and propagation of different species as well as on the quantitative and qualitative characteristics of its agar continues to be a popular subject. The effects of irradiance, salinity, U.V., temperature and different types of nutrients are being studied with different life cycle stages of species such as *Gracilaria edulis, G. tenuistipitata, G. changii* and *G. chilensis*. This combination of studies are gradually broadening the basis for successful

cultivation and farming of economically interesting species in various geographic areas.

Several types of studies are evaluating developments that could increase the consumption of *Gracilaria* and its agar. For example, development of new products are anticipated to be the factor that could substantially modify the market, perhaps allowing for better product differentiation. Better raw material management would increase higher yields in agar production. Facing the climatic changes ahead, there is a need for more industry/government sponsored research into strain improvement that might be more tolerant of environmental changes.

It is also recognized that in the future, food applications will continue to increase driven by the growth of processed foods in developing countries. Therefore, the farming of species highly productive for food-grade agar will have to be increased or domestication and cultivation of new species will be needed. The development of seaweed farming may have negative and positive effects. The development of seaweed farms inevitably involves alteration of natural habitat. This may include alteration of the substratum or removal of other seaweed species or invertebrates to give way to the farm. In many places there is a complete absence of studies addressing this problem and its consequences. Often the farming communities lack the capacity to evaluate the changes or to figure out the long-term ecological consequences of these changes. In other cases, the economic benefits of *Gracilaria* farming have quickly overcome any possible concern for environmental impact. In countries and regions with enough economic development (e.g. USA, New Zealand, and Australia) visual obstructions that will lower the value of coastal properties or conflict of interest with recreational uses of the sea have limited the development of farming areas.

Seaweed in a farm can sometimes utilize the nutrients from an environment that already might not be very rich in nutrients, causing nutrient depletion. In addition, the invertebrates and fishes (herbivores) that might increase in density during intense algal growth, probably suffers significant reductions after seaweed harvesting.

A final aspect to be considered is the significant habitat modification that might be produced by the introduction of farming implements, such as plastic tubes (in the bottom farming system), monolines, plastic straw used to tie the seaweed, bamboo sticks and similar. Whenever farming stops for whatever reason, farming refuse (e.g. old monolines and so on) often are abandoned in the area without attempts to clean the contaminants or to restore the original habitat.

On the positive effects of farming, seaweeds may be used as nutrient sink, especially relevant in association with invertebrate farming or multitrophic types of farming. Also they can increase primary productivity and to expand the habitat area and food supply for herbivorous fish and invertebrates.

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MORMPHOMETRIC ANALYSIS OF MORPHOFORMS FROM *DOLCIHOS BIFLORUS* L.: A SEARCH

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ABSTRACT

Three distinct morphoforms of horse gram (*Dolichos biflorus* Linn.) are recognized from the colour of the seed. The common morphoform is one with the buff seed coat (brownish colour). Others are cream and black seed coat types. The black seeded type is generally shorter in duration and low in vigour. In this context an attempt was made regarding the preliminary analysis of the morphoforms of *D. biflorus*. Morphometric characteristics were analyzed both in the seedlings and mature plants which includes length of the seedling, primary root, number of lateral roots, R/S ratio and vigour index. The mean data thus obtained are tabulated. There was no marked variation in seedling between the morphoforms. The variation between these was not significant statistically. Mean morphometric data of mature plants were statistically significant at 99.9% probability level. Foliar characteristics such as length of leaf varied from 9.1 to 9.7 cm, which were not statistically significant. However, the mean length of rachis shows significant variation at $P < 0.05$. Although there were slight variations in leaflets characteristics, they were not statistically significant between morphoforms. The duration of flowering ranged from 67 to 69 days. The black seed morphoform flowered a little earlier. However, there was no significant variation related to flowering period between the morphoforms.

Keywords: Horse gram (*Dolichos biflorus,* morphoforms, morphometric characters.

1. INTRODUCTION

The contribution of wild plants as foods has always been recognized as part of the local knowledge, which forms a greater part of the complex cultural system. Research has shown that many wild plants are rich in specific constituents, other than primary metabolites, referred as phytochemicals, which may have health promoting effects. The major plant-derived chemical groups, now recognized as having potential health promoting effects, at least under some circumstances, are the flavonoids, alkaloids, carotenoids, pre and pro-biotics, phytosterols, tannins, fatty acids, terpenoids, saponins and soluble and insoluble dietary fibres. These phytochemicals have the potential to be incorporated into foods or food supplements as nutraceuticals.

The term nutraceuticals can be summarized as "any non-toxic food extract supplement that has scientifically proven health benefits for both disease treatment and prevention". It has been generally stated that the health promoting effects of nutraceuticals and other functional foods are likely due to biochemical and cellular interactions, which together promote the overall health of an individual. In the global market place, nutraceuticals and functional foods have become a multi-billion dollar industry. *D. biflorus* commonly called Horse Gram or Kulatha is a branched sub erect or trailing annual leguminous herb. The medicinal uses of this plant date back to the Vedic period. It is most commonly used in Ayurveda and other traditional systems of medicines. This is a pulse crop of great potential values and is commonly designated as the 'poor man's pulse.' It is more prevalent in certain pockets of Kerala. However, the yield per unit area is low and also in the face of enhancing competition from other pulses, this crop has been replaced by other pulse crops, like green gram, black gram etc. Not much has been done in the improvement of production and productivity of this valuable crop. Moreover, morphoform categorization was not even tried in this pulse crop, though there was distinct differentiation in seed colour such as black, cream and brown. Thus, the present investigation has been undertaken to evaluate morphometric characterization of different morphoforms in terms of seed germination, growth and development.

2. MATERIALS AND METHODS

2.1. Plant materials

Kulatha or Horse gram (*Dolichos biflorus* Linn.) is a slender, sub erect low growing common twining creeper, native of most parts of India, and is found up to altitudes of 1000 m. They are succulent, pubescent, annual bushy herb. The plant is profusely

branched at the base, the branches intervening among themselves or with the plants of the companion crop (Kochar, 1992). The seeds of *Dolichos biflorus* for the present study were collected from Kerala Agricultural University, Trissur, Kerala. On the basis of seed colour, they were grouped into three morphoforms, viz., black, brown, and cream. The dry, uniform seeds of each morphoforms were sown separately in earthern pots, filled with farm-yard-manure and soil (Fig-1). The plants were raised for the production of pure seeds. The present investigations were carried out using these seeds, thus raised from pure breeding plants.

Fig. 1. *Dolichos biflorus* L. plant

2.2. Morpho-histometric analysis

2.2.1. Morphometric analysis of seedling and mature plants

Morphometric analysis of *Dolichos biflorus* L., was conducted during seed gemination (seedling characteristics) and then on mature plants. The analyses during germination were done from the date of sowing to the fifth day of germination. In the present study, the characteristics of seedling analysed were length of the seedling, radical, number of lateral roots, root/shoot (R/S) ratio, and the vigour index.

The length of seedling and the radicle was measured in centimeter. The R/S-ratio was calculated by dividing the radicle length to the length of seedling. The vigour index was computed using the formula.

Vigour index = (Length of shoot + Root length) x 100

The morphometric data of the mature plants were recorded after 50 percent flowering. The characteristics studied were the total height of plant, the mean length of internode, the total number of nodes, the length of leaf, petiole (rachis), first lateral petiolule (LP), median petiolule (MP), second lateral petiolule (LP), first lateral leaflet (LL), mid leaflet (ML), second lateral leaflet (LL), breadth of first lateral leaflet (LB), mid leaflet (MB), second lateral leaflet (LB),duration of flowering, length of flower, pedicel, sepal, standard petal, wing petal, keel petal, anthers, carpel, pod, breadth of pod and the number of seeds/pod. To avoid statistical error, the data were computed from ten plants and the mean was calculated.

3. RESULTS AND DISCUSSION

3.1. Morpho-histometric analysis

Many morphological features, which have enormous scope for research, are in fact simply neglected. However, in the present study on *Dolichos biflorus,* both vegetative and floral features were studied, to draw additional features that may help in discrimination of the three morphoforms (Fig. 2).

Morphometric characteristics were analyzed both in the seedlings and in mature plants.

Fig. 2. Flowers of different morphoforms of *Dolichos biflorus*

Fig. 3 Seeds morphoforms of horse gram (a) Black, (b) Cream and (c) Brown

3.2. *Morphometric analysis of seedling and mature plants*

In the present study, five days old seedlings were evaluated to discriminate and assess the interrelationship between three morphoforms of horse gram. The characters analyzed include length of the seedling, length of primary root, number of lateral roots, R/S ratio and vigour index. The mean

data thus obtained were tabulated (Table-1). There was no marked variation in seedling morphology between the morphoforms. However, the length of seedling varied from 1.18 cm in cream seeded variety to 1.20 cm in black, and to 1.21 cm in brown (Fig.3). The variation between these morphoforms was not significant statistically, as the calculated F-value was much lesser than that of tabulated index (Table-1). R/S ratio was lowest in cream and highest in brown seed morphoform. However, the vigour index was highest in black and lowest inbrown. They showed signifi cant variation between morphoforms, because the calculated F-value was far exceeding the tabulated value at $P < 0.05$ (Table-1).

Table 1. Mean morphometric data of seedling

Morphoforms	Characters			R/S Ratio	Vigour Index
	Length of Seedling (cm)	Length of radicle (cm)	No. of lateral Roots		
1	1.20	3.11	2.60	2.50	43.8
2	1.18	2.77	3.00	2.44	444
3	1.21	3.34	2.60	2.88	422
Calculated F - value	0.4777	0.1916	0.7576	1.1363	5.4927
Level of Significance	NS	NS	NS	NS	*

1 = Black; 2 = Cream; and 3 = Brown Morphoforms; NS = Not significant' * = siginificant at $p < 0.05$.

Interrelationship between these characteristics was computed and tabulated. Length of seedling was positively correlated to length of radicle and R/S ratio and negatively correlated to the number of lateral roots and vigour index. Radicle length showed positive correlation with R/S ratio and negative relationship with the number of lateral roots and vigour index. However, the number of lateral roots was positively correlated to vigour index. Variations in seedling characteristics to some extent are environmentally controlled. But phenotypic plasticity of seedling is, no doubt, an important factor in enabling a species to become widely distributed, but should not provide a basis for taxonomic discrimination. Although genetics and environmental variability on seed germination in soyabean (Sen and Ghosh, 1959) and in horse gram (Ram *et al.,* 2000) were studied, the study at morphoform level seems to be a new report in horse gram (Prasanthi *et al.,* 1999). mature plant characteristics were also calculated and tabulated (Table-2, 3, 4, 5, 6).

The genotypic and phenotypic variance in plant height was reported earlier (Ramakrishnan *et al.,* 1978; Suraiya, 1980). The heritability of various vegetative characteristics was also studied earlier by (Birari *et al.,* 1987). Foliar characteristics like the length of leaf (trifoliate leaf) varied from 9.1 cm in both black and brown morphoforms to 9.7 cm in the cream, which were not statistically varied from each other. The length of rachis ranged from 6.3 to 6.5 cm in the brown and black/cream morphoforms respectively. However, the mean length of rachis showed signifi cant variation at $P < 0.05$ (Table-3).

Table 2. Morphometric data of mature plants

Characters			
Morphoforms	Height (cm)	No. of nodes	Length of internode (cm)
1	112	12	9.0
2	113	16	5.6
3	120	18	9.0
Calculated F- value	36.46	7.6502	47.745
Level of Significance	***	*	***

1 = Black ; 2 = Cream; and 3 = Brown, * = Significant at p = 0.05;*** = Siginificant at $p < 0.001$

Table 3. Interrelationship of various characters in mature plant.

Characters	Height	No. of Node	Legume Internode
Height	1		
No. Node	0.826033188	1	
Leg. Inten	0.397359707	-0.18898224	1

Table 4. Mean data on foliar characteristics in different morphoforms of *D. biflorus* .

Characters											
Morphoforms	Length of leaf (cm)	Length of rachis (cm)	Length of petiole (cm)			Length of leaflet (cm)			Breadth of leaflet (cm)		
			LP 1	MP	LP 2	LL 1	ML	LL 2	LB 1	MB	LB 2
1	9.1	6.5	0.3	0.3	0.3	2.4	2,6	1.5	1.5	1.3	0.9
2	9.7	6.5	0.2	0.3	0.25	2.6	2,4	1.5	1.5	1.5	1.2
3	9.1	6.3	0.3	0.3	0.3	2.7	2,5	2.6	1.7	1.7	1.3
Calculated F-Value	0.833	8.66	0.3478	0.01	0.1235	0.2314	0,3	2.5	0.1429	1.8507	1.8072
Level of Significance	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS

1 = Black; 2 = Cream and 3 = Brown; NS = Not significant, * = Significant at $p < 0.05$.

Table 5. Mean data on floral characteristics and duration of flowering in different morphoforms of *D. biflorus.*

Morphoforms	Duration (days)	Flower length (cm)	Length of pedicel (cm)	Length of sepal (cm)	Length of petal			Length of anther (cm)	Length of carpel (cm)
					Standard (cm)	Wing (cm)	Keel (cm)		
1	67	2.70	0.371	0.30	1.30	1.30	0.90	0.20	1.20
2	68	2.73	0.40	0.25	1.20	1.10	1.20	0.20	1.20
3	69	2.76	0.40	0.20	1.25	1.20	1.00	0.20	1.10
Calculated F-value	1.5004	0.7058	0.1886	0.5892	1.5613	2.9701	0.2698	0.0683	0.0529
Level of Significance	NS	NS	NS	NS	NS	NS	NS	NS	NS

1 = Black ; 2 = Cream; and 3 = Brown morphoforms ; NS = Not significant.

Besides the length of rachis, the length of petiole was also analysed individually from each trifoliate leaf and were designated as LP1, VP and LP2, in clock-wise direction. The length of LP1 varied from 2 to 3 mm, showed the lowest reading in the cream seed morphoform. The mean length of median petiole was 3 mm, irrespective of morphoform demarcation. Similarly, the length of LP2 was also more or less the same in all the morphoforms studied. Moreover, the variation in the length of petiole between morphoforms was not statistically significant (Table-3). The length and breadth of leaflet also showed no significant variance between

morphoforms analysed. However, the mean length of second lateral leaflet (LL2) was the lowest in the black and cream seed morphoforms. The breadth of LL was much lower than the rest of the leaflets and was least in the black morphoform, averaging 9 mm. Although there were slight variations in leaflets characteristics, they were not statistically signifi cant between morphoforms (Table-3).

Interrelationships between different foliar characteristics were computed by ANOVA and the results were tabulated .The mean length of the trifoliate leaf was correlated to the length of rachis, mean length of lateral leaflet (LL) and also to breadth of second lateral leaflet (LB). All other foliar characteristics showed negative correlation to leaf length. The mean length of rachis showed negative correlations to other features. However, the length of petioles exhibited strong interrelationship with other features and also to the length of median leaflet. But the mean length of median leaflet was negatively correlated to most of the characters analysed. The breadth of leaflets, however, showed strong interrelationship with one another. The black seed morphoform flowered a little earlier. The duration of flowering ranged from 67 to 69 days (Table - 4). However, there was no significant variation related to flowering period between the morphoforms. The length of pedicel was uniform in all the morphoforms studied (4 mm). The mean length of sepals in horse gram was 2-3 mm. However, they did not reveal any significant variance. The length of petals was also uniform in terms of percentage length of standard, wing and keel petals. The variations noticed in the present study were not statistically significant (Table-4).Similarly the length of anthers and the length of carpels were also more or less uniform and showed no significant variation.

The interrelationship between floral characteristics was analysed by ANOVA. Duration of flowering was found to be related strongly to length of flower, length of pedicel and length of keel petal, and it was negatively correlated to other floral parts. The interrelationship between duration of flowering and other agronomic characteristics was reported earlier by several investigators (Ghorpade, 1985; Birari *et al.,* 1987). An investigation on morphometric characteristics of fruit in three morphoforms of *Dolichos biflorus* with distinct seed colour was analysed. The length of pod ranged from 5-6 cm, the breadth 0.59 cm to 0.62 cm and the number of seeds/pod was 5 (Table-5 and 6). Between the morphoforms, these features showed no significant variation. Interrelationships between various characteristics of pods revealed that the breadth of the pod was positively related to the number of seeds/pod. However, the length of fruit was negatively correlated with the breadth of the fruit. In horse gram, Aggarwal and Kang (1976) observed significant positive correlation of pod length with grain number, grain weight. Suraiya (1980) reported that pod length exhibited genotypic correlations with seed yield in 15 morphoforms of horse gram. The present observation is in conformity with earlier investigations.

Table 6. Mean data on characters of pod in horse gram

Morphoforms	Length of pod (cm)	Breadth of pod (cm)	No. of seeds/pod
1	6.0	0.61	5
2	5.0	0.62	5
3	5.5	0.59	4
Calculated F-value	0.54407	0.50	0.5767
Level of Significance	NS	NS	NS

1= Black; 2=Cream; and 3 = Brown; NS= Not Significant.

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INSIGHT INTO PHARMACEUTICAL IMPORTANCE OF BRYOPHYTES

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ABSTRACT

Historically, Bryophytes were accounted to be a monophyletic group and were placed in an inclusive Bryophyta. Some species are aquatic though some can adapt and live in arid regions. Bryophytes size ranges from microscopic to 12 inches in length, the average size is between 0.5 – 2 inches long and colors vary from green to black and sometimes colorless. Bryophytes plays a vital role in the biosphere even their size is insignificant. As a biotic factor in the environment, they provide food for numerous herbivorous birds and animals. They prevent soil erosion by carpeting the soil. Bryophytes cause the outer portion of rock to slowly crumble as they grow with lichens on rock surfaces. And because of it they contribute and help to soil formation. When mixed with the soil, bryophytes increase the water-holding capacity of the soil and the amount of organic matter in the soil. Some bryophytes like sphagnum or peat moss has some economic importance. It is used as packing material for breakable or fragile objects such as figurines and dinnerware's. It is also used as packing materials for transporting plants and plant parts, since sphagnum holds water and hence prevent plants from drying during transport. As a whole, bryophytes are of little economic importance to man.

Keywords: Bryophytes, medicinal, non-vascular, microhabitat.

1. INTRODUCTION

Bryophytes are the ancient terrestrial chlorophyllous spore-forming amphibians, although no valid scientific documents for this have been appeared in the literature. This concept was fundamentally based on the similarity of the present-day liverworts the first land plant fossils, the spores of which date back almost 400 million years. They are taxonomically kept between the algae and the pteridophytes and there are appx. 14000 species of Bryophyta, 6000 of Marchantiophyta (liverworts), 300 of Anthoceratophyta, species and 5000 are mosses. Generally bryophytes are not infected or attacked by pathogens, insects, snails, slugs and mammals, however, studies on their phytochemistry have been neglected for centuries. Bryophytes are considered to be useless for human diets, difficulty of identification and collection of a large amount, although several liverworts have been used as medicinal plants in China to cure cut, burns, bruises, pulmonary tuberculosis, neurasthenia, fractures, convulsions, ulopathy, neurasthenia etc.

2. REVIEW OF THERAPEUTICAL IMPORTANCE

2.1. Phytochemicals from bryophytes

Bryophytes are known to produce diverse secondary metabolites to combat a number of biotic and abiotic stress such as predation, UV radiation, extreme temperature and microbial decomposition. 40 new carbon skeletal acetogenins, phenolic compounds and terpenoids have been found in this class, for example, marchantin A, marchantin E and riccardin C and diterpene dialdehyde, sacculatal, by recent development of spectroscopic apparatus, particularly by high resolution NMR techniques. The most interesting chemistry of liverworts is that most of sesqui- and diterpenoids are enantiomers of those found in higher plants and some different species of the same genus, like *Frullania* produce both normal and its enantiomeric sesquiterpenoid. They are the source of large variety of secondary metabolites and thus provide a great potential for biotechnological and biopharmaceutical applications. In past few years more than 400 novel chemical compounds were isolated from bryophytes and they were structurally elucidated. Some of biologically active compounds isolated from mosses includes biflavonoids, terpenes and terpenoids (like di- and triterpenoids) and flavonoids whereas liverworts reported to contain a large variety of lipophilic mono-, di- and sesquiterpenoids as well as aromatic compounds like bibenzyls, benzoates, cinnamates and naphtalenes. Flavonoids such as quercetin (182.5 µg/g), luteolin (464.5 µg/g), and apigenin (297.5 µg/g) were reported to be present in the liverwort *Marchantia linearis* (Remya *et al.,* 2014). Two moss species *Thuidium tamariscellum and Brachythecium buchananii* were reported to be rich in secondary metabolites like flavonoids, terpenoids

and phenols (Greeshma *et al.,* 2016). In spite of the fact that many plant secondary metabolites are the potential therapeutic introduction of novel drugs in the market has decreased in past few years. In fact, higher plants and bryophytes have similar evolutionary history but search for novel therapeutic compounds within biodiversity of bryophyte remained neglected due to small size and lack of awareness among people. These small plants remained unexploited so far in drug discovery process in spite of few reports from the past depicts some of their ethnomedicinal uses. Studies on secondary metabolites of bryophytes have revealed the presence of few original compounds, some of which are not synthesized by higher plants (Rashmi *et al.,* 2014).

2.2. Antimicrobial aspects of Bryophytes

Bryophytes have been reported as antibiotics (Tedela *et al.,* 2014). Various organic solvent extracts of bryophytes have been investigated in past. Literature emphasizes that the alcoholic and the aqueous extracts or the various compound isolated from different species of bryophytes (hepatic and mosses) have shown antimicrobial effects against various group of fungi, as well as Gram negative and Gram positive bacteria. Recently extracts of few of the selected species of bryophytes (seven mosses and three liverworts) viz the *Radula flacida, Cyatodium africanum, Frullania spongiosa, Thuidium gratum, Ectropothecium aeruginosum, Sematophyllum caespitosum, Stereophyllum radiculosum, Babulalam berenensis, Campilopusa spericuspis* and *Calympereserosumlam berenensis, Campilopusa spericuspis* and C*alymperes erosum* have shown interesting antimicrobial activity (Rashmi *et al.,* 2014). Similarly, the methanolic and water fractions of *Targionia hypophylla* possess strong microbicidal activity (Remya *et al.,* 2012). The methanolic and aqueous extracts of *Plagiochilla beddomei, Leucobryum bowringii* and *Octoblepharum albidum* exhibited different degree of growth inhibition against bacterial species such as *Salmonella typhimurium*, *Staphylococcus aureus*, *Klebsiella pneumonia, E.coli, Bacillus cereus* and *Pseudomonas aeruginosa* and fungal species like *Candida albicans, Cryptococcus neoformans, Trichophyton rubrum, Mucor indicus, Apergillus niger* and *A. flavus* (Manoj, 2012). Therefore, the potential antimicrobial properties of bryophyte can be harnessed for the therapeutic purpose against the respective pathogen. The antibacterial and antifungal activities of bryophytes have been discussed in below.

2.3. Microbicidal potential

In recent year extensive studies have been conducted for the search of antibacterial properties in different species of plant. Organic extracts of various medicinal plants containing flavonoids have been reported to show antimicrobial activity (Praveen Dahiya *et al.,* 2012). The antibacterial activities of isofavonoids and flavonoids, and glycosides of luteolin and apigenin have also been reported. But most of the investigations were centered on angiosperms. Few data are presently available about these smaller groups of plants, bryophytes. However, few of the recent study on bryophytes has shown some of the antibacterial activity against gram-positive and gram-negative bacteria (Meenakshi *et al.,* 2011).

Also, various phenolic compounds isolated from *Atrichum, Dicranum, Mnium, Polytrichum*, and *Sphagnum* spp. are known to shown antimicrobial properties. Apart from this the antimicrobial activity for three moss species *Eurhynchium angustirete, Rhytidia delphussquarrosus* and *Rhodo bryumroseum*, and for two liverwort species *Frullania dilatata* and *Lophocolea heterophylla* has been reported for the first time. Similarly, aqueous extract of few bryophytes have some inhibitory effect on the growth of *Escherichia coli* as tested on plates. However, this antibacterial activity seems to be specific for certain bryophyte species, as the extracts of *Marchantia polymorpha, Porella platyphylla* and the moss *Dicranum scoparium* showed antimicrobial effects on the gram-positive bacteria namely *Bacillus subtilis, Staphylococcus aureus* and *Sarcina lutea*, but no activity against gram-negative *E. coli* (Rashmi *et al.,* 2014).

Many of the bryophyte species are also known to show antifungal property (Abhijit *et al.*, 2011). Different crops growing in greenhouses, like tomatoes, wheat and green pepper were infected with the pathogens *Phytophora infestans, Erysiphe graminis* and *Botrytis cinerea* and later treated with alcoholic extracts from different bryophytes. All bryophyte extracts showed a species-specific antifungal activity against the plant pathogenic fungi depending on the concentration. Ethanolic extract *of Marchantia linearis* exerted a potent fungicidal activity against *Botrytis cinerea* and marginally in *Rhizoctonia solani* i.e., showing varied levels of growth inhibition with different concentrations. (Remya *et al.,* 2014). Furthermore, extracts from *Neckera crispa* and *Porella obtusata* showed antifeeding effects against the Portuguese slug *Aarion lusitanicus*. In view of the above features that bryophyte extracts showed fungicidal and

antifeedant effects, a commercial product was developed and is sold as natural pesticide. Apart from this studies conducted in the past have revealed few of the compounds isolated from bryophytes extracts have shown reversal of conventional antibiotic resistance development in pathogenic fungi. Therefore, the problem of drug resistance development in pathogenic fungi can be solved easily (Rashmi *et al.,* 2014).

Also, in addition to this studies conducted on one of the model species of moss *Physcomitrella patens* revealed that this moss under axenic condition produces a tetracyclic diterpene, namely 16α–hydroxykaurane (16 α-hydroxy-ent-kaurane, Kaurenol, $C_{20}H_{34}O$) (Anna *et al.,* 2010). Although, the utility of 16 α–hydroxykaurane is not yet revealed, but it is presumed to be bioactive. This, compound is known to be produced by lichen species and fungi and it is commonly known from *Gibberella fujikuroi*, a plant pathogenic fungus that infects rice plants and causes foolish rice seedling disease. As recently shown, 16α–hydroxykaurane is involved in spore germination in *Physcomitrella patens* and leads to a complete inhibition of spore germination when applied in high concentrations (2-3 μM) (Anna *et al.,* 2010). Also, few bryophyte extracts have been found to be effective on human pathogenic fungi although the bioactive compounds may cause allergenic effects and dermatitis in few cases. Nevertheless due to risk of allergic reactions, bryophyte extracts were not recommended for scientific medicinal use so far.

2.4. Phytochemicals vs pharmacological potential

Since bryophytes are the reservoir of complex secondary metabolites, their vast application in traditional medicine is not astonishing. A large number of bryophytes are used as medicines in homeopathy. About 3.2% of mosses and 8.8% of liverworts taxa have been chemically investigated. Species like *Sphagnum, Marchantia, Riccia, Barbula, Bryum, Octeblepharum and Fontinalis* are used to treat different diseases, including cardiovascular diseases, inflammation, fever, lung diseases, infections, wounds and skin diseases (Rashmi *et al.,* 2014). In China more than 30 species can be bought at the local pharmacist 66 and around 40 different kinds of bryophytes have been used to treat diseases of cardiovascular system, tonsillitis, tympanitis cystitis and bronchitis and to cure skin disease and burns. Many of the species for example *Polytrichum commune* which is used as antipyretic and anti-inflammatory agent or boiled as a tea for treating the cold. *Rhodobryum giganteum* is another species traditionally used to treat, other diseases like cardiovascular diseases or angina (Rashmi *et al.,* 2014). According to the some of the recent report bryophytes are the source of numerous chemical compounds of biotechnological and biopharmaceutical interest. Several secondary metabolites have been isolated so far from different species but the mechanisms behind their activity are still widely unexplored.

2.5. Antioxidant potential

Few of the bryophyte species have been studied in context to antioxidant activity. Recent study suggests that some of the liverworts and moss possesses strong antioxidative machinery which helps them to survive in the extreme climate and stress condition. Heavy metal, desiccation and ultraviolet radiation have been found to trigger an array of different enzymes in bryophytes (Rashmi *et al.,* 2014). Few of the bryophyte species have been found to hyper accumulate metals and few others were able to sequester the toxic metals. The study conducted on antioxidant activity of the Antarctic mosses *Sanionia uncinata* and *Polytrichastrum alpinum* var. alpinum has indicated their potential to be used as antioxidants for medicinal and cosmetic purpose. The methanolic extracts of *Plagiochilla beddomei, Leucobryum bowringii* and *Octoblepharum albidum* possess potential antioxidant properties (Manoj, 2012). Also, in addition the antioxidant activities of some of the species of bryophyte like *Atrichum undulatum*. *Polytrichum formosum*, *Pleurozium schreberi* and *Thudium tamariscinium* has been screened, and all tested species have showed antioxidant effects lower than the positive control, caffeic acid (Boris *et al.,* 2012). Moreover, the screening for the antioxidant property of the aqueous extract of the three moss namely *Brachythecium rutabulum*, *Calliergonella cuspidate* and *Hypnum mammillatum* in context of their ABTS (2, 2- azino- bis (3- ethlybenzthiozoline- 6 sulphonic acid) cation scavenging activities and phenolic content have known to show some positive response. Out of the three extracts, *Brachythecium rutabulum* have shown the highest of the phenolic content which further suggested potential of this extract in search of many other novel antioxidant compounds in this moss. Apart from this methanolic and and ethyl acetate extract of *Marchantia polymorpha* have also shown antioxidant property. Summing up, bryophyte could be the source of many novel antioxidants if screened which could be used for novel drug discovery.

2.6. Biopharming for phytochemicals

Bryophytes have indeed penetrated the fore front of modern medicines. Although a vast variety of biopharmaceuticals has been produced in microbial

or mammalian cells but plants based production system possesses several advantages over the mammalian and microbial system thus, making them interesting alternatives. Microbial systems are favored because of easy cultivation and high productivity, whereas mammalian cell lines (preferentially Chinese Hamster Ovary cells) are favored for complex multimeric proteins or those requiring posttranslational modifications (Rashmi *et al.,* 2014). In contrast to these currently used systems, plants based production system possesses several advantages over these system thus making them interesting alternatives. As higher eukaryotes, they perform posttranslational modifications closely resembling those of humans, thus minimizing the risk of product contamination by infectious agents derive from the used cells or media (Rashmi *et al.,* 2014). Also bryophytes offer the researchers and the company a high production system which can be grown without antibiotics, hence avoiding the danger of contamination of the final product. Apart from these advantages, mosses are the only plants known to show a high frequency of homologous recombination. They allow stable integration of inserted genes into the host cell. Furthermore, the highly complex moss system, compared to bacteria and fungi, permits a much wider array of expression than is possible in other systems. In view of the above advantages of mosses over other production system, today, many complex biopharmaceuticals are being produced by moss bioreactors. The Chair of plant Biotechnology from the University of Freiburg, Germany, and the biopharmaceutical company Greenovation Biotech Gmbh in Heilbronn, Germany; have started a cooperation to enhance the yield of recombinant proteins from moss. The moss *Physcomitrella patens* has been successfully grown in a bioreactor which require only water and minerals to nourish the moss, in the presence of light and CO_2 (Greenovation). Consequently many complex proteins can be produced in moss bioreactor. Other products are human growth factor that is required by the researcher for tissue culture. This plant has successfully been able to produce human proteins and is the only plant being used to produce the blood-clotting factor IX for pharmaceutical use.

3. CONCLUSION

Use of medicinal plants has been appreciated due to low cost and lesser side effects. Herbal drugs have been used successfully in the treatment of various ailments over the last few decades. Development of drug resistance in pathogens is one of the major problems in medicine. Natural products derived from the botanicals can be used as a substitute to solve the problem. A number of herbal compounds have been discovered with immense therapeutic potential. Therefore, to meet the potential future demand for various bioactive compounds used as drugs, a new production system is required significantly. Bryophyte, a small and apparently insignificant group of plants may serve as a source of some unique biologically active molecules. Many of the bryophytes are important source of medicine, antibacterial and antifungal agents. Antifungal efficacy of certain liverworts and mosses can substitute the conventional synthetic fungicides used in crop protection especially in the countries where fugal invasion in the crop fields is a common phenomenon. The problem of development of drug resistance in common human pathogenic fungi can be solved by using antifungal compounds harvested from uncommon sources like bryophytes. Several bryophytes are able to produce antifungal compounds. Furthermore, the use of moos bioreactor has opened new possibilities for the production of many plant and animal metabolites.

Future scope: In the past few years, rapid progress has been made to isolate various plant based therapeutic compounds. Bryophytes being rich source of a variety of secondary metabolites could be a promising source of the bioactive compounds with immense therapeutic potential. Being present in varied niche and occupying the most diverse group of plant kingdom, they could be the source of various evolved metabolic pathways that could be wisely manipulated for the development of various novel therapeutic compounds. Therefore, bioprospecting of bryophytes is required to discover the natural wealth of bryophytes. Creation and development of production system by using bryophyte cells could solve the future demand of novel plant based production system. Hence, engineering of metabolic pathway for production of novel metabolites, and strategies for the development of the bioprocess for bryophyte cell system is the need of time to dig out some more information to satisfy the thirst of novel drug discovery.

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RESILIENCE OF FERNS: WITH REFERENCE TO DESICCATION AND REHYDRATION STRESS OFFER NEW INSIGHTS

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ABSTRACT

Ferns are one of the oldest vascular plants in existence and they are the second most diverse group of vascular plants followed to angiosperms. To unravel fern success has focused on the eco-physiological power and stress tolerance of their sporophyte and the gametophyte generations. In this context, those insights encompass plant water relations, as well as the tolerance to and recovery from drought or desiccation stresses in the fern life cycle are reviewed. Lack of secondary xylem in ferns is compensated by selection for efficient primary xylem composed of large, closely arranged tracheids with permeable pit membranes. Protection from drought-induced hydraulic failure appears to arise from a combination of pit membrane traits and the arrangement of vascular bundles. Features such as tracheid-based xylem and variously sized megaphylls are shared between ferns and more derived lineages, and offer an opportunity to compare convergent and divergent hydraulic strategies critical to the success of xylem-bearing plants. Similarly the synthesis and accumulation of sugar, proline and stress proteins along with the production of pool of polyphenols add strength to desiccation stress. Thus, it can possible to suggest that selection acted on the physiology in a synchronous manner that is consistent with selection for drought tolerance in the epiphytic niche, and the increasingly diverse habitats of the mid to late Cenozoic.

Keywords: Ferns, desiccation, rehydration stress, osmolytes, vasculature, polyphenols.

1. INTRODUCTION

Phylogenetic analysis reveals that land plants evolved from simple aquatic algal progenitors (Bennici, 2008). The radiation of once aquatic species on to dry terrestrial habitat required the evolution of adaptive features suites that permitted life in land. To accommodate in this habitat, plants have developed critically two modes of surviving desiccating conditions. One *via* the avoidance of desiccation i.e., common in modern terrestrial vascular plants and has been accomplished by the development of internal water conductance and characters such as highly organized cuticles and effective stomatal mechanism. Meanwhile, some land plants relied on the radical mechanism of survival from desiccation: by desiccation tolerance (DT) i.e., those that which can lose from their vegetative structures all internal water and enter into, and recover from, anhydrobiosis, the cessation of metabolic activity as a result of low intracellular water content (Bewley, 1979). An environment that brings a plant to an air-dried anhydrobiotic state is sufficiently lethal to kill all modern agricultural crops and > 99% of all vascular land (Alpert, 2000; Alpert and Oliver, 2002). The DT in vascular plants is rare phenomenon. But many of the characters that facilitated desiccation tolerance in ancestral land plants are still commonly found in algae and bryophytes (Alpert, 2005; Oliver *et al.*, 2000). Reports point out that some bryophytes recover from over 20 years of desiccation in herbaria (Alpert, 2000; Stark *et al.*, 2005).

Desiccation tolerance can exist in some phase but be completely absent in another part of an organism's life cycle. The gametophytes of some bryophytes exhibit high degree of tolerance than sporophytes. Desiccation tolerance requires the complex and organized shut-down of metabolism and the occurrence of DT in many distantly related lineages and life stages indicates that there may be significant variation in the mechanisms behind this phenomenon (Alpert and Oliver, 2002).

DT of spores and seeds is well known in vascular plants, but much less is known about vegetative DT in lineages with two separate free-living stages. Ferns exhibit two separate free-living generations which alternate between independent gametophyte and sporophyte generations. The gametophyte is the site of fertilization, is relatively small, lacks vascular tissue, and either completely lacks or has a poorly developed cuticle. The sporophyte, the primary stage for dispersal, has a well-developed vascular system and a waxy cuticle complete with stomata. These differences alone

result in unique life-cycle-mediated ecological strategies, especially as they relate to water relations and demography (Watkins *et al.*, 2007).

Because of the well-known resurrection fern like *Selaginella* sp, the presence of DT in the vascular sporophytes of ferns has been reported to be more common than in other vascular plants. Proctor and Pence (2002) recorded that 64 species of ferns exhibited DT and estimated that less than 1% of all ferns possess such ability.

2. MECHANISMS

2.1. Water transport

Ontogenetically, fern fronds are megaphylls arising from rhizome, although in tree ferns the fronds emerge from an apical region of trunk comprised of pith parenchyma, fibers and adventitious roots. Fern vascular tissue is meristeles that are surrounded by an endodermis ;although vessels have been reported in *Pteridium aquilinum* and members of *Astrolepis, Marsilea*, and *Woodsia* (Pittermann *et al.*, 2011), the majority of ferns transport water by means of primitive tracheids the walls of which are perforated by reticulate, homogenous pit membranes. The organization of the vascular bundles within the stipe is highly variable, ranging from the solitary vascular central bundle i.e., haplostele to the multiple bundles - dictyosteles of some species. Fern dictyosteles are most commonly reticulate. In contrast to the typically short and narrow conifer tracheids, which evolved to transport water as well as support the canopy, fern xylem evolved solely for the movement of water leaving support to an outer ring of schlerenchyma fibers (Pittermann *et al.*, 2011; Watkins *et al.*, 2010). Similarly, the length and diameter of fern tracheids varies greatly with conduits in excess of 4 cm observed in scrambling and weedy species (Pittermann *et al.*, 2011). The ability of fern xylem to explore a broader morph space within the constraints imposed by unicellular conduits probably shaped the competitive ability and persistence of the modern pteridoflora, and may have factored into the evolution of pseudo-woody vascular strategies characteristic of extinct Carboniferous fern taxa (Wilson and Knoll, 2010). That said, the absence of a bifacial vascular cambium and its derivative secondary xylem has led to a developmental scheme that limits not only the hydraulic capacity of pteridophytes, but also branching and the overall architecture of the fern canopy.

2.2. Free radicals and reactive oxygen species (ROSs)

Free radicals are atoms or molecules with an unpaired electron, which is readily donated and are highly reactive. Oxygen is a highly oxidizing molecule and readily forms radicals such as singlet oxygen ($_1O^2$), superoxide ($O_2^{\bullet-}$), the hydroxyl radical (•OH) and nitric oxide (NO•). These are known as reactive oxygen species (ROS) (Halliwell and Gutteridge 1999). ROS cause damage to all macromolecules and subcellular components (Vicre *et al.*, 2004; Berjak, 2006) and being the most damaging consequence of desiccation stress. Because of their highly reactive nature, the accumulation of the products of ROS-associated damage together with the up-regulation of antioxidants to quench ROS activity is normally assayed. However, there is also recent convincing evidence for a role for ROS in intracellular signalling (Bailly, 2004; Laloi *et al.*, 2004). While we have little information on how ROS might play a role in signalling associated with desiccation tolerance, resurrection plants appear to go to great lengths to minimize ROS formation and to quench their activity. It is also evident that the ability to maintain antioxidant potential in the dry state is essential for recovery upon rehydration. For example, Illing *et al.* (2005) and Farrant (2007) have shown that antioxidant enzymes remain undenatured during desiccation, so that the same enzymes can function to prevent ROS damage during rehydration. In all plants, ROS form as a natural consequence of metabolic processes involving electron transport and thus mitochondria and chloroplasts are major sites of ROS production. Under hydrated conditions, their activity is neutralized and homeostatic control realized by what has been referred to as the "classical" (Kranner and Birtić, 2005) antioxidants such as the water-soluble glutathione (γ-glutamyl-cysteinylglycine; GSH) and ascorbic acid (Asc) (Noctor and Foyer, 1998), the lipid soluble tocopherols and β-carotene (Munne-Bosch and Alegre 2002) together with enzymes such as superoxide dismutase (SOD), ascorbate peroxidase (APX), other peroxidases, mono- and dehydroascorbate reductases, glutathione reductase (GR) and catalase (CAT). However, under severe water stress conditions, disruption of electron transport results in excess ROS production. While ROS accrue mainly from respiratory metabolism in seeds, there is an additional critical contribution from disruption of photosynthesis in vegetative tissues. Excess energy from excited chlorophyll molecules rapidly results in formation of ROS (Smirnoff, 1993) which are inadequately dealt with by desiccation-sensitive plants, ultimately causing loss of viability. The total antioxidant potential, the

extent of up-regulation of antioxidant enzymes together with the potential polyphenol antioxidant capacity and anthocyanin protection of the homoiochlorophyllous species is greater than that of the poikilochlorophyllous species. This supports the contention that homoiochlorophyllous resurrection plants might require greater protection against ROS than the poikilochlorophyllous plants, since the latter better avoid ROS formation due to their dismantling the photosynthetic apparatus (Farrant, 2000; Farrant *et al.*, 2003).

Dicranopteris linearis (Burm.F.) Underw. is a desiccation-tolerant forked fern that can tolerate drought. Studies regarding the desiccation rehydration stress in the fern revealed remarkable level of phenolic compound deposits in the tissues. (Kavitha and Murugan, 2016a).Fractionation of phenols by RP-HPLC reveals the waxing and waning pattern of phenolic acids such as ferulic acid, hydroxy benzoic acid and phloroglucinol. The total phenolic content, and phenolic acid profile suggest the protective role of polyphenolics against environmental stress. It seems to act as barrier in the species against desiccation.

Desiccation and rehydration induced greater accumulation of ROS like hydrogen peroxide (H_2O_2) and superoxide radical ($O_2^{\cdot-}$) in *D.linearis* (Kavitha and Murugan, 2016b). The active accumulation of these reactive oxygen species (ROS) indicates the severe oxidative stress felt by the fern during desiccation stress. The activity of peroxidases (POX) enzyme (both cytosolic and cell wall bound) examined in the fern revealed that the activity of cytosolic peroxidase was significantly higher throughout the periods of desiccation compared to wall bounded POX. The role of scavenging potential of this enzyme against ROS generated during stress has been established (Kavitha and Murugan, 2016c).

The initial response to ROS production is at the level of plasma membrane. Lipid peroxidation disrupts the membrane integrity of the plant cell. Activation of peroxidation of lipids is one of the earliest response of plants against stress particularly drought stress. H+ adenosine triphosphatases (H+ ATPases) located on the plasma membrane is largely affected by the changes in the membrane lipids.These pumps establish an H+ electrochemical potential across their respective membranes, which can be used as the driving force for secondary active transport. These enzymes maintain homeostasis inside the cell and thus help the cell to compact stress (Małgorzata Janicka-Russak, 2011).The changes in the lipid peroxidation rate studied in *D.linearis* indicates the presence of an effective anti-oxidative mechanism operating in the plant. Plasma membrane bound H+ATPase is a tightly bound integral membrane protein which helps to generate electro chemical proton gradient across the membrane. Thus, this enzyme play critical role in several physiological processes inside the plant and more over it help to resist stress under extreme environmental conditions. Desiccation rehydration stress in D.linearis showed a regulation in H+ATPase activity due to abrupt changes in the lipid peroxidation rate related with stress. Prolonged desiccation in D.linearis resulted in almost a constant H+ATPase activity at par with the control indicating the normal functioning of the plasma membrane even in the stressful conditions. (Kavitha and Murugan, 2016b).

2.3. Denaturation and sub-cellular perturbations

As water is progressively lost, the cytoplasm becomes increasingly viscous. Moreover loss of water promotes protein denaturation and membrane fusion, processes that start to occur at water contents of below 50% RWC or 0.3 $g.g^{-1}$ (loss of type III and some of type II water) (Walters, 1998). Upon further water loss to 10% RWC, ≤0.1 $g.g^{-1}$ (loss of type II and some type I water) the hydrophobic effect of water that is essential in the maintenance of macromolecular and membrane structure is lost and irreversible sub-cellular denaturation occurs. It is generally thought that desiccation-tolerant systems substitute water with hydrophilic molecules that form hydrogen bonds to stabilize macromolecular interactions in their native configuration (Crowe *et al.*, 1998). In addition to this water replacement, further stabilization of the sub-cellular milieu is thought to be brought about by vitrification of the cytoplasm by the same water replacement molecules (Hoekstra *et al.*, 2001). Typical water replacement molecules include sugars, particularly sucrose together with oligosaccharides (Berjak, 2006), hydrophilic proteins, particularly late embryogenesis abundant (LEA) proteins (Mtwisha *et al.*, 2006) and small heat shock proteins (Mtwisha *et al.*, 2006) and compatible solutes, including amino acids such as proline (Gaff and McGregor, 1979) and amphiphiles (Hoekstra *et al.*, 2001). Kavitha and Murugan (2016d) previously reported that the FTIR spectroscopic study of the fern *D.linearis* under desiccation rehydration stress revealed the sensitivity of the carbohydrate metabolism in the fern leaves. In the desiccated fern the band strength was altered suggesting the change of carbohydrate from one form to another i.e., monomeric forms were converted in to sucrose or dimers. After ten days of stress, decrease in band area and band strength indicated decrease in carbohydrate

synthesis along with conversion of monomers to disaccharides like sucrose/trehalose which act as osmolytes. The increased concentration of reducing sugars in the fern leaves as revealed by the present biochemical analysis suggest the accumulation of sucrose which act as osmolyte. It has been observed that in *D.linearis* the biosynthesis of proline is activated under dehydration whereas rehydration induces the opposite pathway.

But till now exhaustive metabolomic studies on the various resurrection plants and the role of sugars, sucrose in particular, in subcellular protection against desiccation is not fully elucidated. Sucrose is apparently accumulated in the leaves and roots of all angiosperm resurrection plants examined to date (Whittaker *et al.*, 2004; Peters *et al.*, 2007). Oligosaccharides also accumulate in resurrection plants during drying, but always to a lesser extent than that of sucrose. Sucrose accumulation and trehalose are water replacement molecule add boon to desiccation.

Studies in primitive vascular resurrection plant, *Selaginella tamariscina* revealed dynamic expression changes of the desiccation-responsive proteins suggesting the plant has developed a specific desiccation tolerant mechanism (Wang *et al.*, 2010). Similarly desiccation rehydration treatment in the fern *Polypodium virginianum*, showed transient expression of polypeptides necessary during the early stages of rehydration when the rapid initiation of physiological and repair processes are essential (Reynolds and Bewley., 1993). According to Daniel and Gaff (1979), changes in the soluble protein composition were found in all desiccation tolerant and sensitive species of angiosperms after dehydration, but there was no consistent pattern of compositional change within either type of plant. *In vivo* changes in protein synthesis in desiccation sensitive and tolerant species of grass *Sporobolus* showed an increase in the protein content of the desiccation tolerant species which is due to increase in the activity of enzymes involved in the tolerant mechanism of the plant(Ghasempour and Kianian., 2007).

Thakur and Bhatla, (2015) employed proteomic approach to analyze sunflower seed development stage revealed specific qualitative expressions of diverse classes of desiccation tolerant low mass proteins, has put forward new information which can be explained further to investigate their respective physiological relevance. Chakrabortee *et al.*, (2007) characterized polypeptides - the late embryogenesis abundant (LEA) proteins as marker for desiccation tolerance.

In beet plants, Gzik (1996) observed that increased levels of amino acids were related to osmotic adjustment for stabilization of water state in tissues under water deficit conditions. In the resurrection plant *Barbacenia purpurea* the increase of hydroxyproline, serine, valine, histidine, and tyrosine can be an indicative of deposition of extensins in cell wall which provide stability to the cell wall. Moreover, the increase in tryptophan and decrease in shikimic acid observed in *B. purpurea* suggest a change in metabolism toward the secondary metabolite production, which develops an important role on desiccation tolerance (Suguiyama *et al.*, 2014). In *D.linearis* increase in serine, valine, tyrosine observed are corroborating with the above results. Up regulation of phenyl alanine indicates the activation of secondary metabolite synthetic pathway. Accumulation of amino acids can be also associated to storage of available substrate for protein synthesis and quick recovery of the plant metabolism after rehydration. In a more recent study using the resurrection lycophyte *S. lepidophylla*, more than half of the amino acids were more abundant in the dry as compared to the hydrated state (Wone *et al.*, 2013).

3. CONCLUSION AND FUTURE OUTLOOK

There is good reason to suspect that physiological and morphological traits in the ferns are in a coordinated manner that was consistent with the Cenozoic diversification of the fern epiphytic flora, and possibly other fern radiations across the post-Eocene landscape. Future research will seek to elucidate the structure-function trade-offs associated with variable stealer structure and pit membrane traits with respect to hydraulic function and cavitation resistance, since so little is known about the functional value of the myriad of bundle arrangements characteristic of fern taxa. Since the fern vascular system is anatomically tractable, future work will explore the relationships between stele arrangements, frond venation and gas-exchange, with the goal of placing the results in a broad phylogenetic context. Working toward a concurrent understanding of how gametophyte physiology parallels adaptive traits in the sporophyte will be critical toward building a comprehensive picture of fern radiations since gametophyte establishment may push the physiological and niche boundaries of their associated sporophytes.

Due to their seedless, treeless habit and a lifestyle seemingly resigned to the shady forest understory, pteridophyte physiology has long lingered in the shadow of conifers and angiosperms. However, literature survey has shown ferns to

physiologically competitive, resistant to stress, highly diverse and extremely adaptive, so it is time for this ancestral lineage to step into the spotlight of evolutionary eco-physiology. Indeed, the understanding of the evolution of plant water transport would be incomplete without a vigorous examination of the physiological traits that contributed to the 400 million year-long success of these persistent plants. With the advent of more transcriptome, proteome and metabolome studies, these similarities will probably become increasingly apparent.

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CHANGES IN SPECIES COMPOSITION AND ECOLOGICAL ATTRIBUTES OF PLANT SPECIES IN THE *BRACHIARIA RAMOSA* (STAPF.) DOMINATED GRASSLAND AS INFLUENCED BY DISTURBANCE

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ABSTRACT

The present study on the influence of disturbance in the dominated grassland near Bharathiar University, Coimbatore was studied over a period of one year from September, 2014 to August, 2015. The study was made during three seasons such as winter, summer and rainy so as to find out the seasonal changes as influenced by disturbance. The studied grassland is a semi-arid community containing most number of mesophytes with few xerophytes. To study the impact of disturbance, two sites such as undisturbed and disturbed ones spread over an area of 10 and 12 ha respectively were selected in the grassland. The floristic analysis showed that the undisturbed community was registered with 71 species and the disturbed community with 51 species. The family, Poaceae was represented by the high number of 14 and 13 species respectively in undisturbed and disturbed communities. Of the 71 species encounted, a sizable number of 66 species (92 %) harbour medicinal uses. It indicates that the study site was a potential habitat of medicinal plants with wide diversity. The quantitative ecological characters have been varied widely between the two sites due to the influence of disturbance. The resource apportionment for various species present in both study sites indicates that the grass, *Brachiaria ramosa* shared higher amount of resources than any other species present in the communities. The study suggested that the studied *Brachiaria ramosa* dominated grassland near Bharathiar University must be given conservation priority to protect the valuable medicinal species.

Keywords: Dominated grassland, Bharathiar University, *Brachiaria racemosa*.

1. INTRODUCTION

Grassland may be defined as a plant community which is dominated by perennial grasses, where there are few or no shrubs and trees are absent (Moore, 1964). Grasslands may be of two kinds, natural and artificial (Speeding, 1976). If origin is ignored, grasslands can be divided into cultivated and uncultivated (Davies, 1960). Grasslands are located across contrasting climatic and management gradients, which results in considerable variation in ecosystem structure, environmental conditions and disturbances regions (White *et al.,* 2000; Gilmanov *et al.,* 2010). The world's grasslands have been classified in many ways, chiefly on the basis of climatic factors (Moore, 1964) because this is often considered to be the major factor in determining the distribution of grasslands. Apart from the climate, edaphic, physiographic and the biotic factor, including fire play a major role in deciding the distribution of grasslands (Thomas, 1960). Throughout the world in the tropics, temperate and alpine regions the grassland occupy approximately 45.0 million sq.km. area and it is approximately 24% of the vegetation cover of the globe (Shantz, 1954). Based on the physiognomy and habitat, the grasslands of the world are classified under 7 types (Misra, 1968).

In tropical and subtropical India there are no examples of temperate or subtropical climax grassland and the typical tropical savannah type is also absent, the deciduous forest grading into thorn forest without any open park light stage. Even the alpine meadows are presumably secondary, owing to the existence of turf to grazing and lopping of the bushes. In the tropical zone grassland is common enough as a secondary seral stage and may be very stable preclimax under the influence of fire and grazing. In several regions of limited extent grassland also occurs as an edaphic climax. In all these cases the typical form is savannah or scrub, with or without scattered trees.

Grasslands of southern India exist due to biotic interferences, moisture stress, poor and shallow soils and long dry season on the plateau. Ranganathan (1938) considered the upland grass areas of Nilgiris as 'climatic climax' with species of *Andropogon pertusus, Themeda imberbis, Cymbopogan polyneuros* and *Eragrostis nigra* as common grasses. Meher – Homji (1969) has dicussed the phytogeographical aspects of the shola grasslands and considers frost as a controlling factor

for the perpetuation of these grasslands. Bor (1938) opines that once that we admit the existence of grazing and burning in the area, we cannot apply the term 'climatic climax' and so these grasslands should be considered as biotic climax.

Ecological studies in grassland ecosystem are comparatively easier than those of forest ecosystem. Grasslands are easily manoeuvreable and are more uniform in physiognomy and composition which conditions provide ample opportunities for extensive ecological investigations. Besides, the grasslands have been the centre of agronomic and industrial activities of man and so study of this system has received his early attention. Phytosociological analysis of a plant community is the first and foremost basis of the study of any piece of vegetation as it is a pre-request to the understanding of community structure and organization. In the following account, a case study of grassland near Bharathiar University has been discussed. The study includes floristic composition, life-forms, ecologically important species, distribution level of constituent species, their numerical strength and cover, the relative importance of all constituent species, dominance and diversity of the community. The disturbance index has been calculated on basis of local biotic influence and it has been discussed with the species richness.

2. MATERIALS AND METHODS

2.1. Description of study sites

The present study sites the semiarid grasslands are located near Bharathiar University, Coimbatore at the latitude 11° 13' N and the longitude 76° 38' E at an elevation of 426 m above M.S.L (Fig. 1). The undisturbed and the disturbed grassland taken for the present study are situated adjacently each other and they spread over an area of ca. 10ha (undisturbed) and ca.12ha (disturbed). The study was carried out for a period of 12 months from September, 2014 to August, 2015.

The climatic data of the study area is given in Table 1. The temperature generally ranged between 17°C (January) and 36°C (March). During the study period the lowest and highest minimum temperatures have varied between 17.1 (January, 2015) and 22.9°C (August, 2015). On the other hand, the lowest and highest maximum temperatures have ranged between 27.9 (December, 2014 and 35.5°C (March, 2015). From January to March was a dry season and less rainfall occurred during April and May months. Adequate rainfall usually occurs from June through August during south- west monsoon. The north-east monsoon starts from October. The average rainfall for the past 20 years was as much as 600 mm/year. The relative humidity generally ranged from 80 (July) to 90% (December). The velocity of the wind was generally moderate.

The vegetation of the undisturbed study area was *Brachiaria ramosa* – dominated grassland composed by thirteen other grasses, one sedge and fifty six forbs. In disturbed study area, the same grass, *Brachiaria ramosa* was dominant and the associated species comprised twelve grasses, one sedges and thirty seven forbs. No natural woody vegetation was found in the study area. However, attempts were made to raise some tree species. The fauna of the region included some domestic animals like cattle, sheep, goats etc which usually graze on the pasture land. A few peacock, jackles, snakes, rats etc. were also found in the study area.

2.2. Phytosociological studies

The minimum quadrat size of 1 × 1m was fixed by the species – area curve method for phytosociological observations. Each time 100 quadrats were laid by the randomized method in each site. The minimum number of quadrat required (ie.100) was determined as described by Griez – Smith (1964). For this, the mean number of individuals of the first two, four, six, eight and so on quadrat were calculated and plotted against the number of observations. It will be seen that the mean at first fluctuates, steadying as the required number of quadrats was reached.

The number and type of each species occurring in each quadrat were recorded. For grasses, each tiller was counted as an individual because it is impossible to decide from aerial shoots wheather it is separated or connected in the subterranean region, especially in perennial grasses. Different workers have used arbitrary units to represent individual. Armstrong (1907) and Stapledon (1913) have counted the entire individuals as far as possible in the case of erect plants, but in creeping grasses each rooting unit has taken as an individual. Stove and Fryer (1935) have considered an independent root system, as nearly as this could be determined without actually lifting the plant, to be a unit for counting. In the case of creeping plants, any portion of the plant upto 5 cm in length and having functional root was counted as one plant. Only the plants beyond seedling stage (ie., more than 2 cm height in case of monocots and beyond first leaf stage in dicots) were counted. The basal area at the point of emergence for the constituent species were measured. From the observations, the quantitative characters such as frequency, density, abundance, relative frequency,

relative density, relative dominance, importance value index and relative value of importance were calculated.

Frequency, density and abundance were calculated using the following formulae:

$$\text{Frequency} = \frac{\text{Number of quadrats in which the species present}}{\text{Total number of quadrats studied}} \times 100$$

$$\text{Density} = \frac{\text{Total number of individuals of the species in all quadrats}}{\text{Total number of quadrats studied}}$$

$$\text{Abundance} = \frac{\text{Total number of individuals of the species in all quadrats}}{\text{Number of quadrats of occurrence of the species}}$$

Basal area = Πr^2

Where,

Π = 3.14 and 'r' is the radius of the stem at the point of emergence.

Relative frequency, relative density and relative dominance were calculated from the following formulae:

$$\text{Relative Frequency} = \frac{\text{Number of occurence of the species}}{\text{Number of occurence of all species}} \times 100$$

$$\text{Relative density} = \frac{\text{Number of individuals of the species}}{\text{Number of individuals of all species}} \times 100$$

$$\text{Relative dominance} = \frac{\text{Total basal area of the species}}{\text{Total basal area of all species}} \times 100$$

Important Value Index (I.V.I) is the sum of quantities of relative frequency, relative density and relative dominance expressed per 300.

Relative value of importance (RVI) was calculated by using the formula: $\text{RVI} = \frac{\text{IVI}}{3}$

The frequency index community coefficient (FICC) was calculated by using the formula as given by Gleason (1920):

$$\text{FICC} = \frac{P}{Q} \times 100$$

Where,

P = $\frac{1}{2}$ of the frequencies of occurence of common species.

Q = $\frac{1}{2}$ of the frequencies of occurance of common species (P) + frequencies of occurance of exclusive species in all the four communities studied.

The similarity index (SI) has been calculated by the formula of Sorensen (1948):

$$SI = 2C / A + B$$

Where,

SI = Similarity index

C = Number of common species in both the sites

A and B = Number of species in the 1st and 2nd sites respectively.

Dominance index was determined by the following formula as given by Simpson (1949):

$$C = \Sigma (ni/N)^2$$

Where,

C = Dominance index

ni = Number of individuals of a species over unit area

N = Corresponding total number of individuals of all species over the same unit area

Σ = Summation

The Shannon – Wiener's index of species diversity was worked out by the following formula as given by Margelf (1968):

$$\overline{H} = \Sigma \, Pi \ln Pi$$

Where,

$\overline{H}$ = Shannon – Wiener's index of species diversity

Pi = S/N

S = Number of individuals of one species

N = Total number of all individuals in the sample

ln = The logrithim to the base 'e'

The disturbance index in both sites was calculated as per the method of Gunaga *et al*. (2013). To assess the biotic interference or the disturbance factors on vegetation, we considered scrapping, manual ploughing, litter collection, removal of soil, fire, gardening, grazing, building construction, trampling and collection of plants as the main parameters. For each site, the level of disturbance indicated by each of these ten parameters was scored from 0 (Undisturbed) to 3 (disturbed). The ten scores were added, and the sum multiplied by 100/30 to give a percentage Combined Disturbance Index (CDI).

3. RESULTS

The present study on the sociological attributes of various plant species present in the *Brachiaria ramosa* dominated grassland community was carried out over a period of one year from September 2014 to August 2015. The influence of disturbance over the community composition was

also assessed. The climatic data of the study area was given in Table 1. The range of temperature over the study period was existing between 17.1°C (January 2014) and 35.5°C (March 2015). The total rainfall during the study period of 2014-15 was 831.7 mm / year. Most of the rainfall was occurring during north-east monsoon (Oct - Dec). The south -west monsoon (June - July) was also brought certain rainfall but not at the level of north east monsoon during the study period. The relative humidity was always above 80 % with the peak of 90% during the month of December, 2014. The total number of plant species present in both the study areas was varying greatly higher number of 71 species was noted to be present in undisturbed site, whereas in the disturbed site, the species richness was drastically reduced through various anthropogenic activities to greater level of 28% loss.

The family-wise contribution of plant species to both disturbed and undisturbed study sites is given in Table 2. Among the 71 species present in the undisturbed site, a higher number of 14 species are belonging to the family, Poaceae. The other families such as Amaranthaceae and Euphorbiaceae have contributed 7 and 6 species respectively to the community. The remaining 20 families have contributed little number of less than 5 species only to the community. In the disturbed site, the Poaceae contributed the higher number of 13 species followed by Amaranthaceae with 6 species and Fabaceae and Convolvulaceae with 4 species each, the remaining 11 families contributed less number of species only to the disturbed community. The medicinal uses, parts used and mode of administration of various plants species present in both study area are given in Table 2. Of the 71 species, 66 (92%) harbour various medicinal uses. Majority of the plant species reported to have the medicinal uses for snake bite, kidney problem, hair growth, diabetes, jaundice and cancer. This wide usage of the plant species present in the study areas showed its potentiality for economic species.

The species composition during three different seasons such as winter (December), summer (March) and rainy (July) for the two study areas is given in Tables 3 and 4. Among the 71 species available in undisturbed site, 14 were grasses, 1 sedge and 56 forbs. In disturbed site, 13 were grasses, 1 sedge and 37 were forbs. The variation in species composition indicates that the members of Poaceae were known to be resistants against disturbance. Similarly the sedge, *Cyperus rotundus* was not distributed even in both sites. However, the forbs were disturbed drastically as 19 species such as *Abutilon indicum*, *Aerva lanata* , *A. tomentosa, Alternanthera pungens, A. sessilis, Boerhaavia erecta, Cardiospermum halicacabum, Commelina benghalensis, Corchorus tridens, Euphorbia hirta, Evolvulus alsinoides, Ipomea dissecta, Leucas aspera, Mirabilis jalapa, Oldenlandia umbellata, Passiflora foetida, Phyllanthus amarus, P. maderaspatensis* and *Plumbago zeylanica* have completely vanished in the disturbed site.

The quantitative ecological characters such as frequency, abundance, density and basal cover and synthetic characters such as relative frequency, relative density, relative dominance, importance value index and relative value of importance for all the study species present in the undisturbed and disturbed study sites for 3 different seasons are given in Tables 5 and 6 respectively. Generally, the grasses were disturbed more or less evenly in the respective communities than the sedges and forbs. In undisturbed site, the grasses like *Brachiaria ramosa, Cynodon dactylon, Chloris barbata* and *Sporobolus heterolepis* were distributed evenly than any other species in both the communities as they secured more than 50% of frequency value.

The sedge *Cyperus rotundus,* despite its consistency between the seasons in both study areas was determined to have restricted distribution. Among the forbs in undisturbed site, the species like *Boerhaavia diffusa, Achyranthes aspera, Parthenium hysterophorus* and *Alysicarpus monilifer* have higher frequency value than the rest of the species. However, in disturbed site, *Parthenium hysterophorus* was the only species having better distribution in terms of frequency percentage obtained. In general, it was observed that the rainy season was characterized by higher number of species with better distribution followed by winter and summer seasons. This fact shows that rainfall is the primary factor in this region having influence over the community composition and the distribution level as well.

In undisturbed site, the grasses such as *Brachiaria ramosa, Chloris barbata, Cynodon dactylon* and *Sporobolus heterolepis* and in forbs, the species like *Aerva tomentosa, Alysicarpus monilifer, Gomphrena decumbens* and *Macrotyloma uniflorum* have distributed abundantly than the other constituent species (Tables 5, 6 and 7). In the disturbed site, the same grass species as in undisturbed site and the forbs like *Acalypha indica*, *Amaranthus spinosus*, *Bidens pilosa*, *Euphorbia hirta*, *Evolvulus nummularius, Indigofera enneaphylla*, *Macrotyloma uniflorum, Merremia tridentate*, *Spermacoce hispida* and *Trichodesma indica* were present abundantly (Tables 8, 9 and 10).

As determined for frequency, the grass species such as *Brachiaria ramosa, Chloris barbata, Cyanodon dactylon* and *Sporobolus heterolepis* were registered higher density in undisturbed site. In the similar fashion, the species like *Achyranthes aspera, Alysicarpus monilifer, Boerrhavia diffusa, Euphorbia hirta, Parthenium hysterophorus, Tridax procumbens* and *Vernonia cinera* were determined to have higher density among the forbs in undisturbed site. In disturbed site generally the density of all species were reduced greatly (Tables 8, 9 and 10). The species such as *Brachiaria ramosa, Chloris barbata, Cyanodon dactylon* and *Sporobolus heterolepis* were the species of higher densities in undisturbed site. The species like *Alysicarpus monilifer, Euphorbia hirta, Tridax procumbens* and *Vernonia cinerea* were registered comparatively higher density among the forbs in the disturbed site.

Based on the basal cover, the grass species, *Brachiaria ramosa* was considered to be the dominant species in both the undisturbed and disturbed sites (Tables 5-10). Between the three studied seasons this grass secured the basal cover of 124.62 mm^2/m^2-245.92 mm^2/m^2. Similarly in undisturbed site, this grass species secured the basal cover between 29.57 and 95.05 mm^2/m^2. It shows that this grass species is a resistant against the various kinds of disturbances excerted over the community than the other species recorded in study site. Next to the dominant grass *Brachiaria ramosa*, the other perennial grass *Chloris barbata* occupied the high basal area in both study sites. When the forbs are considered altogether, this species such as *Acalypha indica*, *Achyranthes aspera, Alysicarpus monilifer, Boerrhavia diffusa, Calotropis gigantea, Commelina benghalensis, Croton bonplandianum, Eupatorium odoratum*, *Gomphrena decumbens, Indigofera enneaphylla, Lantana camera, Parthenium hysterophorus, Solanum torvum, Tephrosia purpurea, Trichodesma indicum, Tridax procumbens* and *Vernonia cinerea* were occupied higher basal cover than the other forbs in the undisturbed site (Tables 5-7). Similarly, in disturbed site also the species of forbs such as *Achyranthes aspera, Alysicarpus monilifer, Boerrhavia diffusa, Calotropis gigantea, Commelina benghalensis, Croton bonplandianum, Eupatorium odoratum*, *Indigofera enneaphylla, Lantana camera, Parthenium hysterophorus, Datura metel, Trichodesma indicum, Tridax procumbens* and *Vernonia cinerea* were have higher basal area (Tables 8-10).

The relative position of constituent species in terms of frequency, density and basal cover in the undisturbed and disturbed sites are presented in Tables 5-7 and 8-10 respectively. In undisturbed site the grass species such as *Brachiaria ramosa, Chloris barbata, Cyanodon dactylon* and *Sporobolus heterolepis* and the forbs such as *Eupatorium odoratum*, *Achyranthes aspera, Alysicarpus monilifer, Boerrhavia diffusa, Euphorbia hirta, Parthenium hysterophorus, Tridax procumbens* and *Vernonia cinera* were registered higher values of relative frequency, relative density and relative dominance. The same grasses such as *Brachiaria ramosa, Chloris barbata, Cyanodon dactylon* and *Sporobolus heterolepis* and the forbs such as *Eupatorium odoratum*, *Achyranthes aspera, Alysicarpus monilifer, Boerrhavia diffusa, Euphorbia hirta, Lantana camera Parthenium hysterophorus, Tridax procumbens* and *Vernonia cinera* have registered appreciated values of relative frequency, relative density and relative dominance. The same grass species mentioned for these relative values and the majority of the forbs mentioned for this purpose have in turn secured higher importance value index (IVI) (Tables 5-10) which indicates that all these species have received the maximum impact of environment in their respective site. The relative value of importance (RVI) was also determined to be higher for these mentioned species of higher IVI in their respective sites (Tables 5-10). The presence of higher ecological importance for these species in both sites showed that they are having well adaptive mechanism against the disturbance.

Table 1. Climatic data of the study area for the study period.

Year and month	Temperature (°C)		Rainfall (mm)	Relative humidity (%)
	Max.	Min.		
2014				
Sep	30.8	22.6	170.5	83
Oct	31.2	22.3	30.0	84
Nov	29.9	20.9	303.7	89
Dec	27.9	20.0	161.6	90
2015				
Jan	29.8	17.1	-	89
Feb	32.5	19.5	3.0	87
Mar	35.5	20.3	-	85
Apr	34.8	22.5	29.0	86
May	33.5	23.4	33.0	81
Jun	30.6	22.4	24.0	81
Jul	30.8	23.3	51.5	80
Aug	31.4	22.9	25.4	85

Table 2. List of plant species with their families and their medicinal uses in the *Brachiaria ramosa*dominated grassland.

S.No.	Species	Family	Medicinal uses	Part used
	Grasses			
1.	*Andropogon virginicus*	Poaceae	A decoction of the roots is used in the treatment of backaches. A tea made from the leaves is used in the treatment of diarrhea. Externally, it is used as a wash for frostbite, sores, itching, piles and poison ivy rash.	Root and leaves
2.	*Apluda mutica*	Poaceae	Fodder	Entire Plant
3.	*Brachiariaramosa*	Poaceae	Palatable during lean period.	Grain, leaf
4.	*Chloris barbata*	Poaceae	Skin diseases, fever, diarrhoea and diabetes. Fodder when young.	Leaf
5.	*Cymbopogon caesius*	Poaceae	Cereal and grass forages Forage plants.	Leaf
6.	*Cynodon dactylon*	Poaceae	Eye disorders, weak vision, pungent, bitter, fragrant, heating, appetizer, vulnerary, anthelmintic, antipyretic, alexiteric. It destroys foulness of breath, useful in leucoderma, bronchitis, piles, asthma, tumors, and enlargement of the spleen. Laxative, brain and heart tonic, aphrodisiac, alexipharmic, emetic, emmenagogue, expectorant, carminative and useful against grippe in children, and for pains, inflammations, and toothache; palatable.	Stem and leaf
7.	*Digitaria eriantha*	Poaceae	It is used to cure weak bones, infections.	Leaf
8.	*Eragrostis aspera*	Poaceae	Grains are eaten for asthma.	Grains
9.	*Heteropogon contortus*	Poaceae	In fever, muscle pain, atrophy and toothache, Asthma (Plant oil).	Whole plant
10.	*Melinis repens*	Poaceae	-	-
11.	*Pennisetum alopecuroides*	Poaceae	-	-
12.	*Perotis indica*	Poaceae	Good fodder.	Leaf
13.	*Setaria pumila*	Poaceae	It can be eaten as a sweet or savoury food in all the ways that rice is used, or ground into a powder and made into porridge, cakes, puddings etc.	Seed
14.	*Sporobolus heterolepis*	Poaceae	Native Americans ground the seeds of the grass to make a tasty flour, and many species of birds eat the seeds.	seeds
	Sedges			
15.	*Cyperus rotundus*	Cyperaceae	Acrid, cooling, astringent, appetizer, stomachic, anthelmintic and useful in treatment of leprosy, thirst, fever, blood diseases, biliousness, dysentery,	Tubers

			pruritus, pain, vomiting, epilepsy, opthalmia, erysipelas etc. According to the Unani system of medicine, the root is diuretic, emmenagogue, diaphoretic, anthelmintic, vulnerary and useful for ulcers and sores, fevers, dyspepsia, urinary concretions.	
Forbs				
16.	*Abutilon indicum*	Malvaceae	Various parts of the plant are used as a demulcent, aphrodisiac, laxative, diuretic, sedative, astringent, expectorant, tonic, anti-inflammatory, anthelmintic, and analgesic and to treat leprosy, ulcers, headaches, gonorrhea, and bladder infection.	whole plant
17.	*Acalypha indica*	Euphorbiaceae	Leaves - laxative, anthelmintic. Leaf juice is a safe and speedy emetic for children, and is useful in chronic bronchitis and asthma. Decoction is employed in ear-ache. Leaves used for ulcers, snake- bites, skin diseases, rheumatism, scabies, headache and root acts as a cathartic.	Leaf, root, stalks (young shoots) and flower.
18.	*Achyranthes aspera*	Amaranthaceae	Pungent, laxative, stomachic, carminative and useful in the treatment of vomiting, bronchitis, heart diseases, piles, itching, abdominal pains, ascites, dyspepsia, dysentery, blood diseases etc. Useful for reclamation of wastelands. Leaf is consumed as pot herb. Seeds rich in protein, cooked and eaten. Used in religious ceremonies in India.	Leaf, seed
19.	*Aerva lanata*	Amaranthaceae	Diuretic and demulcent. The whole plant, especially the leaves are edible. The leaves are put into soup or eaten as spinach or as a vegetable. The plant provides grazing for stock, game in and chickens. A leaf- decoction is prepared as a gargle for treating sore-throat and used in various complex treatments against guinea-worm, smoke from the burning plant is inhaled. The leaf-sap - eye-complaints; infusion - diarrhoea and in an unspecified manner at childbirth, and on sores; root is used in snake-bite treatment. For pains in the lower part of the back leaves and flowers are reduced to ash which is rubbed into cuts on the back.	Leaf, root, flower.
20.	*A. tomentosa*	Amaranthaceae	Roots are chewed to form brush for cleaning teeth. Seeds are said to relieve head ache. They are also used against rheumatism. The herb is diuretic and demulcent. Its decoction is used to remove swellings.	Root and Seed
21.	*Alysicarpus monilifer*	Fabaceae	Roots - for the treatment of leprosy and urinary troubles. The decoction of root is being used for cough. Boiled leaves purgative. It has antiproliferation activity against tumor cells. The whole plant - antipyretic, antiperiodic and has expectorant properties. The leaves -to treat jaundice and stomach pain. Leaf paste - for coetaneous problems.	Leaf, stem, root, whole plant.
22.	*Alternanthera pungens*	Amaranthaceae	Purification of blood, and all sorts of impurities, decongestant, diuretic, anti-inflammatory, anti liver ailments, kidney problems, diarrhea in	Leaf

			children and teething problems in children.	
23.	*A. sessilis*	.Amaranthaceae	Diuretic, tonic and cooling. Juice of this plant deemed beneficial to eyes; an ingredient in the making of medicinal hair oils and used for simple stomach disorders, diarrhoea, dysentery and as a plaster for diseased or wounded skin parts and against fever, vomiting blood, headache and vertigo; Leaf sap is sniffed up the nose to treat neuralgia. Paste is used to draw out spines or any other object from the body and it is also used to cure hernia.	Whole plant
24.	*Amaranthus spinosus*	Amaranthaceae	Diabetes. The seed is used as a poultice for broken bones, internal bleeding, diarrhoea and excessive menstruation. Root - effective diuretic, gonorrhea, emmenagogue and antipyretic, toothaches. The bruised leaves are considered a good emollient and applied externally in cases of ulcerated mouths, eczema, burns, wounds, boils, earache and hemorrhoids. Leaves are also for gastroenteritis, gall bladder inflammation, abscesses, colic menorrhagia, arthritis and for the treatment of snakebites.	Root, leaf, seed, tender shoot.
25.	*Bidens pilosa*	Asteraceae	Antibacterial, antidysenteric, anti-inflammatory, antimicrobial, antimalarial, diuretic, hepato-protective and hypotensive activities.	Leaves, Root and Seed.
26. b	*Boerhaavia erecta*	Nyctaginaceae	Diuretic, stomachic, cardiotonic, hepatoprotective, laxative, anthelmintic, febrifuge, expectorant and, in higher doses, as an emetic and purgative. As a diuretic it is useful in cases of strangury, jaundice, enlarged spleen, gonorrhoea and other internal inflammations, asthma. Decoction of the whole plant - gastro-intestinal, liver and infertility problems and to treat convulsions in children. Paste of the root used to cure ulcers. Sap from the leaves is squeezed into the eye to treat conjunctivitis.	Whole plant, Root, leaves.
27.	*B. diffusa*	Nyctaginaceae	Cooling, astringent to bowels, useful in biliousness, blood purification, leucorrhoea, anaemia, inflammations, heart diseases and asthma. Leaves dyspepsia, tumours, spleen enlargement, abdominal pains. They are appetizer, alexiteric, in opthalmia,to treat joint pains. Seeds are tonic expectorant, carminative, useful in lumbago, scabies, and blood purifier.	Root, leaf, seed
28.	*Calotropis gigantean*	Asclepiadaceae	Used to treat common diseases like fevers, rheumatism, indigestion, cough, cold, eczema, asthma, elephantiasis, nausea, vomiting, diarrhea etc. Dried whole plant is a good tonic, expectorant, depurative, and anthelmintic. The root bark is febrifuge, anthelmintic, depurative, expectorant and laxative. The powdered root used for asthma, bronchitis, and dyspepsia. Leaves for the treatment of paralysis, arthralegia, swellings and intermittent fevers. Flowers are bitter, digestive, astringent, stomachic, anthelmintic and tonic.	Leaf, root bark, flower, seed.
29.	*Cardiospermum halicacabum*	Sapindaceae	It is used for arthritis and other painful conditions of the body. They can be used as a ear drops for ear ache, purulent discharge from ears. Root decoction can be given for haemorrhoids. Whole plant used for	Whole plant, Leaf.

			constipation and abdominal discomfort. The oil prepared from the paste of the leaves with gingilly oil can be used as a hair tonic and cure for dandruff.	
30.	*Cleome pentaphylla*	Capparidaceae	Plant pacifies vitiated kapha, intestinal worms, colic, stomach upset, cardio myopathy, headache, diarrhea, fever and dyspepsia.	Whole plant
31.	*Clitoria ternatea*	Fabaceae	Blood purifier, abortifacient, astringent, demulcent, emetic, purgative and in the treatment of anaemia, impetigo, menorrhagia and psoriasis. Seeds - antispermatogenic, anti-ovulatory and contraceptive activities.	Seed, arial parts
32.	*Coccinia indica*	Cucurbitaceae	It is used to treat ring worm, psoriasis and itch; when mixed with ghee cures sores, skin diseases, skin eruptions of small pox; causes cooling effect to eyes, heals big ulcers, small lesions of scabies, anuria and alleviate body heat and has antispasmodic effect. Green fruit when chewed cures sores on tongue; raw fruit used as vegetable; dried fruit removes eczema. Dried bark has cathartic properties. Juice of tuberous roots, stem and leaves cure diabetes, intermittent glycosuria, enlarged glands and skin diseases like pityriasis and urinary tract infection. Used in treating gastro – intestinal disturbances, liver weakness, dysentery, vomiting, infestation, purifies blood, curb infection in the body, effective against chronic cough and cold and gives good results for bronchitis and asthma. Tubers remove pain in joints, diabetes, skin lesions (Tenia), apthous ulcers, wheezing and phlegm. Decoction of stem and leaf cures bronchitis.	Leaf, stem, fruit, bark, root.
33.	*Commelina benghalensis*	Commelinaceae	For mouth thrush, inflammation of the conjunctiva, psychosis, epilepsy, nose blockage in children, insanity and exophthalmia; diuretic, febrifuge and anti- inflammatory; animal fodder, vegetable; laxative and to cure inflammations of the skin; leprosy.	Whole plant
34.	*Corchorus tridens*	Tiliaceae	Leaves - vegetable in stews eaten with starchy staple foods, and in soups and sauces.	Leaf
35.	*Crotalaria verrucosa*	Fabacaea	Juice of leaves diminishes salivation, juice used for scabies and impetigo, dyspepsia, blood impurities, diarrhea, dysentery, leprosy.	leaves
36.	*Croton bonplandianum*	Euphorbiaceae	Whole plant has been credited with potential to cure liver diseases and swelling of the body, cure against ring worms and skin diseases. Bark and roots - alternative and chologogue. Leaves - controlling B.P and for the treatment of skin diseases and cut and wounds and it is antiseptic and antidote.	Whole plant, Leaf, Bark, root.
37.	*Datura metel*	Solanaceae	Seeds along with other substances are used as a remedy for the symptoms of madness based on homeopathic principle; decoction of seeds - eye diseases. The seeds - potential source for hyoscine, pain relief, asthma and other illnesses. The seed extract to treat wounds, tooth decay and leprosy due to hyoscine.	Seeds, leaves, Flowers.

38.	*Erigeron annuus*	Asteraceae	Epilepsy, cough, cold, venereal disease, skin diseases.	Root
39.	*Eupatorium odoratum*	Asteraceae	It is good for colds. Boil the tea leaves in some water and serve as tea. Sweeten with honey or as people do, serve with a dash of salt.	Leaf
40.	*Euphorbia hirta*	Euphorbiaceae	Decoction or infusion, to treat gastrointestinal disorders, including intestinal parasites, diarrhoea, peptic ulcers, heart burn, vomiting and amoebic dysentery. It is used to treat respiratory system disorders, including asthma, bronchitis, hay fever, laryngeal spasms, emphysema, coughs and colds. Leaves - diuretic to treat uro-genital like kidney stones, menstrual problems, sterility and venereal diseases. The plant is also used to treat infections of the skin and mucous membranes, including warts, scabies, tinea, thrush, aphthae, fungal afflictions, measles, Guinea -worm and as an antiseptic to treat wounds, sores and conjunctivitis. The plant has a reputation as an analgesic to treat severe headache, toothache, rheumatism, colic and pains during pregnancy; axial parts palatable.	Leaf, tender shoot
41.	*E. microphylla*	Euphorbiaceae	Plant extract – jaundice; lower the elevated levels of serum bilirubin; antiulcer.	Leaf
42.	*Evolvulus nummularis*	Convolvulaceae	Cough cold, venereal disease, spermopiotic, fever, epilepsy, insanity, nervous debility, and loss of memory.	Whole plant
43.	*E. alsinoides*	Convolvulaceae	Roots - nerve tonic. Whole plant is widely used in ayurveda medicinal practice.	Root, Whole plant.
44.	*Gomphrena decumbens*	Amaranthaceae	Plant part is used for the treatment of diabetes.	Whole plant
45.	*Heliotrophium indicum*	Boraginaceae	Treating abdominal pains, dysmenorrhoea, hypertension, convulsion, post-partum inflammatory disorders, wounds and infections and skin rashes.	Whole plant
46.	*Hibiscus vitifolius*	Malvaceae	Treatment of jaundice in the folklore system of medicine in India and anti-tubercular drug induced hepatotoxicity.	Root
47.	*Indigofera enneaphylla*	Convolvulaceae	Wound healing.	Whole plant
48.	*Ipomea dissecta*	Convolvulaceae	Diuretic, fever, headache, pimples.	Leaves and Root.
49.	*I. obscura*	Convolvulaceae	Diabetes, hypertension, dysentery, constipation, fatigue, arthritis, rheumatism, hydrocephaly, meningitis, kidney ailments and inflammations.	Whole plant
50.	*Justicia tranquebariensis*	Acanthaceae	Used in the traditional system of medicine for the treatment of fever, pain, inflammation, diabetes, diarrhea and liver diseases; antitumoral, antiviral, analgesic and anti-inflammatory activities.	Leaf
51.	*Lantana camara*	Verbenaceae	Leaves - relieve itching, flu, colds, coughs, fevers, yellow fever, dysentery and jaundice. Roots - gonorrhea. Lantana oil - skin itches, antiseptic for	Leaf, bark, root,

			wounds and externally for leprosy and scabies. Plant extracts - anticancers, chicken pox, measles, asthma, ulcers, swellings, eczema, tumors, high blood pressure, bilious fevers, catarrhal infections, tetanus, rheumatism, malaria and atoxy of abdominal viscera.	flowering tops.
52.	*Leucas aspera*	Lamiaceae	Nasal congestion, cough, cold, fever, headache.	Whole plant
53.	*Macrotyloma uniflorum*	Fabaceae	Dysentery, venereal diseases, fever and diabetes.	Leaves and root.
54.	*Martynia annua*	Martyniaceae	Root decoction is administered for snakebite.Juice of leaf for epilepsy, tuberculosis and sorethroat. Stem of the plant is used by Tantriks in some parts of India.	Leaf, stem root.
55.	*Meremmia emarginata*	Convolvulaceae	-	-
56.	*M. tridentate*	Convolvulaceae	-	-
57.	*Mirabilis jalapa*	Nyctaginaceae	Parts of the plant may be used as a diuretic, purgative, and for vulnerary purposes. The leaves are used to reduce inflammation. A decoction of leaf is used to treat abscesses. Leaf juice may be used to treat wounds.The root is believed an aphrodisiac as well as diuretic and purgative. It is used in the treatment of dropsy.	Leaf, root.
58.	*Oldenlandia umbellata*	Rubiaceae	Decoction of the entire plant bronchial asthma. Decoction of the root is a febrifuge.	Whole plant
59.	*Parthenium hysterophrous*	Asteraceae	Decoction of root – dysentery; sub lethal doses of parthenium - antitumour activity.	Root
60.	*Passiflora foetida*	Passifloraceae	Leaves and fruits - asthma and biliousness. Leaf and root decoction is emmenagogue, used in hysteria and leaf paste is applied on the head for giddiness and headache. The herb is used in the form of lotions or poultices for erysipelas and skin diseases with inflammation.	Whole plant
61.	*Pavonia indicum*	Malvaceae	Nervous debility, hysteria and other nervous disorders and atomic dyspepsia.	Seed
62.	*Peristrophe bicalyculata*	Acanthaceae	Traditional healers are using this species in the treatment of many skin related problems; antidote for snake poison when macerated in an infusion of rice; insect repellant; used as horse feed; green manure; analgesic, anti-inflammatory and antibacterial.	Whole plant
63.	*Phyllanthus amarus*	Euphorbiaceae	Hair, teeth, bones, kidneys, memory, sight, and hearing.	Whole plant
64.	*P. maderaspatensis*	Euphorbiaceae	The plant sap and leaf decoction are credited with emetic and purgative activities. The whole plant is powdered and the solution is applied to scabies. Root decoction constipation, diarrhoea, appetite, intestinal pain, menstrual problems, gastrointestinal disorders, testicular swelling, chest complaints and snake bites. Gastrointestinal trouble in infants is treated by	Whole plant, root.

			giving them a root decoction. Plant sap is used as no se drops to treat toothache. Ground leaves are rubbed on the skin with lemon juice to treatment for rheumatism; palatable.	
65.	*Plumbago zeylanica*	Plumbaginaceae	treatment of toothache, applied externally to treat swellings, rheumatism, leprosy, tumors, and ringworm.	Root
66.	*Solanum torvum*	Solanaceae	The juice to treat fever and alleviate pain; fruit - cosmetic; as rubbing its seeds on the cheeks helps remove freckles; diabetes liver-related ailments, jaundice; juice of the herb certain skin problems and tumors; decoction of the stalk, leaves, and roots - wounds and cancerous sores. Freshly prepared extract of the plant is effective in treating cirrhosis of the liver and also works as an antidote to poisoning by opium.	Fruit, whole plant.
67.	*Spermacoce hispida*	Rubiaceae	Seeds – cooling, demulcent and given in diarrhea and dysentery; recommended as a substitute for coffee; Crushed into paste and taken orally to treat stomach problems; antihypertensive.	Whole plant
68.	*Tephrosia purpurea*	Fabaceae	The plant is reported to cure diseases of the kidney, liver, spleen, heart and blood. The dried herb - tonic, laxative, and diuretic; Used in the treatment of bronchitis, bilious febrile attack, boils, pimples, and bleeding piles. The roots and seeds - insecticidal, pesticidal, and vermifugal properties; roots - leprous wounds and root juice to skin eruptions. Aerial plant parts-anticancer activity against a human nasopharyngeal epidermoid tumor cell line.	Leaf, root, root bark, aerial parts, seed.
69.	*Trichodesma indicum*	Boraginaceae	Leaves and roots are remedy for snake bites; diuretic. Cold infusion of leaves - depurative. Crushed roots, dysentery in children, diarrhea and fever, swollen joints, inflammations and superficial skin injuries; used for arthralgias, inflammations, dyspepsia, diarrhea, dysentery, dysmenorrhea.	Root, leaf, flower.
70.	*Tridax procumbens*	Asteraceae	Plant pacifies vitiated pitta, inflammation, wound, ulcers, anal fistula, and hemorrhoids. Anticoagulant, antifungal and insect repellent; in bronchial catarrh, diarrhoea and dysentery. Wound healing activity and promotes hair growth.	Whole plant
71.	*Vernonia cinerea*	Asteraceae	Plant pacifies vitiated vata, pitta, tonsillitis, stomach pain, diarrhea, intermittent fever, eczema, herpes, ringworm, and elephantiasis. Leaves - conjunctivitis and in lacrimation. Seeds - worm infestation , cough, psoriasis, leukoderma and for other skin diseases. Plant possess anticancer property. Used for abortion, cancer and various gastrointestinal disorders.	Whole plant

Table-3. Species with their individuals in undisturbed grassland during three different seasons.

S. No	Species	Winter (Dec)	Summer (Mar)	Rainy (Jul)
	Grasses			
1.	*Andropogon virginicus*	12 (4)	10 (2)	21(6)
2.	*Apluda mutica*	10(2)	8(2)	18(6)
3.	*Brachiaria ramosa*	563(85)	413(61)	815(100)
4.	*Chloris barbata*	598(79)	411(55)	965(100)
5.	*Cymbopogon caesius*	52(13)	40(8)	76(18)
6.	*Cynodon dactylon*	996(84)	632(71)	1332 (100)
7.	*Digitaria eriantha*	19(8)	12(5)	31(11)
8.	*Eragrostis aspera*	33(10)	21(6)	55(11)
9.	*Heteropogon contortus*	8(3)	6(3)	32(9)
10.	*Melinis repens*	9(2)	6(2)	16(3)
11.	*Pennisetum alopecoroides*	15(5)	10(3)	28(8)
12.	*Perotis indica*	11(3)	7(2)	19(5)
13.	*Setaria pumila*	89(26)	75(16)	120(48)
14.	*Sporobolus heterolepis*	903(70)	753(48)	1519 (100)
	Sedges			
15.	*Cyperus rotundus*	16(9)	13(4)	43(18)
	Forbs			
16.	*Abutilon indicum*	2(2)	2(1)	5(4)
17.	*Acalypha indica*	3(2)	2(2)	19(9)
18.	*Achyranthes aspera*	55(29)	48(15)	66(50)
19.	*Aerva lanata*	11(3)	-	18(6)
20.	*A. tomentosa*	8(2)	5(2)	15(3)
21.	*Alysicarpus monilifer*	34(12)	24(10)	71(19)
22.	*Alternanthera pungens*	6(2)	3(2)	15(5)
23.	*A. sessilis*	5(3)		9(4)
24.	*Amaranthus spinosus*	7(3)	4(2)	6(16)
25.	*Bidens pilosa*	3(2)	2(1)	5(2)
26.	*Boerhaavia erecta*	2(4)	2(1)	3(8)
27.	*B. diffusa*	54(40)	43(15)	89(63)
28.	*Calotropis gigantea*	3(2)	2(1)	6(5)
29.	*Cardiospermum halicacabum*	2(2)	-	4(3)
30.	*Cleome pentaphylla*	-	-	5(2)
31.	*Clitoria ternatea*	4(3)	3(3)	5(4)
32.	*Coccinia indica*	2(1)		3(2)
33.	*Commelina benghalensis*	-	-	3(2)
34.	*Corchorus tridens*	-	-	3(2)
35.	*Crotalaria verrucosa*	-	-	4(3)
36.	*Croton bonplandianum*	6(2)	3(1)	16(9)
37.	*Datura metel*	3(2)	2(1)	5(3)
38.	*Erigeron annuus*	4(1)	-	7(4)
39.	*Eupatorium odoratum*	15(6)	8(3)	26(16)
40.	*Euphorbia hirta*	36(10)	21(8)	52(20)
41.	*E. microphylla*	5(4)	3(3)	13(9)
42.	*Evolvulus nummularius*	8(3)	-	13(5)
43.	*E. alsinoides*	11(6)	-	21(10)
44.	*Gomphrena decumbens*	18(6)	7(4)	39(11)
45.	*Heliotrophium indicum*	6(3)	-	15(5)
46.	*Hibiscus vitifolius*	-	-	2(2)
47.	*Indigofera enneaphylla*	26(11)	9(16)	40(25)
48.	*Ipomea dissecta*	2(1)	-	6(3)
49.	*I. obscura*	-	-	5(3)
50.	*Justicia tranquebariensis*	4(2)	2(1)	8(5)
51.	*Lantana camara*	3(2)	3(1)	4(4)
52.	*Leucas aspera*	7(3)	-	14(6)
53.	*Macrotyloma uniflorum*	-	-	5(3)
54.	*Martynia annua*	4(2)	2(1)	7(3)
55.	*Meremmia emarginata*	5(2)	3(1)	11(4)
56.	*M. tridentata*	-	2(1)	6(3)
57.	*Mirabilis jalapa*	2(1)	2(1)	4(2)
58.	*Oldenlandia umbellata*	-	-	4(2)
59.	*Parthenium hysterophrous*	65(20)	49(15)	89(45)
60.	*Passiflora foetida*	2(1)	-	3(3)
61.	*Pavonia*	-	-	3(3)

	indicum			
62.	*Peristrophe bicalyculata*	9(2)	5(2)	17(14)
63.	*Phyllanthus amarus*	4(2)	6(2)	10(5)
64.	*P. maderaspatensis*	2(2)	2(1)	6(3)
65.	*Plumbago zeylanica*	-	-	3(2)
66.	*Solanum torvum*	4(2)	-	8(5)
67.	*Spermacoce hispida*	-	-	5(5)
68.	*Tephrosia purpurea*	6(2)	3(2)	16(8)
69.	*Trichodesma indicum*	17(6)	9(2)	26(15)
70.	*Tridax procumbens*	40(11)	21(5)	69(38)
71.	*Vernonia cinerea*	48(14)	30(8)	59(41)

Figures in parenthesis are the number of quadrats in which the species present out of 100 quadrats studied.

Table 4. Species with their individuals in disturbed grassland during three different seasons.

S.No	Species	Winter (Dec)	Summer (Mar)	Rainy (Jul)
	Grasses			
1.	*Andropogon virginicus*	6(3)	4(3)	13(8)
2.	*Apluda mutica*	4(3)	2(1)	8(5)
3.	*Brachiaria ramosa*	186(65)	98(49)	315(79)
4.	*Chloris barbata*	211(58)	109(31)	412(90)
5.	*Cynodon dactylon*	315(59)	165(30)	518(79)
6.	*Digitaria eriantha*	5(3)	3(2)	11(5)
7.	*Eragrostis aspera*	13(6)	9(4)	27(11)
8.	*Heteropogon contortus*	5(3)	3(2)	19(7)
9.	*Melinis repens*	5(3)	3(2)	13(6)
10.	*Pennisetum alopecuroides*	8(4)	5(3)	19(10)
11.	*Perotis indica*	7(5)	4(2)	10(5)
12.	*Setaria pumila*	43(15)	21(8)	89(30)
13.	*Sporobolus heterolepis*	189(48)	111(31)	310(69)
	Sedges			
14.	*Cyperus rotundus*	7(4)	3(2)	26(15)
	Forbs			
15.	*Acalypha indica*	2(1)	1(1)	6(3)
16.	*Achyranthes aspera*	20(13)	13(6)	30(18)
17.	*Alysicarpus monilifer*	15(10)	10(6)	32(18)
18.	*Amaranthus spinosus*	5(3)	3(2)	10(4)
19.	*Bidens pilosa*	1(1)	1(1)	2(1)
20.	*Boerhaavia diffusa*	25(11)	15(8)	42(29)
21.	*Calotropis gigantea*	1(1)	1(1)	4(1)
22.	*Cleome pentaphylla*	-	-	1(1)
23.	*Clitoria ternatea*	2(1)	2(1)	5(3)
24.	*Coccinia indica*	-	-	2(2)
25.	*Crotalaria verrucosa*	-	-	1(1)
26.	*Croton bonplandianum*	3(2)	1(1)	8(6)
27.	*Datura metel*	1(1)	1(1)	3(3)
28.	*Erigeron annuus*	2(2)	-	4(3)
29.	*Eupatorium odoratum*	13(6)	7(3)	22(13)
30.	*Euphorbia hirta*	25(9)	19(5)	36(13)
31.	*E. microphylla*	2(2)	2(1)	6(4)
32.	*Evolvulus nummularius*	2(2)	-	6(3)
33.	*Gomphrena decumbens*	8(6)	3(2)	15(10)
34.	*Heliotrophium indicum*	3(2)	-	9(7)
35.	*Hibiscus vitifolius*	-	-	1(1)
36.	*Indigofera enneaphylla*	9(5)	5(3)	16(8)
37.	*Ipomea obscura*	-	-	2(2)
38.	*Justicia tranquebariensis*	3(2)	1(1)	5(3)
39.	*Lantana camara*	3(2)	2(2)	5(4)
40.	*Macrotyloma uniflorum*	-	-	2(1)
41.	*Martynia annua*	2(1)	1(1)	3(2)
42.	*Meremmia emarginata*	2(1)	1(1)	5(3)
43.	*M. tridentata*	-	-	2(1)
44.	*Parthenium hysterophrous*	33(23)	16(9)	80(46)
45.	*Pavonia indicum*	-	-	1(1)
46.	*Peristrophe bicalyculata*	3(2)	1(1)	7(5)
47.	*Solanum torvum*	1(1)	-	3(2)
48.	*Spermacoce hispida*	-	-	2(1)
49.	*Trichodesma indicum*	5(3)	2(1)	16(8)
50.	*Tridax procumbens*	18(11)	11(9)	38(25)
51.	*Vernonia cinerea*	25(16)	10(9)	46(26)

Figures in parenthesis are the number of quadrats in which the species present out of 100 quadrats studied.

Table 5. Species composition in undisturbed grassland: frequency, abundance, density and basal cover and their relative values withimportance value index (IVI) and relative value of importance (RVI) during winter (Dec. 2014).

S.No	Species	Quantitative attributes				Synthetic attributes				
		Frequency (%)	Abundance (individuals/m^2)	Density (individuals/m^2)	Basal cover (mm^2/m^2)	R.F (%)	R.DE (%)	R.DO (%)	IVI	RVI
	Grasses									
1.	*Andropogan virginicus*	4	3	0.12	1.22	0.62	0.30	0.21	1.13	0.37
2.	*Apluda mutica*	2	5	0.1	1.38	0.31	0.25	0.24	0.79	0.26
3.	*Brachiaria ramose*	85	7	5.63	169.88	13.19	14.44	30.60	58.23	19.41
4.	*Chloris barbata*	79	7.56	5.98	82.8	12.26	15.34	14.91	42.51	14.17
5.	*Cymbopogon caesius*	13	4	0.52	18.11	2.01	1.33	3.26	6.6	2.2
6.	*Cynodon dactylon*	84	11.85	9.96	20.01	13.04	25.55	3.60	42.19	14.06
7.	*Digitaria eriantha*	8	2.37	0.19	0.49	1.24	0.48	0.08	1.8	0.6
8.	*Eragrostis aspera*	10	3.3	0.33	1.04	1.55	0.84	0.18	2.57	0.85
9.	*Heteropogon contortus*	3	2.66	0.08	0.21	0.46	0.2	0.03	0.96	0.32
10.	*Melinis repens*	2	4.55	0.09	0.18	0.31	0.23	0.03	0.57	0.19
11.	*Pennisetumalopecuroides*	5	3	0.15	0.68	0.77	0.38	0.12	1.27	0.42
12.	*Perotis indica*	3	3.66	0.11	0.59	0.46	0.28	0.10	0.84	0.28
13.	*Setaria pumila*	26	3.42	0.89	4.03	4.03	2.28	0.73	7.04	2.34
14.	*Sporobolusheterolepis*	70	12.9	9.03	22.97	10.86	23.17	4.13	38.26	12.75
	Sedges									
15.	*Cyperus rotundus*	9	1.77	0.16	2.22	1.39	0.41	0.40	2.2	0.73
	Forbs									
16.	*Abutilon indicum*	2	1	0.02	1.64	0.31	0.05	0.29	0.65	0.21
17.	*Acalypha indica*	2	1.5	0.03	1.37	0.31	0.07	0.25	0.63	0.21
18.	*Achyranthes aspera*	29	1.89	0.55	14.52	4.5	1.41	2.61	8.52	2.84
19.	*Aerva lanata*	3	3.66	0.11	1.39	0.46	0.28	0.25	0.99	0.33
20.	*A. tomentosa*	2	4	0.08	1.22	0.31	0.2	0.22	0.73	0.24

21.	*Alysicarpus monilifer*	12	2.83	0.34	5.65	1.86	0.87	1.01	3.74	1.24
22.	*Alternanthera pungens*	2	3	0.06	1.82	0.31	0.15	0.32	0.78	0.26
23.	*A. sessilis*	3	1.66	0.05	1.42	0.46	0.12	0.25	0.83	0.27
24.	*Amaranthus spinosus*	3	2.83	0.07	2.26	0.46	0.17	0.40	1.03	0.34
25.	*Bidens pilosa*	2	1.5	0.03	1.16	0.31	0.07	0.20	0.58	0.19
26.	*Boerhaavia erecta*	4	0.5	0.02	0.49	0.62	0.05	0.08	0.75	0.25
27.	*B. diffusa*	40	1.35	0.54	14.26	6.21	1.38	2.56	10.15	3.38
28.	*Calotropis gigantean*	2	1.5	0.03	6.03	0.31	0.07	1.08	1.46	0.48
29.	*Cardiospermum halicacabum*	2	1	0.02	0.77	0.31	0.05	0.13	0.49	0.16
30.	*Clitoria ternatea*	3	1.33	0.04	0.36	0.46	0.1	0.06	0.62	0.20
31.	*Coccinia indica*	1	2	0.02	0.28	0.15	0.05	0.05	0.25	0.08
32.	*Croton bonplandianum*	2	3	0.06	4.35	0.31	0.15	0.78	1.24	0.41
33.	*Datura metel*	2	1.5	0.03	6.49	0.31	0.07	1.16	1.54	0.51
34.	*Erigeron annus*	1	4	0.04	1.99	0.15	0.1	0.35	0.6	0.2
35.	*Eupatorium odoratum*	6	2.5	0.15	22.43	0.93	0.38	4.04	5.35	1.78
36.	*Euphorbia hirta*	10	3.6	0.36	1.14	1.55	0.92	0.20	2.67	0.89
37.	*E. microphylla*	4	1.25	0.05	0.08	0.62	0.12	0.01	0.75	0.25
38.	*Evolvulus nummularis*	3	2.66	0.08	0.37	0.46	0.2	0.06	0.72	0.24
39.	*E. alsinoides*	6	1.83	0.11	0.35	0.93	0.28	0.06	1.27	0.42
40.	*Gomphrena decumbens*	6	3	0.18	3.54	0.93	0.46	0.63	2.02	0.67
41.	*Heliotrophium indicum*	3	2	0.06	1.93	0.46	0.15	0.34	0.95	0.31
42.	*Indigofera enneaphylla*	11	2.36	0.26	8.36	1.7	0.66	1.50	3.86	1.28
43.	*Ipomea dissecta*	1	2	0.02	1.69	0.15	0.05	0.30	0.5	0.16
44.	*Justicia tranquebariensis*	2	2	0.04	1.92	0.31	38.97	0.34	39.62	13.20
45.	*Lantana camara*	2	1.5	0.03	10.58	0.31	0.07	1.90	2.28	0.76
46.	*Leucas aspera*	3	2.33	0.07	1.73	0.46	0.17	0.16	2.79	0.93
47.	*Martynia annua*	2	2	0.04	16.28	0.31	0.1	2.93	3.34	1.11

48.	*Meremmia emarginata*	2	2.5	0.05	2.52	0.31	0.12	0.45	0.88	0.29
49.	*Mirabilis jalapa*	1	2	0.02	1.06	0.15	0.05	0.19	0.39	0.13
50.	*Parthenium hysterophrous*	20	3.25	0.65	29.48	3.1	1.66	5.31	10.07	3.56
51.	*Passiflora foetida*	1	2	0.02	0.28	0.15	0.05	0.05	0.25	0.08
52.	*Peristrophe bicalyculata*	2	4.5	0.09	2.55	0.31	0.23	0.45	0.99	0.33
53.	*Phyllanthus amarus*	2	2	0.04	0.51	0.31	0.1	0.09	0.5	0.16
54.	*P. maderaspatensis*	2	1	0.02	0.49	0.31	0.05	0.08	0.44	0.14
55.	*Solanum torvum*	2	2	0.04	4.83	0.31	0.1	0.87	1.28	0.42
56.	*Tephrosia purpurea*	2	3	0.06	7.48	0.31	0.15	1.34	1.8	0.6
57.	*Trichodesma indicum*	6	2.83	0.17	8.12	0.93	0.43	1.46	2.82	0.94
58.	*Tridax procumbens*	11	3.63	0.4	19.11	1.7	1.02	3.44	6.16	2.05
59.	*Vernonia cinerea*	14	3.42	0.48	15.44	2.17	1.23	2.78	6.18	2.06

R.F: Relative Frequency, R.DE: Relative Density, R.DO: Relative Dominance.

Table 6. Species composition in undisturbed grassland: frequency, abundance, density and basal cover and their relative values with importance value index (IVI) and relative value of importance (RVI) during summer (April,2015).

S.No	Species	Quantitative attributes				Synthetic attributes				
		Frequency (%)	Abundance (individuals/m^2)	Density (individuals/m^2)	Basal cover (mm^2/m^2)	R.F (%)	R.DE (%)	R.DO (%)	IVI	RVI
	Grasses									
1.	*Andropogon virginicus virginicus*	2	5	0.1	1.01	0.48	0.36	0.25	1.09	0.36
2.	*Apluda mutica*	2	4	0.08	1.1	0.48	0.29	0.27	1.04	0.34
3.	*Brachiaria ramose*	61	6.77	4.13	124.62	14.8	15.01	31.41	61.22	20.40
4.	*Chloris barbata*	55	7.47	4.11	56.91	13.34	14.94	14.34	42.62	14.20
5.	*Cymbopogon caesius*	8	5	0.4	13.67	1.94	1.45	3.44	6.83	2.27
6.	*Cynodon dactylon*	71	8.9	6.32	12.7	17.23	22.98	3.20	43.41	14.47
7.	*Digitaria eriantha*	5	2.4	0.12	0.3	1.21	0.43	0.07	1.71	0.57
8.	*Eragrostis aspera*	6	3.5	0.21	0.65	1.45	0.76	0.16	2.37	0.79

9.	*Heteropogon contortus*	3	2	0.06	0.15	0.72	0.21	0.03	0.96	0.32
10.	*Melinis repens*	2	3	0.06	0.12	0.48	0.21	0.03	0.72	0.24
11.	*Pennisetumalopecuroides*	3	3.33	0.1	0.45	0.72	0.36	0.11	1.19	0.39
12.	*Perotis indica*	2	3.5	0.07	0.37	0.48	0.25	0.09	0.82	0.27
13.	*Setaria pumila*	16	4.68	0.75	3.39	3.88	2.72	0.85	7.43	2.48
14.	*Sporobolus heterolepis*	48	15.68	7.53	19.15	11.65	27.38	4.82	43.85	14.61
	Sedges									
15.	*Cyperus rotundus*	4	3.25	0.13	1.86	0.97	0.07	0.46	1.50	0.5
	Forbs									
16.	*Abutilon indicum*	1	2	0.02	1.63	0.24	-	0.41	0.65	0.21
17.	*Acalypha indica*	2	1	0.02	0.9	0.48	0.07	0.22	0.77	0.25
18.	*Achyranthes aspera*	15	3.2	0.48	12.67	3.64	1.74	3.19	8.57	2.85
19.	*Aerva tomentosa*	2	2.5	0.05	0.75	0.48	0.18	0.18	0.84	0.28
20.	*Alysicarpus monilifer*	10	2.4	0.24	3.98	2.42	0.77	1	4.19	1.39
21.	*Alternanthera pungens*	2	1.5	0.03	0.9	0.48	0.1	0.22	0.80	0.26
22.	*Amaranthus spinosus*	2	2	0.04	1.28	0.48	0.14	0.32	0.94	0.31
23.	*Bidens pilosa*	1	2	0.02	0.76	0.24	0.07	0.19	0.50	0.16
24.	*Boerhaavia erecta*	1	2	0.02	0.49	0.24	0.07	0.12	0.43	0.14
25.	*B. diffusa*	15	2.86	0.43	11.35	1.64	1.56	2.86	6.06	2.02
26.	*Calotropis gigantean*	1	2	0.02	4.01	0.24	0.07	1.01	1.32	0.44
27.	*Clitoria ternatea*	3	3	0.03	0.73	0.72	0.1	0.18	1.0	0.33
28.	*Croton bonplandianum*	1	3	0.03	2.17	0.24	0.1	0.54	0.88	0.29
29.	*Datura metal*	1	2	0.02	4.32	0.24	0.07	1.08	1.39	0.46
30.	*Eupatorium odoratum*	3	2.66	0.08	11.98	0.72	0.29	3.01	4.02	1.34
31.	*Euphorbia hirta*	8	2.62	0.21	0.65	1.94	0.76	0.16	2.86	0.95
32.	*E. microphylla*	3	1	0.03	0.04	0.72	0.1	0.01	0.83	0.27
33.	*Gomphrena decumbens*	4	-	0.07	1.37	0.97	0.25	0.34	1.56	0.52
34.	*Indigofera enneaphylla*	6	1.5	0.09	32.15	1.45	0.32	8.10	9.87	3.29

35.	*Justicia tranquebariensis*	1	2	0.02	0.95	0.24	0.07	0.23	0.54	0.18
36.	*Lantana camara*	1	3	0.03	10.58	0.24	0.1	2.66	3.0	1.0
37.	*Martynia annua*	1	2	0.02	0.81	0.24	0.07	0.20	0.51	0.17
38.	*Meremmia emarginata*	1	3	0.03	1.5	0.24	0.1	0.03	0.37	0.12
39.	*M. tridentate*	1	2	0.02	0.9	0.24	0.07	0.22	0.53	0.17
40.	*Mirabilis jalapa*	1	2	0.02	1.05	0.24	0.07	0.26	0.57	0.19
41.	*Parthenium hysterophorus*	15	3.26	0.49	22.21	3.64	1.78	5.59	11.0	3.66
42.	*Peristrophe bicalyculata*	2	2.5	0.05	1.41	0.48	0.18	0.35	1.01	0.33
43.	*Phyllanthus amarus*	2	3	0.06	0.25	0.48	0.21	0.06	0.75	0.25
44.	*P. maderaspatensis*	1	2	0.02	0.49	0.24	0.07	0.12	0.43	0.14
45.	*Tephrosia purpurea*	2	1.5	0.03	3.73	0.48	0.1	0.94	1.52	0.50
46.	*Trichodesma indicum*	2	4.5	0.09	4.29	0.48	0.32	1.08	1.88	0.62
47.	*Tridax procumbens*	5	4.2	0.21	10.02	1.21	0.76	2.52	4.49	1.49
48.	*Vernonia cinerea*	8	3.75	0.31	9.96	1.94	1.12	2.51	5.57	1.85

R.F: Relative Frequency, R.DE: Relative Density, R. DO: Relative Dominance.

Table 7. Species composition in undisturbed grassland: frequency, abundance, density and basal cover and their relative values with importance value index (IVI) and relative value of importance (RVI) during rainy (July,2015).

S.No	Species	Quantitative attributes				Synthetic attributes				
		Frequency (%)	Abundance (individuals/m^2)	Density (individuals/ m^2)	Basal cover (mm^2/m^2)	R.F (%)	R.DE (%)	R.DO (%)	IVI	RVI
	Grasses									
1.	*Andropogon virginicus virginicus*	6	3.5	0.21	2.13	0.54	0.34	0.23	1.11	0.37
2.	*Apluda mutica*	6	3	0.18	2.49	0.54	0.29	0.27	1.1	0.36
3.	*Brachiariaramose*	100	8.15	8.15	245.92	9.09	13.35	27.42	49.86	16.62
4.	*Chloris barbata*	100	9.65	9.65	133.62	9.09	15.8	14.9	39.79	13.26
5.	*Cymbopogon caesius*	18	4.22	0.76	25.98	1.63	1.24	2.89	5.76	1.92
6.	*Cynodon dactylon*	100	13.32	13.32	26.76	9.09	21.8	2.98	33.87	11.29

7.	*Digitaria eriantha*	11	2.81	0.31	0.78	1	0.5	0.08	1.58	0.52
8.	*Eragrostis aspera*	11	5	0.55	1.72	1	0.9	0.19	2.09	0.69
9.	*Heteropogon contortus*	9	3.55	0.32	0.81	0.81	0.52	0.09	1.42	0.47
10.	*Melinis repens*	3	5.33	0.16	0.32	0.27	0.2	0.03	0.5	0.16
11.	*Pennisetumalopecuroides*	8	3.5	0.28	1.26	0.72	0.45	1.41	2.58	0.86
12.	*Perotis indica*	5	3.8	0.19	1.01	0.45	0.31	0.11	0.87	0.29
13.	*Setaria pumila*	48	2.5	1.2	5.42	4.36	1.96	0.6	6.92	2.30
14.	*Sporobolus heterolepis*	100	15.19	15.19	38.63	9.09	24.88	4.3	38.27	12.75
	Sedges									
15.	*Cyperus rotundus*	18	2.38	0.43	5.95	1.63	0.7	0.66	2.99	0.99
	Forbs									
16.	*Abutilon indicum*	4	1.25	0.05	4.08	0.36	0.08	0.45	0.89	0.29
17.	*Acalypha indica*	9	2.11	0.19	8.61	0.81	0.31	0.96	2.08	0.69
18.	*Achyranthes aspera*	50	1.32	0.66	17.42	4.54	0.08	1.94	6.56	2.18
19.	*Aerva lanata*	6	3	0.18	2.26	0.54	0.29	0.25	1.08	0.36
20.	*A. tomentosa*	3	5	0.15	2.27	0.27	0.24	0.25	0.76	0.25
21.	*Alysicarpus monilifer*	19	3.73	0.71	11.79	1.72	1.16	1.31	4.19	1.39
22.	*Alternanthera pungens*	5	3	0.15	4.52	0.45	0.24	0.5	1.19	0.39
23.	*A. sessilis*	4	2.25	0.09	2.54	0.36	0.14	0.28	0.78	0.26
24.	*Amaranthus spinosus*	16	0.37	0.06	1.92	1.45	0.09	0.21	1.75	0.58
25.	*Bidens pilosa*	2	2.5	0.05	1.92	0.18	0.08	0.21	0.47	0.15
26.	*Boerhaavia erecta*	8	0.37	0.03	0.73	0.72	0.04	0.08	0.84	0.28
27.	*B. diffusa*	63	1.41	0.89	23.5	5.73	1.45	2.62	9.8	3.26
28.	*Calotropis gigantean*	5	1.2	0.06	12.05	0.45	0.09	1.34	1.88	0.62
29.	*Cardiospermum halicacabum*	3	1.33	0.04	1.53	0.27	0.06	0.17	0.5	0.16
30.	*Cleome pentaphylla*	2	2.5	0.05	0.98	0.18	0.08	0.1	0.36	0.12
31.	*Clitoria ternatea*	4	1.25	0.05	1.23	0.36	0.08	0.13	0.57	0.19

32.	*Coccinia indica*	2	1.5	0.03	0.41	0.18	0.04	0.04	0.26	0.08
33.	*Commelina benghalensis*	2	1.5	0.16	8.15	0.18	0.26	0.9	1.34	0.44
34.	*Corchorus tridens*	2	1.5	0.03	0.79	0.18	0.04	0.08	0.3	0.1
35.	*Crotalaria verrucosa*	3	1.33	0.04	3.93	0.27	0.06	0.43	0.76	0.25
36.	*Croton bonplandianu*	9	1.77	0.16	11.57	0.81	0.26	1.29	2.36	0.78
37.	*Datura metal*	3	1.75	0.05	10.81	0.27	0.08	1.2	1.55	0.51
38.	*Erigeron annus*	4	1.62	0.07	3.34	0.36	0.11	0.37	0.84	0.28
39.	*Eupatorium odoratum*	16	1.62	0.26	38.86	1.45	0.42	4.33	6.2	2.06
40.	*Euphorbia hirta*	20	2.6	0.52	1.63	1.81	0.85	0.18	2.84	0.94
41.	*E. microphylla*	9	1.44	0.13	0.2	0.81	0.21	0.02	1.04	0.34
42.	*Evolvulus nummularis*	5	2.6	0.13	0.58	0.45	0.21	0.06	0.72	0.24
43.	*E. alsinoides*	10	2.1	0.21	0.65	0.9	0.34	0.07	1.31	0.43
44.	*Gomphrena decumbens*	11	3.54	0.39	7.65	0.18	0.63	0.85	1.66	0.55
45.	*Heliotrophium indicum*	5	3	0.15	4.82	0.45	0.24	0.53	1.22	0.40
46.	*Hibiscus vitifolius*	2	1	0.02	2.49	0.18	0.03	0.27	0.48	0.16
47.	*Indigofera enneaphylla*	25	2	0.4	12.86	2.27	0.65	1.43	4.35	1.45
48.	*Ipomea dissecta*	3	2	0.06	5.09	0.27	0.09	0.56	0.92	0.30
49.	*I.obscura*	3	1.66	0.05	3.61	0.27	0.08	0.4	0.75	0.25
50.	*Justicia tranquebariensis*	5	1.6	0.08	3.82	0.45	0.13	0.42	1	0.33
51.	*Lantana camara*	4	1	0.04	14.11	0.36	0.06	1.57	1.99	0.66
52.	*Leucas aspera*	6	2.33	0.14	3.44	0.54	0.22	0.38	1.14	0.38
53.	*Macrotyloma uniflorum*	3	2.75	0.05	1.92	0.27	0.08	0.21	0.56	0.18
54.	*Martynia annua*	3	2	0.07	2.84	0.27	0.11	0.31	0.69	0.23
55.	*Meremmia emarginata*	4	2	0.11	5.52	0.36	0.18	0.61	1.15	0.38
56.	*M.tridentate*	3	2	0.06	2.72	0.27	0.09	0.3	0.66	0.22
57.	*Mirabilis jalapa*	2	1.97	0.04	2.11	0.18	0.06	0.23	0.47	0.15
58.	*Oldenlandia umbellata*	2	2	0.04	0.1	0.18	0.06	0.02	0.26	0.08
59.	*Parthenium hysterophorus*	45	1.97	0.89	40.35	4.09	1.45	4.49	10.03	3.34

60.	*Passiflora foetida*	3	1	0.03	0.41	0.27	0.04	0.04	0.35	0.11
61.	*Pavonia indicum*	3	1	0.03	1.15	0.27	0.04	0.12	0.43	0.14
62.	*Peristrophe bicalyculata*	14	1.21	0.17	4.8	1.27	0.27	0.52	2.06	0.68
63.	*Phyllanthus amarus*	5	2	0.1	1.25	0.45	0.16	0.13	0.74	0.24
64.	*P. maderaspatensis*	3	2	0.06	1.47	0.27	0.09	0.16	0.52	0.17
65.	*Ricinus communis*	2	1.5	0.03	1.5	0.18	0.04	0.16	0.38	0.38
66.	*Solanum torvum*	5	1.6	0.08	9.65	0.45	0.13	1.07	1.65	0.55
67.	*Spermacoce hispida*	5	1	0.05	4.41	0.45	0.08	0.49	1.02	0.34
68.	*Tephrosia purpurea*	8	2	0.16	19.94	0.72	0.26	2.22	3.2	1.06
69.	*Trichodesma indicum*	15	1.73	0.26	12.41	1.36	0.42	1.38	3.16	1.05
70.	*Tridax procumbens*	38	1.81	0.69	32.95	3.45	1.13	3.67	8.25	2.75
71.	*Vernonia cinerea*	41	1.43	0.69	22.18	3.73	1.13	2.47	7.33	2.44

R.F: Relative Frequency, R.DE: Relative Density, R. DO: Relative Dominance.

Table 8. Species composition in disturbed grassland: frequency, abundance, density and basal cover and their relative values with importance value index (IVI) and relative value of importance (RVI) during winter (Dec. 2014).

S.No	Species	Quantitative attributes				Synthetic attributes				
		Frequency (%)	Abundance (individuals/m²)	Density (individuals/m²)	Basal cover (mm²/m²)	R.F (%)	R.DE (%)	R.DO (%)	IVI	RVI
	Grasses									
1.	*Andropogon virginicus*	3	2	0.06	0.61	0.71	0.46	0.29	1.46	0.48
2.	*Apluda mutica*	3	1.33	0.04	0.55	0.71	0.3	0.26	1.27	0.42
3.	*Brachiaria ramosa*	65	2.86	1.86	56.12	15.51	14.29	27.23	57.0	19
4.	*Chloris barbata*	58	3.63	2.11	29.21	13.84	16.21	14.17	44.22	14.74
5.	*Cynodon dactylon*	59	5.33	3.15	6.33	14.08	24.21	3.07	41.36	13.78
6.	*Digitaria eriantha*	3	1.66	0.05	0.12	0.71	0.38	0.05	1.14	0.38
7.	*Eragrostis aspera*	6	2.16	0.13	0.4	1.43	0.99	0.19	1.61	0.53
8.	*Heteropogon contortus*	3	1.66	0.05	0.12	0.71	0.38	0.05	1.14	0.38

9.	*Melinis repens*	3	1.66	0.05	0.1	0.71	0.38	0.04	1.13	0.37
10.	*Pennisetum alopecuroides*	4	2	0.08	0.36	0.95	0.61	0.17	1.73	0.57
11.	*Perotis indica*	5	1.4	0.07	0.37	1.19	0.53	0.17	0.89	0.29
12.	*Setaria pumila*	15	2.86	0.43	1.94	3.57	3.3	0.94	7.81	2.60
13.	*Sporobolus heterolepis*	48	3.93	1.89	4.8	11.4	14.52	2.32	18.24	6.08
	Sedges									
14.	*Cyperus rotundus*	4	1.75	0.07	0.96	0.95	0.53	0.29	1.46	0.48
	Forbs									
15.	*Acalypha indica*	1	2	0.02	0.9	0.23	0.15	0.43	0.81	0.27
16.	*Achyranthus aspera*	13	1.53	0.2	5.28	3.1	1.53	2.56	7.19	2.39
17.	*Alysicarpus monilifer*	10	1.5	0.15	2.49	2.38	1.15	1.20	4.73	1.57
18.	*Amaranthus spinosus*	3	1.66	0.05	1.6	0.71	0.38	0.77	1.86	0.62
19.	*Bidens pilosa*	1	1	0.01	3.84	0.73	0.07	1.86	2.66	0.88
20.	*Boerhaavia diffusa*	1	2.27	0.25	6.6	2.62	1.92	3.20	5.74	1.91
21.	*Calotropis gigantea*	-	1	0.01	2	0.73	0.07	0.97	1.77	0.59
22.	*Clitoria ternatea*	1	2	0.02	0.49	0.23	0.15	0.23	0.61	0.20
23.	*Croton bonplandianum*	2	1.5	0.03	2.17	0.47	0.23	1.05	1.75	0.58
24.	*Datura metal*	1	1	0.01	2.16	0.23	0.07	1.04	1.34	0.44
25.	*Erigeron annus*	2	2	0.02	0.95	0.47	0.15	0.46	1.08	0.36
26.	*Eupatorium odoratum*	6	2.16	0.13	19.43	1.43	0.99	9.41	11.83	3.94
27.	*Euphorbia hirta*	9	2.77	0.25	0.78	2.14	1.92	0.37	4.43	1.47
28.	*E. microphylla*	2	1	0.02	0.03	0.47	0.15	0.01	0.63	0.21
29.	*Evolvulus nummularis*	2	1	0.02	0.09	0.47	0.15	0.04	0.66	0.22
30.	*Gomphrena decumbens*	6	1.33	0.08	1.57	1.43	0.61	0.76	2.80	0.93

31.	*Heliotrophium indicum*	2	1.5	0.03	0.96	0.47	0.23	0.47	1.17	0.39
32.	*Indigofera enneaphylla*	5	1.8	0.09	2.89	1.19	0.69	1.40	3.28	1.09
33.	*Justicia tranquebariensis*	2	1.5	0.03	1.43	0.47	0.23	0.69	1.39	0.46
34.	*Lantana camara*	2	1.5	0.03	10.58	0.47	0.23	5.13	5.83	1.94
35.	*Martina annua*	1	2	0.02	0.81	0.23	0.15	0.39	0.77	0.25
36.	*Meremmia emarginata*	1	2	0.02	1	0.23	0.15	0.48	0.86	0.28
37.	*Parthenium hysterophorus*	23	1.43	0.33	14.96	5.48	2.53	7.26	15.27	5.09
38.	*Peristrophe bicalyculata*	2	1.5	0.03	0.84	0.47	0.23	0.40	11.0	3.66
39.	*Solanum torvum*	1	1	0.01	1.2	0.23	0.07	0.58	0.88	0.29
40.	*Trichodesma indicum*	3	1.66	0.05	2.38	0.71	0.38	1.15	2.24	0.74
41.	*Tridax procumbens*	11	1.63	0.81	38.68	2.62	6.22	4.16	13.0	4.33
42.	*Vernonia cinerea*	16	1.56	0.25	8.03	3.81	1.92	3.89	9.62	3.20

R.F: Relative Frequency, R.DE: Relative Density, R.DO: Relative Dominance.

Table 9. Species composition in disturbed grassland: frequency, abundance, density and basal cover and their relative values with importance value index (IVI) and relative value of importance (RVI) during summer (April, 2015).

S.No	Species	Quantitative attributes				Synthetic attributes				
		Frequency (%)	Abundance (individuals/m^2)	Density (individuals/m^2)	Basal cover (mm^2/m^2)	R.F (%)	R.DE (%)	R.DO (%)	IVI	RVI
	Grasses									
1.	*Andropogon virginicus*	3	1.33	0.04	0.4	1.22	0.59	0.36	2.17	0.72
2.	*Apluda mutica*	1	2	0.02	0.27	0.4	0.29	0.22	0.91	0.30
3.	*Brachiariaramose*	49	2	0.98	29.57	20	14.67	27.24	43.91	14.63
4.	*Chloris barbata*	31	3.51	1.09	15.09	12.65	16.31	13.90	42.86	14.28

5.	*Cynodon dactylon*	30	5.5	1.65	3.31	12.24	24.7	3.04	39.98	13.32
6.	*Digitaria eriantha*	2	1.5	0.03	0.07	0.81	0.44	0.06	1.31	0.43
7.	*Eragrostis aspera*	4	2.25	0.09	0.28	1.63	1.34	0.25	3.22	1.07
8.	*Heteropogon contortus*	1	2	0.02	0.05	0.4	0.29	0.04	0.73	0.24
9.	*Melinis repens*	2	1.5	0.03	0.06	0.81	0.44	0.05	1.3	0.43
10.	*Pennisetum alopecuroides*	3	1.66	0.05	0.22	1.22	0.74	0.20	2.16	0.72
11.	*Perotis indica*	2	2	0.04	0.21	0.81	0.59	0.19	1.59	0.53
12.	*Setaria pumila*	8	2.62	0.21	0.94	3.26	3.14	0.86	7.26	2.42
13.	*Sporobolus heterolepis*	31	3.58	1.11	2.82	12.65	16.61	2.59	31.85	10.61
	Sedges									
14.	*Cyperus rotundus*	2	1.5	0.03	0.41	0.81	0.44	0.37	1.62	0.54
	Forbs									
15.	*Acalypha indica*	1	1	0.01	0.45	0.4	0.14	0.41	0.95	0.31
16.	*Achyranthes aspera*	6	2.16	0.13	3.43	2.44	1.94	3.16	7.54	2.51
17.	*Alysicarpus monilifer*	6	1.66	0.1	1.66	2.44	1.49	1.52	5.45	1.81
18.	*Amaranthus spinosus*	2	1.5	0.03	0.96	0.81	0.44	0.88	2.13	0.71
19.	*Bidens pilosa*	1	1	0.01	0.38	0.4	0.14	0.35	0.89	0.29
20.	*Boerhaavia diffusa*	8	1.87	0.15	3.96	3.26	2.24	3.64	9.14	3.04
21.	*Calotropis gigantean*	1	1	0.01	2	0.4	0.14	1.84	2.38	0.79
22.	*Clitoria ternatea*	1	2	0.02	0.49	0.4	0.29	0.45	1.14	0.38
23.	*Croton bonplandianum*	1	1	0.01	0.72	0.4	0.14	0.66	1.2	0.40
24.	*Datura metal*	1	1	0.01	2.16	0.4	0.14	1.99	2.53	0.84
25.	*Eupatorium odoratum*	3	2.33	0.07	10.46	1.22	1.04	9.63	11.89	3.96
26.	*Euphorbia hirta*	5	3.8	0.19	0.59	2.04	2.84	0.54	5.42	1.80
27.	*E. microphylla*	1	2	0.02	0.03	0.4	0.29	0.02	0.71	0.23
28.	*Gomphrena decumbens*	2	1.5	0.03	0.58	0.81	0.44	0.53	1.78	0.59
29.	*Indigofera enneaphylla*	3	1.66	0.05	1.6	1.22	0.74	1.47	3.43	1.14
30.	*Justicia tranquebariensis*	1	1	0.01	0.47	0.4	0.14	0.43	0.97	0.32

31.	*Lantana camara*	2	1	0.02	7.05	0.81	0.29	6.49	7.59	2.53
32.	*Martynia annua*	1	1	0.01	0.4	0.4	0.14	0.36	0.90	0.30
33.	*Meremmia emarginata*	1	1	0.01	0.5	0.4	0.14	0.46	1.0	0.33
34.	*Parthenium histeroporus*	9	1.77	0.16	7.25	3.67	2.39	6.68	12.74	4.24
35.	*Peristrophe bicalyculata*	1	1	0.01	0.28	0.4	0.14	0.25	0.79	0.26
36.	*Trichodesma indicum*	1	2	0.02	0.95	0.4	0.29	0.87	1.56	0.52
37.	*Tridax procumbens*	9	1.22	0.11	5.25	0.67	1.64	4.83	7.14	2.38
38.	*Vernonia cinerea*	9	1.11	0.1	3.21	3.67	1.49	2.95	8.11	2.70

F: Relative Frequency, R.DE: Relative Density, R.DO: Relative Dominance.

Table 10. Species composition in disturbed grassland: frequency, abundance, density and basal cover and their relative values with importance value index (IVI) and relative value of importance (RVI) during rainy (July, 2015).

S.No	Species	Quantitative attributes				Synthetic attributes				
		Frequency (%)	Abundance (individuals/m^2)	Density (individuals/m^2)	Basal cover (mm^2/m^2)	R.F (%)	R.DE (%)	R.DO (%)	IVI	RVI
	Grasses									
1.	*Andropogon virginicus*	8	1.62	0.13	1.32	1.13	0.57	0.32	2.02	0.67
2.	*Apluda mutica*	5	1.6	0.08	1.1	0.71	0.35	0.27	1.33	0.44
3.	*Brachiariaramose*	79	3.98	3.15	95.05	11.22	13.88	23.7	48.8	16.26
4.	*Chloris barbata*	90	4.57	4.12	57.05	12.78	18.16	14.22	45.16	15.05
5.	*Cynodon dactylon*	79	6.55	5.18	10.4	11.22	22.83	2.59	36.64	12.21
6.	*Digitaria eriantha*	5	2.2	0.11	0.27	0.71	0.48	0.06	1.25	0.41
7.	*Eragrostis aspera*	11	2.45	0.27	0.84	1.56	1.19	0.2	2.95	0.98
8.	*Heteropogon contortus*	7	2.71	0.19	0.48	0.99	0.83	0.11	1.93	0.64
9.	*Melinis repens*	6	2.16	0.13	0.26	0.85	0.57	0.06	1.48	0.49
10.	*Pennisetum alopecuroides*	10	1.9	0.19	0.85	1.42	0.83	0.21	2.46	0.82
11.	*Perotis indica*	5	2	0.1	0.53	0.71	0.44	0.13	1.28	0.42
12.	*Setaria pumila*	30	2.96	0.89	4.02	4.26	3.92	1	9.18	3.06

13.	*Sporobolus heterolepis*	69	4.49	3.1	7.88	9.8	13.66	1.96	25.42	8.47
	Sedges									
14.	*Cyperus rotundus*	15	1.73	0.26	3.6	2.13	1.14	0.89	4.16	1.38
	Forbs									
15.	*Acalypha indica*	3	2	0.06	2.72	0.42	0.26	0.67	1.35	0.45
16.	*Achyranthes aspera*	18	1.66	0.3	7.92	2.55	1.32	1.97	5.84	1.94
17.	*Alysicarpus monilifer*	18	1.77	0.32	5.31	2.55	1.41	1.32	5.28	1.76
18.	*Amaranthus spinosus*	4	2.5	0.1	3.21	0.56	0.44	0.8	1.8	0.6
19.	*Bidens pilosa*	1	2	0.02	0.76	0.14	0.08	0.18	0.4	0.13
20.	*Boerhaavia diffusa*	29	1.44	0.42	11.09	4.11	1.85	2.76	8.7	2.9
21.	*Calotropis gigantean*	4	1	0.04	8.03	0.56	0.17	2	2.73	0.91
22.	*Cleome pentaphylla*	1	1	0.01	0.19	0.14	0.04	0.04	0.22	0.07
23.	*Clitoria ternatea*	3	1.66	0.05	1.23	0.42	0.22	0.3	0.94	0.31
24.	*Coccinia indica*	2	1	0.02	0.27	0.28	0.08	0.06	0.42	0.14
25.	*Crotalaria verrucosa*	1	1	0.01	0.98	0.14	0.04	0.24	0.42	0.14
26.	*Croton bonplandianum*	6	1.33	0.08	5.78	0.85	0.35	1.44	2.64	0.88
27.	*Datura metal*	3	1	0.03	6.48	0.42	0.13	1.61	2.16	0.72
28.	*Erigeron annus*	3	1.33	0.04	1.91	0.42	0.17	0.47	1.06	0.35
29.	*Eupatorium odoratum*	13	1.69	0.22	32.88	1.84	0.97	8.19	11	3.66
30.	*Euphorbia hirta*	13	2.76	0.36	1.13	1.84	1.58	0.28	3.7	1.23
31.	*E. microphylla*	4	1.5	0.06	0.09	0.56	0.26	0.02	0.84	0.28
32.	*Evolvulus nummularis*	3	2	0.06	0.27	0.42	0.26	0.06	0.74	0.24
33.	*Gomphrena decumbens*	10	1.5	0.15	2.94	1.42	0.66	0.73	1.42	0.47
34.	*Heliotrophium indicum*	7	1.28	0.09	2.89	0.99	0.39	0.72	2.81	0.93
35.	*Hibiscus vitifolius*	1	1	0.01	1.24	0.14	0.04	0.3	0.48	0.16
36.	*Indigofera enneaphylla*	8	2	0.16	5.14	1.13	0.7	1.28	3.11	1.03
37.	*Ipomea obscura*	2	1	0.02	1.44	0.28	0.08	0.35	0.71	0.23
38.	*Justicia tranquebariensis*	3	1.66	0.05	2.38	0.42	0.22	0.59	1.23	0.41

39.	*Lantana camera*	4	1.25	0.05	17.64	0.56	0.22	4.39	5.17	1.72
40.	*Macrotyloma uniflorum*	1	2	0.02	0.76	0.14	0.08	0.18	0.4	0.13
41.	*Martynia annua*	2	1.5	0.03	1.22	0.28	0.13	0.3	0.71	0.23
42.	*Meremmia emarginata*	3	1.66	0.05	2.51	0.42	0.22	0.62	1.26	0.42
43.	*M.tridentate*	1	2	0.02	0.9	0.19	0.08	0.22	0.05	0.01
44.	*Parthenium hysterophorus*	46	1.73	0.8	36.27	6.53	3.52	9	19.05	6.35
45.	*Pavonia indicum*	1	1	0.01	3.84	0.14	0.04	0.95	1.13	0.37
46.	*Peristrophe bicalyculata*	5	1.4	0.07	1.97	0.71	0.3	0.49	1.5	0.5
47.	*Solanum torvum*	2	1.5	0.03	3.62	0.28	0.13	0.9	1.31	0.43
48.	*Spermacoce hispida*	1	2	0.02	1.76	0.14	0.08	0.43	0.65	0.21
49.	*Trichodesma indicum*	8	2	0.16	7.64	1.13	0.7	1.9	3.73	1.24
50.	*Tridax procumbens*	25	1.52	0.38	18.14	3.55	1.67	4.52	9.74	3.24
51.	*Vernonia cinerea*	26	1.76	0.46	14.79	3.69	2.02	3.68	9.39	3.13

R.F: Relative Frequency, R.DE: Relative Density, R.DO: Relative Dominance.

Table - 11. Similarity index (SI) and frequency index community coefficient (FICC) obtained for the study sites during three different seasons.

S.No.	Season	Attributes	
		SI	FICC
1.	Winter	0.68	95.28
2.	Summer	0.63	96.26
3.	Rainy	0.83	94.30

Table 12. Simpson index of dominance and Shanno-Wiener's index of diversityfor the study sites during three different seasons.

S.No	Study sites	Attributes					
		Dominance index			Shanno-Wiener's index of diversity		
		Winter	Summer	Rainy	Winter	Summer	Rainy
1.	Undisturbed	0.14	0.12	0.13	1.72	1.5	1.44
2.	Disturbed	0.12	0.10	0.09	1.78	1.74	1.7

Table 13. Combined Disturbance Index (out of 30) scored by the study sites.

S. No.	Disturbance factors	Scores in study areas	
		Disturbed	Undisturbed
1.	Scrapping	2	0
2	Manual ploughing	1	0
3.	Litter collection	1	0
4.	Removal of soil	2	0
5.	Fire	2	0
6.	Gardening	1	1
7.	Grazing	3	1
8.	Building construction	3	0
9.	Trampling	3	1
10.	Collection of plants	2	2
	Total Score	20	5

Fig.1. Location of the study sites

Of the various plant species available in the two different study areas, the grass species *Brachiaria ramosa* secured the highest value of IVI of above 49.86 in undisturbed site (Figs. 2-4). The same species also showed its higher ecological importance in undisturbed site by securing higher IVI of more than 43.91 (Figs. 5-7). The other species like *Boerhaavia diffusa, Cynodon dactylon* and *Parthenium hystoporous* were also showing higher IVI in both sites. The species of least significance (lowest IVI) was varied in both sites across the seasons studied (Figs. 8-11). In undisturbed site, the species such as *Ipomea dissecta* and *Phyllanthus amarus, Merremia emarginata* and *Coccinia indica* were present with lowest IVI during winter, summer and rainy season respectively. In disturbed site, the species such as *Clitoria ternatea, Heteropogon contortus* and *Merremia tridenteta* were secured lowest IVI during winter, summer and rainy season respectively. Based on the IVI score made by these species, it is understood that these are poorly established species in the communities of the study sites.

The resource apportionment for the various species present during different seasons is explained by dominance – diversity curves (Figs. 8-10 and 11-13 respectively for undisturbed site and disturbed site). For both sites the geometric curve obtained exhibited that single species dominance was more pronounced during all seasons. The dominant species, *Brachiaria ramosa* received the higher impact of environment and could able to draw more resources from both the study sites.

The similarity index between the two sites studied for the respective seasons is given in Table 11. The species composition was determined to be more highly similar (83%) during rainy season between the two sites. However, during winter and summer also the similarity was above 60 %. This fact showed that both the study areas were originally contained similar species composition. However, due to disturbance factor, the composition of the species has been changed.

Many constituent species in both study sites have established very poorly, based on their distribution level, density and the ecological importance (Tables 5-10). The grass species such as *Melines repens, Perotis indica, Andropogon virgincus and Apluda mutica* and the forbs such as *Coccinia indica, Oldenlandia umbellata, Passiflora foetida* and *Cleome pentaphylla* have scored very lower values of frequency, density, basal cover and importance value index in the undisturbed site. Therefore, these species are requiring special care to increase their population in undisturbed study site. Similarly, in disturbed study site also, many species were present with poor perpetuation. The grass species such as *Apluda mutica, Digera arvensis, Melines repens* and *Perotis indica* and the forbs like *Cleome pentaphylla, Hibiscus vitifolius, Merremia tridentate* and *Spermacoce hispida* have occurred with least ecological perpetuation, as they obtained very lower values for quantitative ecological characters. Therefore these species also need special care for protection.

On basis of frequency index community co-efficient (FICC), it is known that both the study sites were homogenous to each other (> 94 % FICC) at all

seasons (Table 12). The dominance index obtained in both sites is less than 0.14. So both sites are not dominated by any single species on basis of number of individuals contributed (Table 12). The Shannon-Wiener's index of diversity obtained for the two study areas is given in Table 12. As the index value was around 1.5 in both study areas during all the three studied seasons, the diversity of species was not note worthy. This score showed that both the study sites were not having contusive environment for the development of saturated community.

The combined disturbance index (CDI) scored by the two sites is presented in the Table 13. It exhibited that the disturbed site scored 20 CDI whereas, undisturbed site scored only 5 CDI. It is known from this fact that the disturbed site studied was highly influenced by many external factors including anthropogenic disturbances due to which many of the species lost in the disturbed site.

4. DISCUSSION

The effect of disturbance on the changes in species composition and certain quantitative ecological attributes of the constituent species in *Brachiaria ramosa* dominated grassland were studied for a period of one year. The temperature data shows that there are no well marked seasons in the study area. The differences between summer and winter minimum temperatures were ranging between 4 and 5°C only. The maximum of that was varying from 6 to 8°C only. It is of common fact that in tropical climatic zone, the seasons are not marked well. The rainfall and humidity data exhibited that the study area is having semi–arid habitats which are mainly constituted by mesophytes and zerophytes.

The development of vegetation in terms of the number of individuals in the study area is directly proportional to rainfall. The grasslands of the present study area are having abundant number of individuals of various plant species during south-west monsoon and north-east monsoon months (July – August and October – December respectively). It indicates that the rainfall is the limiting factor mainly influenced in the community development. It is well known that in tropical climatic areas water is the limiting factor as the other climatic factors like intensity of light, photoperiod, humidity, temperature, etc are available enormously, except the rainfall which is occurring during seasons only.

Among the 71 species available altogether in both study sites, a higher number of 66 species (92%) possessed medicinal uses. It indicates the potentiality of study area for the inhabitation of medicinal plants. It may be explained that the semi-arid condition with water stress during most of the months in a year can induce the plant species to produce secondary metabolites as defence mechanism against water stress (Frank *et al.*, 2012; Elisa *et al.,* 2013). The uses of species for diverse medicinal purposes show the production of different kinds of secondary metabolites with rich varieties of bioactive compounds in the study sites.

Phytosociological analysis of a plant community is the first and foremost basis of the study of any piece of vegetation as it is a pre-requisite for the understanding of community structure and organization. The appearance of a plant community is largely dependent upon the life-form of the dominant plants, classification based on the criteria given by Raunkiar (1934) who gave different terms to designate different types of life-forms. In the present study, the life-form classes indicate a chamaephytic flora. This supports the view that the herbaceous flora mainly composed by annuals and fresh vegetative growth takes place every rainy season (Ambasht, 1987). But the usefulness of the Raunkiaer's biological spectrum as indicative of climatic condition is limited as the other ecological factors such as biotic disturbances are not taken into account. The present study area also met with certain biotic disturbances like mild grazing and construction attempts.

For understanding the community structure and organization, species composition is foremost requisite. Species composition is one of the major characters of plant community (Dansereau, 1960). It is evident from the data that the study area comprised a considerable number of herbs among them, forbs contributed more species than grasses. However, the number of individuals contributed by the grass was considerably higher than the forbs. This may be attributed to the presence of wide ecological amplitude in grasses (Misra, 1980; Manorama, 1996).

Of the 71 and 51 species present in the undisturbed and disturbed study sites respectively, the grasses like *Brachiaria ramosa, Cynodon dactylon, Chloris barbata* and *Sporobolus heterolepis* and the forbs like *Boerhaavia diffusa, Achyranthes aspera, Parthenium hysterophorus* and *Alysicarpus monilifer* in undisturbed site and in disturbed site in addition to these forbs, *Euphorbia hirta* and *Tridax procumbens* were distributed evenly by securing more than 70% frequency value. According to Misra (1980) this may be attributed to their high reproductive capacity, quick dispersal of seeds and wind pollination to produce viable seeds. Due to the

lacking of these attributes, the other constituent species may show poor distribution.

In addition to higher distribution, these grasses and forbs in the two study sites were present with higher density and basal cover also. Shantz (1954) opined that the presence of tolerance to poor conditions, adaptability and suit various ecological niches for certain grass species could be the possible reasons for the successful establishment in the grasslands as the dominant and important grasses.

In both study sites many species registered lower values of frequency, density and basal cover and hence the importance value index. They are the grass species such as *Melinis repens, Perotis indica, Andropogon virginicus, Apluda mutica* and the forbs such as *Coccinia indica, Oldenlandia umbellata, Pasiflora foetida* and *Cleome pentaphylla.* The poor distribution mechanism, less seed output and lower viability of seeds may be the factor responsible for the weaker establishment of the above mentioned species in the studied sites (Paulsamy, *et al.,* 2008).

It was further observed that a sizable number of 20 species such as *Abutilon indicum, Aerva lanata, A. tomentosa, Alternanthera pungens, A. sessilis, Boerhaavia erecta, Cardiospermum halicacabum, Commelina benghalensis, Corchorus tridens, Euphorbia hirta, Evolvulus alsinoides, Ipomea dissecta, Leucas aspera, Mirabilis jalapa, Oldenlandia umbellata, Pasiflora foetida, Phyllanthus amarus, P. maderaspatensis* and *Plumbago zeylanica* have disappeared in distributed site. This may be due to poor adaptability of these species against the disturbance caused in the disturbed site. Gunaga *et al.* (2013) also observed the same trend of disappearance of many species in disturbed site as their adaptive variations are determined to be not enough for survival.

Despite the dominance exerted by the grass species, *Brachiaria ramosa,* the dominance index obtained in both sites showed that there was no single species dominance in the study area. This contradiction may be due to the number of individuals contributed by all the remaining species altogether was greater than the total number of individuals contributed by the single species, *Brachiaria ramosa.* However, the resource apportionment by the individual species indicates (Figs. 8-13) that the communities were dominated by single species. Therefore, the functional behaviour of individual species in terms of community metabolism was playing major role in deciding the species importance in the present study areas rather than the numerical strength contributed to communities. This fact is at par with the generalizations made for community metabolism and stability of ecosystem in majority of natural communities at global level (McNaughton, 1985).

The combined disturbance index scored by disturbed site (Table 13) indicates that this site was severely influenced by anthropogenic disturbances which resulted in the drastic changes in species composition and community organization. It is quite clear that greater protection leads to better regeneration of community. From the commercial point of view, these studied grasslands are valuable as they contain many medicinal and other economically important plants. Repeated annual fires, continued grazing, scraping, collection of medicinal plants and other anthropogenic disturbances resulted in the low regeneration as well as low density of the vegetation. Varghese and Menon (1998), Hedge *et al*. (2005), Gunaga *et al*. (2013) have also reported the effect of human disturbance on the community composition and possible management practices to be followed for the effective regeneration of the vegetation.

5. CONCLUSION

In conclusion, it is suggested that the studied *Brachiaria ramosa* dominated grassland near Bharathiar University must be given conservation priority to protect the valuable medicinal species. Despite the seasonal changes, the anthropogenic disturbances were determined to be most influencing factor to affect the species composition and the quantitative ecological attributes of many sensitive species. Therefore, construction activities, over grazing by domestic animals, fire, scraping, collection of medicinal plants etc must be checked so as to protect the species in their habitats. Further, ecosystem - specific management plans must be developed to protect the individual species and the grassland community as well. Protection of such natural grassland will also aid in the regulation of ecological process like energy flow, food chain and food web and cycling of materials which would results in ecological balance and stability of ecosystem.

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SCREENING OF ANTIMICROBIAL ACTIVITY IN *PENTATROPIS MICROPHYLLA* (ROTH) WIGHT (APOCYNACEAE)

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ABSTRACT

The stem bark extracts of the medicinal plant species, *Pentatropis microphylla* Roth Wight by using three alcoholic solvents *viz;* petroleum ether, ethyl acetate and methanol were tested against four human pathogenic bacteria *viz*., *Bacillus subtilis*, *B. thuriengensis, Klebsiella pneumoniae* and *Escherichia coli* and four human pathogenic fungi *viz., Aspergillus niger, A. flavus, A.baumannii* and *Fusarium oxysporum* for assessing the antimicrobial properties by adapting disc diffusion method. The results of the study revealed that all extracts showed varied degree of antimicrobial activity against the tested pathogens. However, the ethyl acetate extracts exhibited higher inhibition zone (17.23 mm) against the bacterium, *Klebsiella pneumoniae* and the fungus, *Aspergillus niger* (19.63 mm). Therefore the result strengthens the existing traditional usage of the plant for the therapeutic use.

Keywords: Medicinal plant, *Pentatropis microphylla*, Disc diffusion, Microorganisms.

1. INTRODUCTION

In Ayurvedic Medicine, there are numerous herbs which have been used historically for treating a large variety of ailments. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Suparna *et al*., 2014). Spices have been used for not only flavor and aroma of the foods but also to provide antimicrobial properties (Nanasombat *et al.,* 2002). Spices may contribute piquancy of foods and beverages (Praveen *et al.,* 2006). In addition to these spices are some of the most commonly used natural antimicrobial agents in foods. Some of the natural compounds found in various spices possess antimicrobial compounds. *Pentatropis microphylla* belongs to the family, Apocynaceae is one such medicinal plant used in traditional system of medicine in Anthiyur forests of Erode district of Tamil Nadu, India. It is a climber, mostly distributed in undisturbed deciduous forest margins of peninsular India, Srilanka and Pakistan (Gibbs *et al.*, 1987). The plant has the properties of antifungal, antiseptic and keratolytic. It is used in the treatment of skin problems also. The plant contains octacosanol, alpha-amyrin, friedelin and beta-sitosterol and an appreciable amount of salicyclic acid has also been isolated from this plant. It also reported to have a cardiac glycoside (Fatope, 1995). The plant is mentioned as cooling and alternative properties and useful in gonorrhea. It is used in constipation, colic and diarrhoea. It is used traditionally in acidity and fever. However, no published works are available for the antimicrobial property of stem bark of this plant. Hence in the present study, an attempt has been made to focus the plant in this angle and hence to assess its therapeutic potency.

2. MATERIALS AND METHODS

2.1. Plant material

Fresh stem barks were collected from the population of *P. microphylla* present in the Anthiyur forests of Erode district and washed under running tap water, air dried and then homogenized to fine powder and stored in air tight bottles.

2.2. Preparation of extracts

250g air-dried stem bark powder was subjected to 250ml of methanol in soxhlet extraction for 8 hours (50-85˚C). The extracts were concentrated to dryness in a flask evaporator under reduced pressure and controlled temperature (50-60˚C) to yield crude residue, which was then stored in refrigerator. To obtain petroleum ether and ethyl acetate extracts, the same method as used to obtain methanol extract was adopted.

2.3. Media used

Freshly prepared nutrient agar medium and PDA medium were used for the culture of bacteria and fungi respectively.

2.4. Microorganisms

In vitro antimicrobial activity was examined for the chemical extracts of stem bark of the study plant, against four bacterial species which include the gram positive strains *viz*., *Bacillus subtilis*, *B. thuriengensis* and gram negative strains *viz., Klebsiella pneumonia* and *Escherichia coli* and four fungal species *viz., Aspergillus niger, A. flavus, A. baumannii*, *Fusarium oxysporum.* All these microorganisms were obtained from the Department of Microbiology, Hindustan College of Arts and Science, Coimbatore. All the microorganisms were maintained at 4˚C on nutrient agar slants (for bacteria) and PDA slants (for fungi) until further use.

2.5. Antimicrobial assay

The alcoholic extracts were tested for their effect against the growth of pathogenic bacteria and fungi by disc diffusion method (Bauer *et al*., 1966). Both the organisms, bacteria and fungi tested were inoculated into nutrient agar and PDA media respectively. After an incubation period of 24 hrs at a temperature of 35˚C, three or four colonies isolated from these media were inoculated into 4ml of nutrient broth and incubated for 2 hrs at 35˚ C. The cultures were adjusted with sterile saline solution to obtain turbidity. Petri dishes containing Muller-Hinton agar medium and PDA medium were streaked with these microbial suspensions of bacteria and fungi respectively. Disks of 6mm diameter were impregnated with the extracts of petroleum ether, methanol and ethyl acetate separately. Tetracycline is used as positive control. After equilibrium at 4˚C, the plates were incubated overnight at 37˚C and the diameter of any resulting zones of inhibition was measured. Each experiment was repeated at least three times.

3. RESULTS AND DISCUSSION

The antibacterial activity of the all the alcoholic stem bark extracts of the study species, *Pentatropis microphylla* generally showed inhibitory activity against the growth of *Bacillus subtilis, B. thuringiensis* and *Klebsiella pneumoniae*. However, towards *Escherichia coli,* all these extracts showed activity with less pronounced manner (Table 1). Further, the ethyl acetate extract has determined to have highest inhibitory activity (17.23 mm diameter inhibitory zone) against the bacterium, *Klebsiella pneumoniae* (gram negative) and followed by the methanol extract showed the higher inhibitory activity against the bacterium, *Escherichia coli* (14.17mm diameter inhibitory zone). It indicates the presence of effective active principle compounds in the ethyl acetate and methanol extracts of stem part of *Pentatropis microphylla* to suppress bacterial activity. It has been observed further that the ethyl acetate extracts showed significantly higher inhibitory activity against the colonial growth of *Klebsiella pneumoniae* than that of the commercially available antibiotic, tetracycline. This fact shows the higher therapeutic potential of ethyl acetate extract of the study species. The petroleum ether extract has comparatively less activity against the tested pathogens. It may be attributed to the presence of respective active compounds with insufficient quantities in this crude extract.

Table 1. Antibacterial activity of certain alcoholic stem bark extracts of the species, *Pentatropis microphylla*.

Plant extract	Diameter of zone inhibition (mm)			
	Gram positive bacteria		Gram negative bacteria	
	Bacillus subtilis	***B. thuringiensis***	***Klebsiella pneumoniae***	***Escherichia coli***
Standard*	20.77± 0.20	18.06 ± 0.56	11.33 ± 0.42	16.12 ± 0.61
Petroleum ether	11.87 ± 0.15	10.16 ± 0.57	9.16 ± 0.37	-
Ethyl acetate	12.13 ± 0.61	11.03 ± 0.61	17.23 ± 0.58	14.16 ± 0.66
Methanol	11.16 ±0.47	14.17 ± 0.60	13.73 ± 0.75	8.06 ± 0.30

* Tetracycline

Table 2. Antifungal activity of certain alcoholic stem bark extracts of the species, *Pentatropis microphylla*.

Plant extract	Diameter of zone inhibition (mm)			
	Aspergillus niger	***A. flavus***	***A. baumanii***	***Fusarium oxysporum***
Standard*	24.67 ± 0.48	23.17 ± 0.67	26.73 ± 0.67	30.73 ±0.67
Petroleum ether	-	10.24 ± 0.78	12.73 ± 0.66	-
Ethyl acetate	19.63 ± 0.53	18.77 ± 0.71	19.45 ± 0.71	15.67 ±0.59
Methanol	10.73 ± 0.54	11.73 ± 0.70	14.63 ± 0.65	12.17 ± 0.38

* Tetracycline

The antifungal activity of various alcoholic stem bark extracts of the study species, *Pentatropis microphylla* against the four studied fungal species is given in Table 2. The results of the study report that the ethyl acetate extract has the highest inhibitory activity (19.63 mm diameter inhibitory zone) against the fungus, *Aspergillus niger*. The methanol extract showed considerable effect to all the tested fungal pathogens. The petroleum ether extract was also found to be better with respect to inhibitory function only against the two fungal species, *Aspergillus flavus, A. baumannii*. Significantly higher inhibitory activity of ethyl acetate extract is nearly to the commercially available antibiotic tetracycline against the fungus, *Aspergillus niger* observed shows the superior healingness of stem bark of *P. microphylla.*

The results of present investigation clearly indicated the antibacterial and antifungal efficacy of the study species *Pentatropis microphylla* stem bark extracts. This activities may probably due to the presence of bioactive compounds like flavonoids (Tsuchiya *et al*., 1996), phenolics and polyphenolics (Mason and Wasserman, 1987), tannins (Ya *et al*., 1988), terpenoids (Scortichini and Pia Rossi, 1991), sesquiterpenes and glycosides (Varshney *et al*., 1996) are effective antimicrobial substances against a wide range of microorganisms.

This fact indicats the existence of strong antifungal activity of leaf part of the study species, *A. caesia* and hence its effective healing property against the infectious diseases. Proper isolation and purification of active compounds by using ethyl acetate solvent would ensure the therapeutic value of this medicinal plant when it will be used commercially.

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CONSERVATION OF RARE MEDICINAL PLANT BY RAPID CALLUS INDUCTION FROM LEAF, NODE AND INTERMODAL EXPALNTS OF *THALICTRUM JAVANICUM* BLUME.

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ABSTRACT

The callus culture of *Thalictrum javanicum* Blume was generated from leaf, node and internodal explants. Different growth regulators are greatly influenced in the growth of callus cultures. The callus was obtained from leaf, node and internodal explants by inoculating them on MS medium supplemented with various concentrations and combinations of growth regulators *viz.*, auxins (IAA, NAA), and cytokinin (BAP) were used. The supplementation of medium with BAP (3.8) and IAA (0.1) was suitable for the efficient callus induction when compared with others.

Keywords: Auxins, cytokinin, callus.

1. INTRODUCTION

The species which are failed in successful propagation through seeds or lacking vegetative reproduction can be multiplied through *in vitro* regeneration by employing tissue culture techniques. In natural communities, the species present with very lower population size may be due to their failure or less efficient in sexual or vegetative reproductions. From the early studies it has been known that such species of lower reproductive efficiency by employing tissue culture technology have been successfully increased their population (Thambiraj and Paulsamy, 2012; Padhmavathy *et al.,* 2013; Prema *et al.,* 2013; Vinitha *et al.,* 2013; Jamuna and Paulsamy, 2014). The study species, *Thalictrum javanicum* Blume is a medium sized erect herb, found in the temperate Himalayas from Kasmir to Sikkim in Khasi hills, and Kodaikanal and Nilgiri hills of Western Ghats in Tamil Nadu, India. At global level, it is generally distributed in the hilly tracts of India, Srilanka, China and Java at the altitude of around 2400 m above msl. The entire plant is said to be medicinally important by having antimicrobial property (Tayung and Kar, 2005; Aravindkumar, 2007; Abinaya *et al.,* 2013a), antioxidant property (Abinaya *et al.,* 2013b, 2014a) and antiinflammatory property (Abinaya *et al.,* 2014b). The roots are diuretic, purgative and tonic (The Wealth of India, 1976; Sharma, 2009). Root part is also reported to have dye. The paste form of root is used for the treatment of haematuria (Tiwari and Pande, 2006). Leaves are germicidial, used in skin disorders and sores. Leaf paste and its decoction can be applied on sores and other skin diseases. Root febrifuge is also used for the treatment of eye troubles. In veterinary medicine, leaves crushed with mustard oil which applied on the bodies of animals as a germicidal (Umberto Quattrocchi, 2012). Owing to these medicinal uses, the belowground and the aboveground parts are being exploited severely in the Nilgiris (Paulsamy, 2007). In the present investigation, the study species, *Thalictrum javanicum*, a traditional medicinal plant species of lower population size was attempted for *in vitro* regeneration by using the explants such as leaf, stem and internode.

2. MATERIALS AND METHODS

2.1. Preparation of plant material

Healthy plants of the study species, *T. javanicum* were collected from the forest margins at high hills of Nilgiris, the Western Ghats, Tamil Nadu, India. The plants were maintained under greenhouse condition in the Kongunadu Arts and Science College campus. The leaf explants were excised into 1 cm long segments and were washed with liquid detergent (5% Teepol, Qualigens, India) followed by Bavistin (1% w/v) for 3 min and then mercuric chloride (0.1% w/v) for 1 min. Finally the explants were sterilized with 70% ethanol followed by three times with sterile distilled water and the explants were aseptically inoculated on Murashige and Skoog medium supplemented with various concentrations and combinations of phytohormones for induction of callus.

2.2. Callus culture

The explants were cultured on MS (Murashige and Skoog) basal medium supplemented with different concentrations of auxins alone and in combinations with cytokinins. Considering the quantity and quality of callus and percentage of response, best explants were selected. Further callus studies were confirmed with that explants only. All

the cultures were incubated at 24±2° C under 16 h photoperiod provided by cool white florescent lights. Leaf explants were excised aseptically and cultured on MS medium supplemented with different concentrations of auxins alone and in combinations with cytokinins. Considering the quantity and quality of callus and percentage of response, best explants were selected. Further callus studies were confirmed with that explants only.

2.3. Data analysis

All the experiments were repeated thrice with 15 replicates. The effect of different treatments was analyzed using one way analysis of variance (ANOVA), and means were compared using the Tukey test at the 0.05 level of significance.

3. RESULTS AND DISCUSSION

3.1. Callus Culture studies

Callus is a dedifferentiated and unorganized mass of parenchyma cells formed by the proliferation of parent tissue. It is a good source of genetic variability and adventitious shoot formation. The number of days required for callus induction after the inoculation of explants was varied between 13 and 29 for the study species, *T. javanicum.* All the explants needed a longer period of around 29 days for the effective initiation of callus from the three explants used *viz*., leaf, node and internode.

Table 1. Effect of different concentrations of growth regulators (BAP, NAA and IAA) on per cent callus formation from leaf, node and internode explants of the species, *Thalictrum javanicum.*

Growth regulators (mg/mL)			Days required for callus formation after inoculation			Callus formation (%)		
BAP	NAA	IAA	Explants			Explants		
			Leaf	Node	Internode	Leaf	Node	Internode
0.5	0.1	0.0	24	26	21	23.02±0.02^{ab}	34.12±0.12^{c}	56.25±0.57^{c}
0.8	0.1	0.0	22	15	24	22.01±0.02^{ab}	23.02±0.55^{b}	58.51±0.01^{c}
1.5	0.1	0.0	25	22	25	21.05±0.01^{b}	59.31±0.15^{d}	63.21±0.50c^{d}
2.0	0.1	0.0	26	23	22	26.04±0.11^{b}	62.14±0.56^{e}	67.01±0.61^{d}
2.5	0.5	0.0	27	21	24	11.04±0.01^{a}	39.01±0.47^{cd}	78.05±0.14^{e}
3.0	0.7	00	21	23	26	26.01±0.03^{b}	26.02±0.59^{bc}	76.58±0.41^{e}
3.8	0.8	0.0	19	22	27	39.36±0.12^{c}	27.22±0.75^{bc}	80.44±0.31^{f}
4.5	1.0	0.0	18	21	24	25.55±0.51^{b}	37.85±0.62^{c}	82.05±0.70^{f}
4.5	1.5	0.0	21	22	23	11.50±0.56^{a}	61.11±0.03^{e}	52.25±0.23^{c}
4.7	1.0	0.0	23	25	29	17.60±0.46^{a}	75.32±0.56^{f}	67.35±0.15^{d}
4.9	1.0	0.0	22	25	13	19.40±0.61^{a}	65.07±0.01^{e}	54..20±0.01^{c}
4.9	1.5	0.0	21	24	14	35.02±0.26^{c}	58.56±0.11^{d}	51.61±0.11^{c}
5.0	1.0	0.0	23	19	25	69.90±0.21^{e}	78.96±0.37^{f}	81.03±0.58^{f}
5.2	1.0	0.0	27	25	21	55.01±0.42^{d}	31.22±0.65^{c}	56.89±0.38^{c}
5.8	1.0	0.0	26	25	26	25.58±0.35^{b}	25.36±0.01^{b}	45.25±0.67^{b}
6.0	1.0	0.0	22	23	21	23.47±0.36^{b}	31.65±0.52^{c}	49.21±0.57^{b}
3.8	0.0	0.1	25	26	25	-	30.45±0.31^{c}	89.99±0.61^{f}
4.0	0.0	0.1	25	29	23	-	14.26±0.57^{a}	25.02±0.64^{a}
4.5	0.0	0.5	24	29	24	21.36±0.29^{b}	18.28±0.61^{ab}	53.85±0.41^{c}
4.8	0.0	0.5	26	25	22	20.16±0.19^{b}	15.08±0.21^{a}	51.25±0.21^{c}

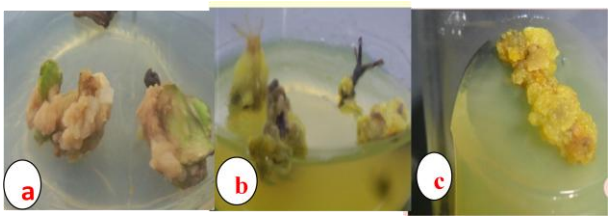

Fig. 1. Proliferation of callus in leaf (a), node(b) and intermodal(c) explants.

A minimum period of 18, 15 and 13 days for leaf, node and internode explants respectively was observed to be enough to produce callus. However, it was noticed that the internode explants responded well for callus formation when cultured onto the MS medium supplemented with the growth regulators, BAP and IAA. A higher level of 89.99 % of internode responded effectively for callus formation in the MS medium containing the cytokinin (BAP - 3.8 mg/L) and the auxin (IAA – 0.1 mg/L) and followed by nodal explants (78.96 %) and leaf explants (69.90 %) in the MS medium containing the cytokinin (BAP - 5 mg/L) and auxin (NAA - 1mg/L) (Table 1, Fig. 1a, b and c).

The Ranunculaceae member, *Thalictrum javanicum* required a longer period of 29 days for effective callus initiation (Table 1). It is explained that the nature of tissues, the degree of totipotency and composition of medium with respect to micronutrients and hormones might be the possible reasons for this fact (Baskaran and Jayabalan, 2005). Rosy and Rosakutty (2011) also reported that higher amount of callus formation was obtained in *Pterospermum reticulatum* after one month from the date of inoculation. Can *et al*. (2008) pointed out that several factors including the culture environments and hormonal and non hormonal regulators act synergistically in determining the proper induction, proliferation of calli and regeneration into plants.

4. CONCLUSION

Callus culture system offer many advantages as a model system for several biological investigations. Here, in the present investigation an efficient protocol has been devised for *in vitro* callus induction of an important medicinal plant, *Thalictrum javanicum* from leaf, node and internodal explants. Callus culture system offer many advantages as a model system for several biological investigations.

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A NEW SPECIES OF *CEROPEGIA* L. (APOCYNACEAE) FROM SOUTHERN WESTERN GHATS OF INDIA

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ABSTRACT

Ceropegia paulsamii Karuppusamy et Ravichandran sp. nov. (Apocynaceae) is described and illustrated from Megamalai wildlife sanctuary in southern Western Ghats of Tamilnadu state, India. It is similar to *Ceropegia decaisneana* Wight but differs in sub-succulent fasciculate roots, flowers with short corolla lobes with middle constriction, outer corona trilobed, whitish, and basally caudate each coronal segment.

Keywords: *Ceropegia paulsamii,* Apocynaceae, western ghats, Megamalai wildlife sanctuary.

1. INTRODUCTION

Ceropegia L. (Apocynaceae, subfamily Asclepiadoideae) is a large, old world genus of about 200 species distributed largely in Africa, South Asia to part of Australia (Bruyns, 2003). In India, about 51 species have been described morphological basis and most of them are endemic to Western Ghats (Jagtap and Singh, 1999; Karthikeyan *et al*., 2009). Northern Western Ghats of Maharashtra region was well explored for genus *Ceropegia* by Prof. S.R. Yadav and his team. They have recognized many new species including *C. anantii* Yadav *et al*., *C. anjanerica* Malpure *et al*., *C. bhatti* Yadav *et al*. and *C. mohanramii* Yadav *et al*. in last decade. Southern Western Ghats are not well explored for this genus since many narrow endemic species are confined only hill peaks and grasslands in high altitudes. Bruyns (1997) have rightly noted that several non-succulent species without tubers from Western Ghats seem to be both vegetatively and florally quite similar to species from West Africa and even to *C. cumingiana* from Australia. Some species are possessing peculiar floral structure and unusual coronal organization. These are possibly modern representatives of the most primitive form in the genus.

During a floristic survey in Theni district of Tamilnadu state, the authors collected a few interesting specimens of *Ceropegia* from Megamalai wildlife sanctuary of southern Western Ghats, that did not match with any other known *Ceropegia* species. After critical examination of these specimens, relevant literature search and consultation with Meve Ulrich, Dept. of Plant Systematics, Univ. of Bayreuth, Germany, it turned out to be a new species which is described and illustrated here.

1.1. Ceropegia paulsamii Karuppusamy et Ravichandran Sp. nov. (Fig. 1-2)

Ceropegia decaisneana Wight similis, rootis sub-succulentis fasciculatis, foliis ovato-oblongo-ellipticis nervis lateralibus 4-5 obliquis, calyxi lobi glabri, corolli lobi brevis 1.2 cm (nec 4 cm) longis, corona exteriori trilobatis (nec bilobatis), albis, segmentis basis caudatis differt.

Type: India, Tamilnadu, Theni district, Megamalai wildlife sanctuary, ca 1300 m m.s.l. 18-12-2010, S. Karuppusamy and V. Ravichandran 1217 (holotype and isotypes: MH; paratypes: S. Karuppusamy and V. Ravichandran 1521, 1568, Herbarium of Department of Botany, The Madura College, Madurai, Tamilnadu)

1.2. Etymology

The new species named in the honor of Dr. S. Paulsamy, professor in department of Botany, Kongunadu Arts and Scinece College, Coimbatore for his valuable contribution in plant ecology and conservation in Nilgiri Biosphere Reserve for more than 25 years.

1.3. Description

Twining perennial herb. Root stock sub-succulent, fasciculate, fibrous, ca. 5-8 cm long, 0.3 mm diameter, brownish, minutely hairy. Stem twining, ca 4 m long, branched, glabrous. Leaves opposite, shortly petiolate, ca 1–1.5 cm long; lamina ovate-elliptic or oblong elliptic, 5 x 10- 2-4 cm, glabrous above, faint below, lateral nerves 4-5, oblique, margin entire, acuminate at apex. Inflorescence axillary, 3-5-flowered cymes, with peduncle ca 1-1.5 cm long, glabrous. Flowers bracteates; pedicel 0.8-1.2 cm long; bracts lanceolate, minute, ca 0.1 mm long, acute, glabrous. Calyx lobes 5, linear, 8-10 x 1.5 mm, acuminate, glabrous.

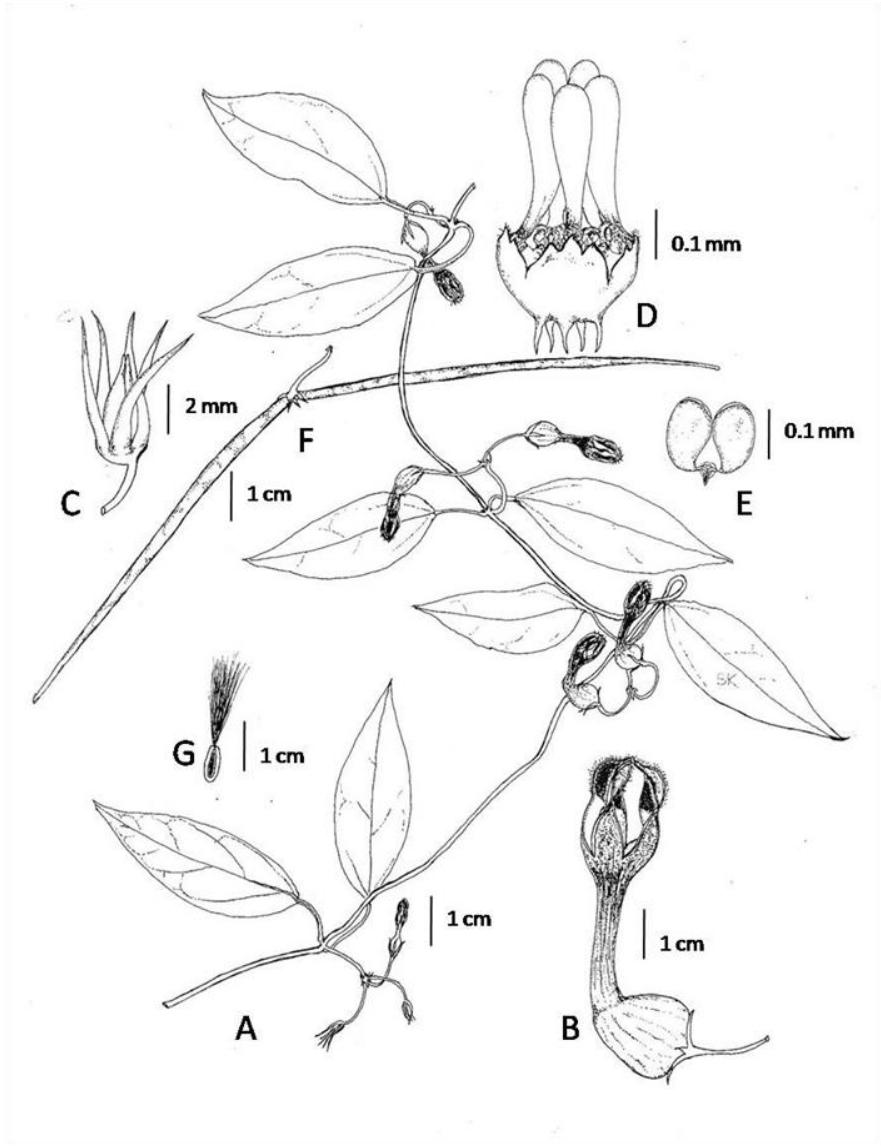

Fig. 1. *Ceropegia paulsamii* sp. nov. (A) twig, (B) flower, (C) calyx with young fruit, (D) corona lateral view, (E) pollinarium, (F) fruit, (G) seed.

Fig. 2. *Ceropegia paulsamii* sp. nov. (a) habit, (b) subsucculent fasciculate root, (c) flower side view, (d) Flower longitudinal sectional view, (e) calyx with young fruit, (f) corona side view with inner side of corolla tube, (g) outer corona and staminal column top view, (h) mature fruit.

Corolla tubular, 3.5-4.5 cm long, curved; base of tube inflated, globose, 1.2-1.9 x 1.0-1.2 cm, whitish with purple striations outside, deep purplish inside; corolla tube narrow cylindrical, slightly widened above, 1.5-2.2 cm long, 0.5 cm wide at base, 1.2 cm wide at mouth, mouth and tube molted dark purple above, deeply purplish inside, glabrous both sides; corolla lobes short, 0.8-1.2 cm, elliptic-lanceolate, base broad, apex acute, folded back along the midrib, erect, margins hairy, constricted at middle, lobes joint to form a case at the apex, inside dark purple background. Corona sessile, creamy whitish, cupular, 0.4 cm diameter, outer corona trilobed, larger middle lobe and smaller side lobes, lobes triangular, margins hairy, each coronal segments basally caudate; inner corona linear, subulate, erect, 0.8-1.2 cm long, whitish, glabrous. Staminal column yellowish, 0.7 mm wide; stigma 0.2 mm wide; pollinarium 0.2 x 0.3 mm, pollen sacs erect with short caudicle. Pollen key shortly winged; corpuscle brown, rounded at apex. Follicle divergent, 7-15 x 0.3 cm, glabrous, terete, obtuse at apex. Seeds ovoid, flattened, 4 x 2 mm, dark brown, glabrous; coma 2.5 cm long, silvery white. Flowering and Fruiting: Throughout the year peak in September to January.

1.4. Ecology and Distribution

Ceropegia paulsamii Karupp. et Ravich. was found growing among bushes of shola borders at an altitude of ±1400 m m.s.l. 9˚36.430, N – 77˚18.50' E., common in valleys and ravines of evergreen forests of Megamalai wildlife sanctuary. The plants are abundantly noticed in type locality about 50 km radius of the hill area from Iravangalaru to Vellimalai estates. The entire hill range is covered tea, coffee, cardamom and pepper cultivation. In between cultivation lands, the relict forest fringes and ravines are holding this new species. It perennates through subsucculent fibrous root stock and sprout onset of monsoon. Flowering year around but fruits set only seen in November to December.

1.5. Similar species

Ceropegia paulsamii is similar to *Ceropegia decaisneana* Wight but differs in its sub-succulent fasciculate roots, leaves ovate-oblong-elliptic with oblique 4-5, lateral nerves, glabrous calyx lobes, short corolla lobes 1.2 cm (vs 4 cm), outer corona trilobed (vs bilobed), whitish, and coronal segments basally caudate (Table 1).

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Table 1. Differences in the *Ceropegia decaisneana* and *Ceropegia paulsamii* Karupp. et Ravi sp. nov.

Characters	*C. decaisneana*	*C. paulsamii* sp. nov.
Root	Tuberous	Subsucculent, fasciculate, 0.3 mm diameter.
Leaves	Linear-lanceolate or oblong-ovate, lateral nerves 5-7-pairs.	Ovate-elliptic, oblong-elliptic, lateral nerves 4-5, oblique.
Calyx lobes	Ciliate	Glabrous
Corolla tube	Much inflated, broadly funnel-shaped above, green with pale purple spots all over	Ca. 2.5 cm long, inflated below, corolla lobes fused above to form a cage and constricted middle, tube molted with purple all over except inflated portion.
Corona	Orange, tipped with purple, outer corona bifid or truncate, erect process linear.	Whitish, outer corona trilobed, basally caudate each outer coronal segments.

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ETHNOBOTANICAL STUDY OF KNOWLEDGE AND MEDICINAL PLANTS USE BY THE KURUMBA TRIBES IN CHEMMANKARAI, NILGIRI DISTRICT, TAMIL NADU

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ABSTRACT

The present study initiated with an aim to highlight and document the traditional knowledge and medicinal plants used by the Kurumba tribes inhabiting at Chemmankarai area of Nilgiri district, Tamilnadu. During the study selected study area was visited frequently and information was collected through semi directive, open ended interview among the informants of Kurumba tribes. The details on vernacular name of the plant, mode of diagnosis, disease they treat, usage of plants, mode of application were collected. The plants were identified and deposited at the herbaria of Nirmala College for Women, Coimbatore. The results revealed that the total 56 plant species belonging to 31 families and 47 genera have been documented in the present study. The highest number plants being used for fever and wound healing (7), Insect bite, migraine, bath, ulcer, immunity and throat pain (4) followed by joint pain, tooth ache and asthma (3). The habit of the species showed that 68 % of the drugs were obtained from the herbs compared with the other habit plants. The reported potential ethnomedicinal plants could be conserved and further validation need for better utilization and provisions of the documented knowledge.

Keywords: Ethnobotany, Kurumba tribe, traditional knowledge and Nilgiri District.

1. INTRODUCTION

India is rich in ethnic diversity and indigenous knowledge that has resulted in exhaustive ethanobotanical studies (Uma priya *et al* 2011). According to the World Health Organization (WHO) about 65-80% of the world's population in developing countries depends essentially on plants for their primary healthcare due to the poverty and lack of access to modern medicine (Sharma *et al*., 2010). In Indian medicine systems, Ayurveda, Sidha and Unani entirely and Homeopathy partially depend either on plant materials or their derivatives for treating human ailments (Joseph and Justinraj, 2011).

The Western Ghats of India is one such high bio-cultural diversity region, which is one of the global biodiversity hot-spots (Myers *et al*., 2000). The Nilgiri district has variegated plants propagating both exotic and native flora of substantial recuperative utility. It consists all in all six ethnic groups of anthropological interest. They are Todas, Kotas, Kurumbas, Irulas, Paniyas and Kattunayakas (Rajan and Sethuraman,1991). Every tribal group in this country is unique in the sense that they are characterized with certain special knowledge and skills about medicinal plants used in their traditional system of medicine to cure a wide range of disease (Rajan *et al* 2003). They subside on food such has honey, fruits and tubers besides other variety of cereals. New medicinal uses of plant have been continuously reported by several workers in different localities (Ranjith and Ramachandran, 2010). The present work is an effort to document and analyze the traditional knowledge regarding the practice and use of plants in treatment for various ailments by Kurumba tribes of Chemankarai, Nilgiri District, Tamilnadu.

2. METHODOLOGY

2.1. Study Area

Blue mountains are some of the most picturesque mountain ranges situated in Southern India. It is located in North Western corner of Tamil Nadu, South India and the district has geographical area of 2,543 sq. kms. Chemmankarai area situated in Coonoor Taluk of the Nilgiri district, Tamil Nadu, India at altitude of 800 to 830 metres above mean sea level (Fig. 1). The places cover a large area of thick forest vegetation which habitats wild animals such as Black panther, Elephants, Deer, Bear, Bison etc. In view of exploitation and conservation of tribal knowledge an attempt has been made to study the ethanobotanical aspect from Chemmankarai area in Coonoor.

2.2. Kurumba Tribe

Kurumba tribes are found in the forest area of Chemmankarai, Nilgiri district. They are skilled people in honey collection, food harvesting and

medicine preparation. They collect medicinal plants from the deep forest area and utilize it efficiently. These people live in forest area in habitat of wild animals and they are able to sense the smell of the animals nearby or on the way. They make money by selling jack fruits, citrus, Guava, coffee bean cultivation, wild chillies etc., to the Burliar shops on the way to Mettupalayam to Ooty. These people build their houses with stones and red soil.

2.3. Data collection

The present investigation was carried out from Chemmankarai area of Nilgiri district to get information from the tribal practitioners and also to cross check the information provided by the practitioners during the earlier visits. The survey was conducted during June, 2016 to November, 2016. The medicinal plants growing in natural habitats of Chemmankarai forest was collected, identified and authenticated with the help of valid references (Hooker, 1875-97; Gamble and Fisher, 1935 and Matthew, 1991). At the same time plant species were collected and herbarium sheets were prepared by traditional method and were deposited in Department of Botany, Nirmala College for Women Coimbatore. The details on vernacular name of the plant, mode of diagnosis, disease they treat, usage of plants, mode of administration were collected from the tribal practitioners through direct interviews and oral conversations. The tribal practitioners have a sound knowledge about the medicinal plants around their place to treat the common diseases in family and neighbourhood.

2.4. Ailment categories

On the bases of the information gathered from the tribal healers in the study area all the reported ailments were categories (Table 1) *viz.,* kidney stones, cancer, circulatory system, dermatological infection, endocrine disorders, eye infection, fever, gastro intestinal ailment, genito urinary infection, hair problems, piles, poisonous bite, respiratory system disorder and skeleto muscular system disorder.

3. RESULTS AND DISCUSSION

3.1. Documentation of Indigenous ethnomedicinal knowledge

The Nilgiri Biosphere Reserve is an international biosphere reserve in the Western Ghats and it is very rich in floral and faunal diversity. Many ethnobotany studies have been carried out in the Nilgiri hills, but the outcome of the study have not reached the local and scientific communities to explore further. The results of the present study revealed that the 56 plant species are used by Kurumba tribes for herbal remedy for the treatment of various ailments. These species belonging to 31 families, the most represented being Solanaceae (5), Asteraceae and Oxalidaceae (4), Malvaceae, Piperaceae, Myrtaceae and Rosaceae (3), Arecaceae, Sapindaceae, Caryophyllaceae, Zingiberaceae, Lamiaceae, Euphorbiaceae, polygonaceae, Rutaceae and Fabaceae (2). Among the genera *Oxalis* (3), *Solanum*, *Piper*, *Leucas* and *Rubus* (2) are the most represented genera in the studied plants (Table 2).

Table 1. Ailment grouped under by different ailment categories.

Ailment Categories	Biomedical terms	Tamil Terms
Cancer	Cancer	Putru noi
Circulatory system	Blood clotting, blood purification, cholesterol	Ratham kattu, Rayha suthigaripu, kozhuppu.
Dermatological infection	Cuts, wounds, itching, skin irritation, burning injury	Vettukayam, aripu, thol noi, arinja pun.
Endocrine disorders	Diabetes	Neer elivu noi
Eye infection	Eye infection	Kan vedanai
Fever	Fever, malaria fever	Kachal, Kosu kadi kachal
Gastro intestinal ailment	Ulcer, dysentery, pitta	Kudal pun, pittam
Genito urinary infection	Sexual weekness, menstrual problems, post natal care.	Mada vidai kolaru,
Hair disease	Hair disease	Thala mudi noi
Kidney stones	Kidney stone	Kal
Liver problem	Jaundice	Manja kamalai
Piles	Hemorrhoids	Mulam
Poisonous bite	Snake bite, centipede bite, bee bite, insect bite	Pambu kadi, pooran kadu, then poochi kadi, poochi kadi.
Respiratory system disorder	Cold, bronchitis, pneumonia fever	Jaladosham, nenju Sali,
Skeleto muscular system disorder	Arthritis, inflammation, muscular pain.	Vatham, veekam, chadai pidipu

Table 2. List of commonly used medicinal plants by Kurumba tribes of Chemmankarai, Nilgiri district, Tamilnadu.

S. No.	Binomial Name	Family	Vernacular name	Life form	Chemical constituents	Mode of administration	Parts Used	Medicinal Uses
1	*Abutilon indicum* Sweet	Malvaceae	Thuthi	Shrub	Abutilin A(1)	Oral and External	Leaves	Leaf decoction taken orally in empty stomach for 48 days to cure bleeding piles. Leaf paste and turmeric are mixed with heated coconut oil and heated for 15 -20 min, filtered and applied externally for piles.
2	*Achyranthes aspera* L.	Amaranthaceae	Naayuruvi	Herb	Triterpenoid saponin	Oral	Root and Leaves	Root decoction taken orally for stomach upset. Leaves are cooked as greens and used to reduce fever.
3	*Aloe vera* (L.) Burm.f.	Liliaceae	Kathalai	Herb	Anthraquinone	Oral and External	Leaves	The pulp is collected and mixed with coconut oil, filtered and used for external application for wounds. The pulp is taken raw orally to control white discharge in women.The plant is cut and applied on insect sting to avoid swelling and itching.
4	*Arisaema tortuosum* (Wall.) Schott	Araceae	Naga chedi	Herb	Arisaimenone	External	Tuber	The paste of tuber is used as anitidote for veterinary purposes.
5	*Bidens pilosa* L.	Asteraceae	Thatha thala vetti poo	Herb	Friedelinol(1)	External	Leaves	The leaves are crushed and applied on cut wounds for clotting of blood.
6	*Biophytum intermedium* Wight	Oxalidaceae	Little tree plant	Herb	Bioflavanoids	External	Whole plant	The plant juice is applied on the injured part and also for bleeding. Plant paste is applied on forehead for migraine.
7	*Cardamine africana* L.	Brassicaceae	Kattu kadugu	Herb	Alkaloid	External External	Whole plant	Used as herbal bath for babies. Crushed leaves are tied over wounds to improve healing.
8	*Cardiospermum halicacabum* L.	Sapindaceae	Mudakathan keerai	Climber	Cyclohexane-1,4,5-triol-3-one-1-carbolic acid.	Oral and External	Whole plant	The plant is collected and boiled with pepper water taken to reduce joint pain and strengthen bones. The leaves are grinded into paste with *Cissus quadrangularis* and

								applied on broken bones.
9	*Catharanthus roseus* (L.) G. Don	Apocynaceae	Nithya kalyani	Herb	Limonene	Oral Oral	Whole plant	The plant extract is grinded with rhizome of turmeric and pinch of salt and given internally to cure ulcer. Flower petals are boiled and regularly intaken to cure cancer
10	*Centella asiatica* (L.) Urban	Apiaceae	Vallarai	Herb	Siddiqui BS(1)	Oral	Leaves	The leaf paste is mixed with goat milk to increase memory power. Leaf powder is mixed with *Solanum nigrum* to control mouth ulcer. Leaf powder with empty stomach is taken to control white discharge in women.
11	*Cestrum aurantiacum* Lindl.	Solanaceae	Pnari elai	Shrub	Paraquai	External	Leaves	The leaves are crushed and applied on cut wounds.
12	*Colacassia esculenta* (L.) Schott	Araceae	Chaman keerai	Herb	B-Sitosterol	Oral	Leaves	The leaves and tubers are Cooked with fruit of *Tamarindus indica*.
13	*Commelina benghalensis* L.	Commelinaceae	Amala chedi	Herb	Anthocyanin	Oral	Leaves	The leaf juice with *Piper nigrum* are orally intaken to reduce fever.
14	*Cynodon dactylon* (L.) Pers	Graminae	Arugu	Herb	Cyanogenic hyperoside	Oral	Whole plant	The plant with cumin is boiled in water and taken regularly every day morning in empty stomach to cure digestive disorders.
15	*Dodonaea viscose* Jacq	Sapindaceae	Vellari chedi	Shrub	Viscosol	External External	Leaves	The leaf paste is applied externally on broken bones. The leaves are boiled in hot water and used for bath to get rid of body pain.
16	*Drymaria cordata* (L.) Willd. *ex* Schult.	Caryophyllaceae	Chick weed	Herb	Sphingoglycolipid	External	Whole plant	The plant extract is applied externally to odemas in small children.
17	*Emilia sonchifolia* (L.)DC.	Asteraceae	Pothu poo	Herb	Rhamnetin	External	Whole plant	Plant paste with salt is applied on throat to get rid of tonsillitis.
18	*Galinsoga*	Asteraceae	Potato weed	Herb	Triacontanol	External	Leaves	The crushed leaves are rubbed on

	parviflora Cav.							the body for treating insect sting and other skin inflammation.
19	*Hedychium spicatum* Sm.in A.Rees	Zingiberaceae	Spiked ginger lilly	Herb	α- Terpineol	External Oral	Leaves and Rhizomes	The leaves are burnt and the ash is applied over night to cure head ache. The rhizome powder is mixed with goat milk and used in treating asthma.
20	*Hydrocotyle javanica* Thumb.	Araliaceae	Water penny worth	Herb	Cardiac glycosides	External	Whole plant	The plant juice with ash is mixed and used to treat fever. The paste of plants is used to treat wounds and boils.
21	*Ipomoea cairica* Sweet	Convolvulaceae	Morning glory	Climber	Ergoline alkaloid	Oral	Root	Root decoction taken internally for urinary infection.
22	*Leucas aspera* Spr.	Lamiaceae	Thumbai	Herb	α and β sitosterol	Nasal	Leaves	1-2 drops of fresh leaf juice are dropped inside the nose to cure one side head ache.
23	*L. hirta* Spr.	Lamiaceae	Sema thumba	Herb	Coumarins	Oral	Root	Root decoction is used to treat bronchial diseases.
24	*Mangifera indica* L.	Anacardiaceae	Maa maram	Tree	Mangiferin	Oral	Seed	Seed powder is given in empty stomach to get rid of stomach worms.
25	*Michelia champaca* L.	Magnoliaceae	Chembakam	Tree	Liriodenine	External	Leaves and Bark	Leaves and bark are boiled in water and used for bath during fever.
26	*Mimosa pudica* L.	Ceasalpineaceae	Thotta churungi	Herb	Corcetin-dimethylester	Oral	Root	Root decoction taken orally to cure kidney stones.
27	*Myristica fragrans* Houtt.	Myristicaceae	Jathika	Tree	Erythrosurinamensin	Oral Oral	Fruits and seeds	The fruit are collected and flesh is made into pickles to cure digestion problems. The seed is scraped with breast milk and given to new born babies to increase immunity.
28	*Nicandra physaloides* Gaertn.	Solanaceae	Kattu kathiri	Herb	Carotenoid	Oral	Seeds	Decoction of seed is used to treat fever.
29	*Oxalis corniculata* L.	Oxalidaceae	Puli keerai	Herb	Methoxyflavones	Oral	Whole plant	The infusion of the plant is said to be a remedy for hook worm.
30	*O. latifolia*	Oxalidaceae	Puliyan keerai	Herb	β- Sitosterol	Oral	Leaves	The intake of leaf juice of plant treats urinary infection.

31	*O. tuberose*	Oxalidaceae	Neer puli keerai	Herb	Fructooligosaccharides	Oral	Leaves	One hand full of leaves is boiled with one glass of water to reduce fever.
32	*Peperomia tetraphylla*	Piperaceae	Othu chedi	Epiphytic herb	Aristololactam AII(1)	External	Leaves	Leaf paste are applied on fore head to cure migraine
33	*Phyllanthus amarus* Schum. and Thonn.	Euphorbiaceae	Keezhanalli	Herb	Phyllanthine	Oral	Leaves	The leaves are grinded with fresh goat milk and taken internally every morning in empty stomach to cure jaundice.
34	*Physalis peruviana* L.	Solanaceae	Thol thakkali	Herb	Cuscohygrine	Oral	Fruits	Fruits edible
35	*Phytolacca octandra*	Phytollacaceae	Poke weed	Herb	Phytolaccic acid	Oral	Roots	One gram of dried root powder have been used as laxtative.
36	*Piper mulesua*	Piperaceae	Kattu milagu	Climber	Piperine	Oral	Seeds	Seed powder is mixed with honey and taken to cure throat infection and cold.
37	*P. nigrum* L.	Piperaceae	milagu	Climber	α-tocopherol	Oral	Leaves	The leaf, seed decoction are used to treat cough, cold, indigestion.
38	*Polygonum chinense* L.	Polygonaceae	Climbing knot weed	Herb	Squalene	Oral	Stem	The stem is directly broken and chewed to get rid of dysentery.
39	*Psidium gujava L.*	Myrtaceae	Koiya	Small tree	Pentacyclic triterpenoid guajanoic	Oral	Leaves	Leaves are chewed with clove to get rid of tooth ache.
40	*Rhodomyrtus tomentosa* W.	Myrtaceae	Thavuthu palam	Shrub	α-tocopherol	External	Leaves	Fresh leaves are crushed and applied externally on the inflammation to treat tooth ache.
41	*Ricinus communis* L.	Euphorbiaceae	Amma nakku	Small tree	Ricinolein	Oral External External	Seeds	Pregnant women intake oil in size of 50 paise coin every day. The oil is applied on boils. The oil with neem is applied on hair to get rid of ring worm disease in head which causes hair fall.
42	*Rubia cordifolia* L.	Rubiaceae	Pambu vada	Climber	Rubiadin	Oral	Stem	Dried stem powder is mixed with honey and taken for insect bite.
43	*Rubus ellipticus* Sm.	Rosaceae	Mullu palam	Climber	β- Carotene	Oral	Fruit	The fruits are regularly taken by pregnant women as it increase the hemoglobin count in mother and fetus.
44	*R. racemosus* Roxb.	Rosaceae	Sema mullu	Climber	Anthocyanin	Oral Oral	Young Shoot &	Fruits are consumed to increase blood count. Shoots edible.

							Fruits	
45	*Rumex nepalensis* Spr.	Polygonaceae		Herb	Anthraquinone	External	Leaves	The leaves are dipped in heated castor oil and places on swollen wounds and tied over night to reduce swelling.
46	*Ruta graveolance* L.	Rutaceae	Aruvatham pachai	Herb	Sesuiterpene hydrocarbon	External	Leaves and fruits	The leaf paste applied on skin externally to cure skin diseases. The fruits are threaded as chain and tied in hands of new born babies for protect from infection.
47	*Saraca asoca* (Roxb.) de Wilde	Caesalpiniaceae	Asoka maram	Tree	*Catechin*	Oral	Leaves	The leaf decoction is taken for 7 days in empty stomach to cure irregular menstruation.
48	*Sida rhomboidea* Mast.	Malvaceae	Kurunthotti	Herb	Cryptolepinone	Oral	Whole plant	Plant decoction is taken internally to cure rheumatism.
49	*Solanum nigrum* L.	Solanaceae	Manatha kali keerai	Herb	Gentisic acid	Oral Oral	Leaves	Fresh leaves are taken raw to cure mouth ulcer. Leaf decoction is mixed with pepper to reduce fever.
50	*Solanum sisymbrifolium* Lam.	Solanaceae	Thakali mullu palam	Herb	β-sitosterol	Oral	Fruits	Fruits edible.
51	*Spergula arvensis* L.	Caryophyllaceae	Dadhi keerai	Herb	-	Oral	Whole plant	The whole plant is cooked and consumed as body cooler.
52	*Spilanthes clava* W.	Asteraceae	Pal vali poo	Herb	Spilanthol	External	Flowers	The flowers are crushed and placed in place of tooth ache.
53	*Syzigium cumini* (L.) Skeels	Myrtaceae	Naval	Tree	Anthocyanins	Oral	Seeds	The seed powder are dried and taken regularly to have control on diabetics.
54	*Trifolium repens* L.	Fabaceae	Neer thamarai	Herb	-	Oral	Whole plant	Boiled with cumin and taken in empty stomach to cure ulcer problem.
55	*Urena lobata* L.	Malvaceae	Nar chedi	Herb	-	External	Whole plant	The twig is cut and soaked in water for few days and fibre is obtained. The leaves are grinded and applied on inflammation to reduce pain.
56	*Zingiber officinale* Rosc.	Zingiberaceae	Inchi	Herb	Gingerol	Oral	Rhizome	Rhizome juice is mixed with honey to cure throat infection

Table 3. Ingredients added for the preparation of herbal medicines by the Kurumba Tribes.

Botanical names	Other plants added in medicinal preparation	Other ingredients added
Abutilon indicum	*Curcuma longa*	Coconut oil
Aloe vera	--	Coconut oil
Catharanthus roseus	*Curcuma longa*	Salt
Centella asiatica	*Solanum nigrum*	Goat milk
Colacassia esculenta	*Tamarindus indicus*	--
Commelina benghalensis	*Piper nigrum*	--
Cynodon dactylon	*Cuminum cyminum*	--
Emilia Sonchifolia	--	Salt
Hedychium spicatum	--	Goat milk
Hydrocotyle javanica	--	Ash
Myristica fragrans	--	Breast milk
Phyllanthus amarus	--	Goat milk
Piper mulesua	--	Honey
Psidium gujava	*Syzygium aromaticum*	--
Rhodomyrtus tomentosa	*Syzygium aromaticum*	--
Ricinus communis	*Azadirachta indica*	--
Rubia cordifolia	--	Honey
Rumex nepalensis	--	Castor oil
Solanum nigrum	*Piper nigrum*	--
Trifolium repens	*Cuminum cyminum*	--
Zingiber officinale	--	Honey

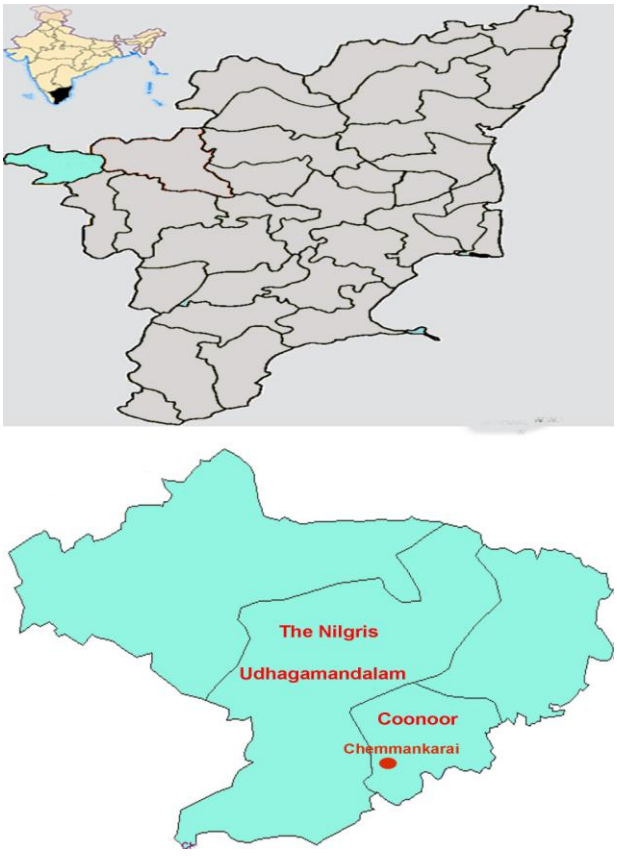

Fig. 1. Showing the study area of Chemmankarai, Nilgiri District, Tamilnadu.

Fig.2. Showing some medicinal medicinal used by the Kurumba tribes of Chemmankarai, Nilgiri District, Tamilnadu. A- *Arisaema tortuosum*, B- *Hedychium spicatum*, C-*Rubia cordifolia*, D- *Spilanthes clava*, E-*Rubus ellipticus*, F- *Rhodomyrtus tomentosa.*

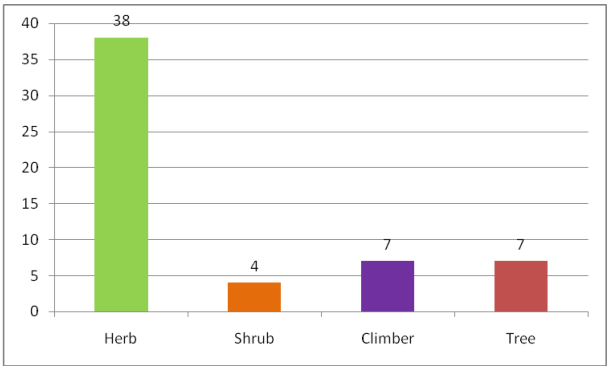

Fig. 3. Analysis of habit with respect to no. of species.

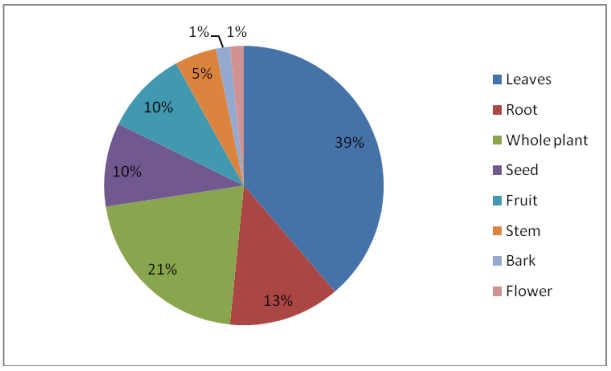

Fig. 4. Statistics of plant parts used.

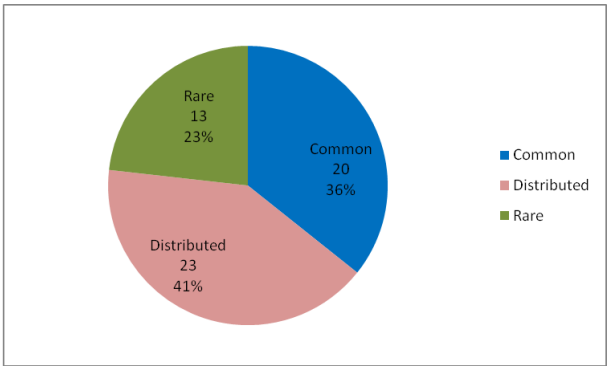

Fig. 5. Status of the plants in the study area.

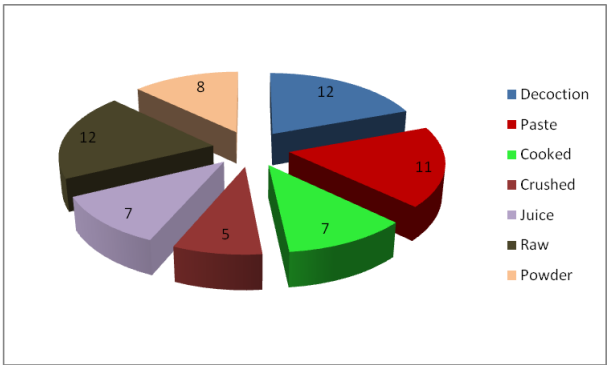

Fig. 6. Categories of Kurumba tribes mode of utilization for the preparation of medicine.

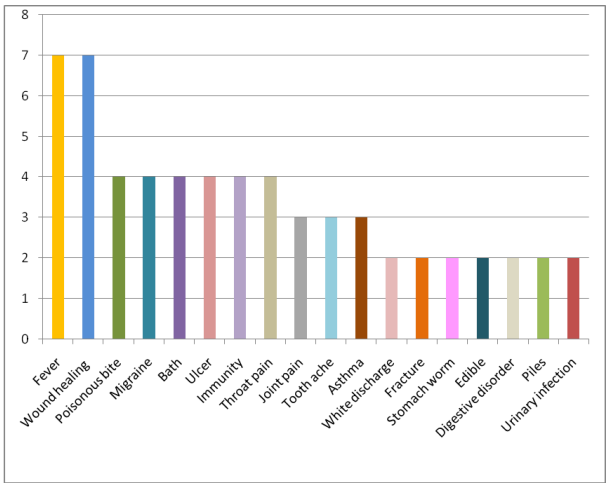

Fig. 7. Plants used for treating various diseases

In the present study more than a single plant used for same ailment, for example *Achyranthes aspera, Commelina benghalensis, Hydrocotyle javanica, Michelia champaca, Nicandra physaloides*, *Oxalis tuberose, Solanum nigrum* (fever), *Bidens pilosa, Cardamine africana, Cestrum aurantiacum, Hydrocotyle javanica, Rumex nepalensis, Aloe vera* (wound) likewise single plant is used for more than 1 disease *Achyranthes aspera* (fever and stomach problems), *Aloe vera* (wound, white discharge and insect sting), *Biophytum intermedium* (bleeding and migraine), *Cardamine africana* (bath and wound healing), *Cardiospermum halicacabum* (joint pain and fracture), *Catharanthus roseus* (stomach ulcer and cancer), *Centella asiatica* (increase memory,white discharge and mouth ulcer), *Dodonaea viscose* (fracture and body pain), *Hedychium spicatum* (Head ache and body pain), *Hydrocotyle javanica* (fever, boils and wounds), *Myristica fragrans* (digestive disorder and immunity), *Ricinus communis* (Ring worm disease and boils), *Ruta graveolance* (skin disease and immunity) and *Solanum nigrum* (mouth ulcer and fever). Several studies have reported the plants used for wound healing, fever, stomach problem, itching, skin irritations and other skin diseases in various parts of the world (Harsha *et al*., 2003; Ayyanar and Ignacimuthu, 2005; Chah *et al*., 2006 and Saikia *et al*., 2006) (Table 2 and Fig. 2).

3.2. Life form and parts used

Analysis of habit forms indicates 38 plants were herbs, 7 plants were trees, 7 plants were climbers and 4 plants were shrubs (Fig. 3). Observations were made earlier studies on ethnobotany have also been reported that the herbs are the dominant life form in their study area (Ayyanar and Ignacimuthu,2005; Xavier *et al,* 2014; Kalaiselvan and Gopalan,2014 and Kannadhasan *et*

al 2016). According to medicinal preparation of plant parts used, leaves are the most preferable part to prepare medicine (39%) followed by whole plant (21%), root (13%), seeds and fruits with 10%, stem (5%) and bark and flowers with 1% (Fig. 4). Similarly Xavier *et al.* 2014 found that leafy crude drug preparations are mostly recommended for ethnomedicine. Fig. 5 shows the number plants used for treating various diseases.

3.3. Method of preparation and mode of administration of plants

The preparation and usage of plant parts were categorized as decoction and raw 12% followed by paste 11%, powder 8%, cooked and juice 7% and raw (5% of the raw materials of plant parts such as fruits, leaf etc.) (Fig. 6). The decoctions were prepared by boiling the plants in water and the water level reduce to about required amount. The preparation of decoction is one of the common ailment practices among some tribal in Tamil Nadu (Ranjith and Ramachandran, 2010; Thirumalai *et al.,* 2012). The paste was prepared by grinding the fresh leaves in water or milk. The mode of administration routes were oral (58%), external application (41%) and Nasal (1%). External application were used to treat piles, skin, wound healing, migraine, broken bones, body pain, head ache, asthma and hairfall. Internal application were preferred to treat fever, ulcer, stomach upset, memory power, digestive disorder, urinary infection, stomach worm, jaundice, cold, tooth ache, rheumatism, diabetics, throat infection and nasal application was for head ache (Fig. 7). The utility of the same was mentioned earlier by Upadhya *et al.,* 2012.

3.4. Ingredients added

The medicines were prepared by the Kurumba tribal healers use more than one plants and other ingredients such as honey, goat milk, breast milk, coconut oil, castor oil, salt and ash to improve the tolerability and medicinal property of certain remedies (Table 3). Xavier *et al.* 2014 have been supported the present findings. Honey and Goat milk are used while intake of prepared medicine in powder forms. Oral medicines are prepared mostly using water, goat milk, breast milk and honey based on the needs.

4. CONCLUSION

The tribes of Chemmankarai area have been using numerous medicinal plants for therapeutic purpose since immemorial times. The people depend on these medicinal herbs for the treatment of various diseases such as fever, kidney stone, white discharge in women, asthma, skin disease etc., these plants are used readily as on when needed and so there is need for documentation and conservation of such Medicinal plants.

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EFFECT OF COCONUT WATER AND ACTIVATED CHARCOAL ON SEED GERMINATION IN AN ENDEMIC ORCHID *RHYNCHOSTYLIS RETUSA* BLUME.

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ABSTRACT

The present study on *in vitro* seed germination of *Rhynchostylis retusa* obtained on nutrient medium made clear pathway for the *in vitro* propagation of orchids by seed culture. The presence of coconut water in the medium enhanced seed germination and growth of seedlings. For enhancing the growth of seedlings further the individual seedlings were cultured on MS medium supplemented with 3 mg/l BAP and 1g/l activated charcoal. These seedlings were sub cultured on rooting medium (IBA) supplemented with activated charcoal. The plantlets were transferred to soil with 90% success. Hence the present investigation made possible for the large scale multiplication of *R.retusa* and thus to conserve this endemic orchid.

Keywords: *Rhynchostylis retusa,* coconut water, activated charcoal, endemic orchid.

1. INTRODUCTION

In vitro culture of orchids is of much importance since the percentage germination in nature is extremely low. Conventional vegetative propagation is very slow and there are chances of virus transfer. The method help to get virus free seedlings at a faster rate. Besides, it is useful in propagating rare and endangered species and conserves them by reintroducing to the natural habitat. Essentially, the orchids are out breeders and they produce very small seeds. The seeds are produced in large numbers ranging from 1,300 to 40, 00,000 per capsule. The great majority of species have relatively undifferentiated seed, no cotyledons and no endosperms. Less than 5% of the seeds germinate in their natural environment.

Orchid embryo is a mass of undifferentiated cells, some with and others without suspensor (Manilal and Sathish Kumar, 1985). Swamy (1949) described 5 types of embryos within the group of suspensorised embryos. Two fundamental types of embryo development occur in Orchidaceae. They are: (1) Zygote divides to form an undifferentiated mass of cells from where the suspensor cells are formed earlier and filamentous proembryo. (2) Zygote divides transversally to form a linear row of four cells. The majority of orchids show the latter type of development. The sub tribes *Cymbidiinae,* and *Crytopodiinae*, where first type is seen (Vijayaraghavan, *et al.,* 1986).

Formation of orchid seedling from the seeds involves 3 sub sequential phases namely germination, protocorm formation and seedling development (Mitra, 1976). The germination of orchid seed begun by the inhibition of water through the testa of seed. Early response of germination was noted by observing the colour and shape of the seed. First visible sign of germination is the swelling of embryo followed by their turning green and emergence of the breasted seed coats. This is called the spherical stage which subsequently develops into protocorm stage and later into the swelling stage (Mitra, 1976).

In the protocorm stage it develops rhizoids confined only to the basal region of protocorm or covers the whole except the meristamatic region. Rhizoids may be simple or branched. The formation of rhizoids in the protocorm is a unique feature of Orchidaceae. Subsequent cell divisions occur both in the apical and basal regions of the protocorm and leaf primordia, morphogenesis is started from the shoot meristem. Later first roots are formed endogenously (Mitra, 1971).

The *in vitro* culture of orchid seeds reveals that the seeds obtained from green pod 8-12 weeks of anthesis, germinate readily in a large number of species. The mature seeds on the other hands are hard to germinate due to some dormancy factors (Mitra, 1976).

Of the various media tried for seed germination the one formulated by Burgeff (1936), Knudson (1946), Vacin and Went (1940), Raghavan and Torrey (1964) and (Mitra *et al.,* 1976) with lesser amounts of ammonium, nitrates, calcium, potassium and phosphate ions along with several vitamins have been found to be most suitable for a large number of orchids. The additional presence of amino acids, urea, casein hydrolysate, yeast extract,

coconut milk, auxins, cytokinins, adenine and gibberellins in the medium has yielded a better germination of embryos and protocorm formation. (Swamy, 1949). Vitamins also play an important role in the germination and in maintaining the development of germinating seeds (Anderson 1967; Arditi 1967). Apart from seed germination and meristem culture, other parts of plants viz. leaves, leaf tip and inflorescence are also used as explants.

Plant tissue culture offers several advantages over conventional propagation vegetative and sexual methods, for large scale propagations. Shoot multiplication can be achieved in small space because miniature plantlets are produced

2. MATERIALS AND METHODS

2.1. Culture media

Murashige and Skoog's (1962) ½ strength basal media was used for the present *in vitro* studies of *Rhyncostylis retusa.* Half strength of medium (Table 1) with coconut water were used for the germination of *R. retusa* seeds.

2.2. Preparation of the media

Chemicals of high purity were used for the media preparation. The stock solutions of macro and micro elements were prepared separately and stored in glass bottles under refrigeration at 5o-10oC. All the stock solutions for MS media were prepared and stored separately. Appropriate quantities of the various stock solutions (Here half strength MS medium used), growth regulators and other supplements were mixed and the final volume of the medium was made up with double distilled water.

pH of the media were adjusted to 5.8 after adding plant growth regulators and coconut milk using O.1N. NaOH and O.1N HCl. Medium was solidified by adding agar. About 15 ml of the medium is dispensed to each culture tube and sterilized in an autoclave at 15 lbs, for 20 minutes. Half strength MS Medium with 20% coconut water was added.

For rooting of the germinating seedlings ½ MS medium supplemented with IBA (1-3 mg) were employed. The media were also supplemented with 0.5-3 gm/l of activated charcoal. Activated charcoal added along with IBA enhanced.

2.3. Preparation of the explant and inoculation

The green immature undehised capsules of *Rhyncostylis retusa* were collected from the field. The capsules were washed in tap water and brought to the inoculation chamber. The capsules were soaked in rectified sprit and surface burned. They were then opened longitudinally with a sterile blade. Seeds were immediately transferred to MS medium under aseptic conditions. The cultures were incubated at 25±2°C temperature conditions.

The percentage of germination and frequency of plant emergence were recorded initially from 45 days and then continue every 15 days interval from the day of inoculation. The changes were observed, studied and recorded with the help of a dissection microscope.

2.4. *Germination rooting and transfer of the plant*

Immature sterile seeds were cultured on MS media supplemented with coconut water (10 - 20%). Cultures were observed initially after 45 days and then continue observation at every 15 days interval. Germination begins from 45 days of culture and on 120th day of inoculation full plantlets developed. After 120 days of culture the plantlets were isolated and cultured on MS media supplemented with BAP at 3 different concentrations (1, 2, 3 mg/l) and three different concentrations of activated charcoal (1, 2, 3 gm/l).

After four weeks the plantlets attained a length of about 3 to 4 cm with 2 to 3 leaves each. These seedlings were sub cultured on to MS medium containing four different concentration of IBA (1, 2, 3 and 4 mg/l) supplemented with four different concentration of activated charcoal (0.5, 1, 2 and 3 gm/l).

After six weeks, plantlets with well developed roots were transferred to soil and grown under green house conditions. The rooted plantlets were thoroughly washed in distilled water to remove the agar and transferred to garden soil mixed with wood charcoal (1 : 1) in plastic cups (under diameter 6 cm × length 8 cm) and placed in a glass house under high humidity (90%). The plantlets were kept under sterile conditions in plastic containers for 2 months after which they were transplanted to field.

3. RESULTS AND DISCUSSION

The present investigation was carried out with the objective of rapid multiplication through *in vitro* seed germination of *Rhynchostylis retusa,* an endemic wild and rare orchid.

The seeds from the fruits collected from the field were cultured on MS medium supplemented with various concentrations of coconut milk after sterilization. The various events occurred during the germination period of the seed were studied with a dissection microscope and is shown in the Table II. The initial sign of seed germination was observed

after 45 days of culture. Within 120 days the seeds were germinated up to a length of 1cm (Fig. 1A and 1B).

The effect of various concentrations of coconut milk on seed germination was studied. The earlier reports suggest that coconut milk plays a very crucial role in seed germination of orchid (Hegarty, 1955; Nimoto and Sagawa, 1961; Cheng and Chua, 1980; Mitra, 1976). An optimum concentration of 15% coconut milk gave maximum response after 120 days of culture (Table III). Hence it was confirmed that coconut milk enhances the seed germination in *R. retusa.*

After 120 days of culture the seedlings were about 1cm in length without roots. For enhancing the growth of the seedlings, the individual seedlings were culture on MS medium supplemented with different concentrations of BAP (1, 2, 3 and 4 mg/l) and activated charcoal (1-3 gm/l), 3 mg/l BAP and 1 gm/l activated charcoal gave optimum results. On this medium seedlings reached an average height of about 2.8cm (Fig. 2A and 2B; Fig. 3A and 3B).

Table 2. Various changes observed during the seed culture in *R. retusa* on three different concentrations of coconut milk.

Days after inoculation	Medium with 10% coconut water	Medium with 15% coconut water	Medium with 20% coconut water
45 days	Embryo enlarged and become globular	Embryo become enlarged; seed coat broken and embryo came outs	Seed coat broken and embryo came out
80 days	Embryo become enlarged; seed coat broken and embryo came out	Prophyll primordium initiated at one direction of the protocorm	Protocorm enlarged in size. Starch grains are uniform in distribution
100 days	Prophyll primordium initiated	Prophyll elongated. Second prophyll formation started at the base of the first	Seed desiccated
120 days	Prophyll elongated	Plantlets with 2-3 cotyledonary leaves were formed	Seed desiccated

Table 3. Effect of three concentrations of coconut milk (10, 15 and 20%) on seed germination 45, 90 and 120 days after inoculation of seed. (Basal medium: MS)

Days	Growth of the Seedling			
	*Control	10%	15%	20%
45	-	**+	+ +	+ +
90	+	+ +	+ + +	+ +
120	+	+ +	+ + + +	+ + +

* Medium without coconut milk; ** The progressive '+' signs indicate an increase in pace of seed growth.

Table 4. Effect of different concentrations of IBA (1-4 mg/l) and activated charcoal (0.5 - 3 g/l) on root induction from the germinated shoots of *R. retusa*. Observations were taken after 45 days of culture.

½ MS IBA mg/l	Charcoal gm/l	% rooting	Average number of roots	Average length of roots (cm)
*Control	0	-	-	-
1	0.5	55	1	1.0
2	1	72	3	1.1
3	2	98	3.8	1.7
4	3	78	3.2	1.4

* Control: ½ MS basal medium

The root development was very poor with the seedlings. Later, for further growth of seedlings, the seedlings were sub cultured on half MS supplemented with different concentrations of IBA (1 to 4 mg/l) and activated charcoal. Maximum response was observed on MS+IBA (3 mg/l) and activated charcoal (2 mg/l) on the medium 98% of the shoots rooted with an average number of 3.8 roots per shoot (Table IV) (Fig. 4A and 4B).

Plantlets were transferred to soil with 90% success (Fig. 5A and 5B). The rooted plantlets were thoroughly washed in distilled water and transferred to plastic cups containing garden soil mixed with charcoal. They were placed in a glass house under high humidity. After two months the plantlets were transferred to the field. The procedure developed here is helpful in rapid multiplication of *R.retusa* and thus to conserve this rare orchid.

Several investigators employed immature seeds from unripe green pods for *in vitro* germination experiments especially in orchids like *Cymbidium, Dendrobium, Epidendum, Oncidium, Vanda* etc. Normally the fertilized ovules were collected 8 to 16 weeks after pollination were used for *in vitro* seed germination on a suitable medium. The difficulty with these experiments is that identifying the critical stage of immature seeds before they pass on to the dormant stage. As the mature and immature seeds can be hardly distinguished morphologically, some biochemical experiments are needed for identifying the right type of immature seeds. Raghavan (1976) described the changes in enzyme compliments as the orchid seed changes from immature to mature stage.

The culture of ovules or seeds from fruits can be extended to study *in vitro* pollination and fertilization leading to hybrid production. The propagation of orchid via. seed germination include three stages ie. germination, protocorm formation and seedling development. The development of a germinated seed via. protocorm into seedlings may be called as "direct" method. In other cases the embryos may form spontaneous callus formation during germination (Curtis and Nichols, 1948; Withner, 1959; Goh, 1970; Mitra *et.al.*, 1976; Vij

et.al., 1981). Here the multiplication pathway is via. organogenesis and it depend upon medium and plant growth regulators. Hence the seed callus via. Protocorm formation developing into seedling can be referred to as "indirect" method. This is basically used for obtaining hybrid orchids from embryos.

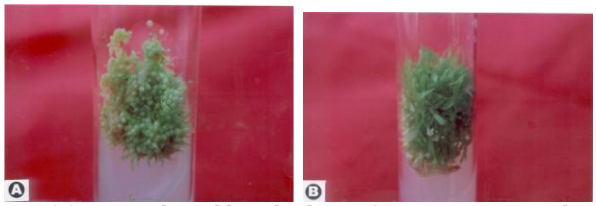

Fig. 1(A): **An 80 days old seed culture of *R. retusa* on MS medium supplemented with 15% coconut water. The seeds have started germinating and produced prophyll primordium. (B): A 100 days old seed culture on MS + 15% coconut milk. The prophylls have elongated and each plantlets can be distinguished.**

Fig. 2(A): The growth of the seedlings on MS medium supplemented with BAP mg/l without activated charcoal. (B): An isolated seedling on MS medium supplemented with BAP (3 mg/l + activated charcoal 1 gm/l) for further growth, one week after inoculation. Comparatively the presence of activated charcoal on the media enhances the growth of the seedlings.

Fig. 3(A): A 45 days old seedling on MS medium supplemented with BAP 3 mg/l + activated charcoal 1 gm/l. The seedlings have reached a size of about 2.5 cm length and rudimentary roots have developed at the base. (B): Seedling on MS medium supplemented with BAP 3 mg/l + activated charcoal 3 g/l for germination. The growth was poor as compared to 1 gm/l activated charcoal.

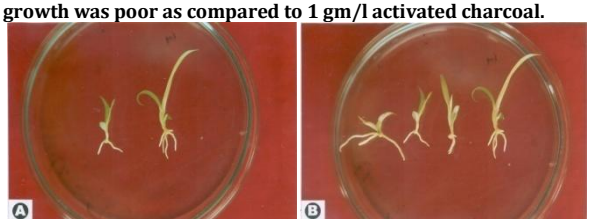

Fig. 4(A): Induction of roots on ½ MS medium supplemented with IBA 3 mg/l and activated charcoal 2 g/l. Well developed healthy roots have developed from the seedling. (B): Four seedlings showing the various stages of root development on ½ MS medium supplemented with IBA 3 mg/l and activated charcoal 2g/l after 45 days of sub culture. The roots have reached an average length of 1.7 cm.

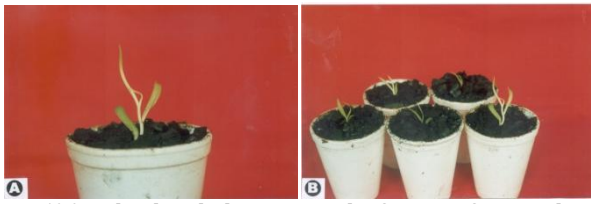

Fig. 5(A): A hardened plant one week after transfer to soil and wood charcoal. (B): Five plantlets transferred to soil growing at various stages of development 2 week after transfer.

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SEED DEVELOPMENT AND GERMINATION STUDIES OF TWO TRUE MANGROVE SPECIES *RHIZOPHORA MUCRONATA* POIR AND *BRUGUIERA CYLINDRICA* (L) BLUME.

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ABSTRACT

The present study was carried out on phenological observations and reproductive characteristics including seed development, maturation and number of days taken for produce mature propagules/seeds by the selected two important mangrove species *Rhizophora mucronata* . Prior *Bruguiera cylindrical* (L) Blume of the family Rhizophoraceae. An interesting adaptation noticed in true mangroves is that or those belonging to the family Rhizhophoraceae reproduce through a unique biological phenomenon called vivipary. In this mode of reproduction in the post fertilization the zygotes stay on the mother plant for a period 3-7 months until they mature in to seedlings or commonly called as propagules.

The physiological maturity of seeds generally determined on the basis of accumulation of higher dry weight with maximum germination. In *Rhizophora macronata* physiological maturity of seed determined as 14th weeks after anthesis. The moisture content of the seed was decreased to with increase of dry weight. In *Brugeiera cylindrica*, the harvestable maturity can be fixed on 12th weeks after anthesis. It was based on the maximum dry weight. 2.4 gm with minimum fresh weight of 4.09 gm. The germination percentage of seeds was also maximum during that period.Seed maturation studies of *Rhizophora mucronata* indicate that the best collection time prevails from April to June and in *Bruguiera cylindrica* the best seed collection time prevails from May to July.

Keywords: Germination studies, mangroves, *Rhizophora mucronata, Bruguiera cylindrica.*

1. INTRODUCTION

Mangroves are halophytes occurring in saline marshy places. The word “mangroves” is considered to be a combination of the Portuguese word “mangue” and the English word “grove”. Mangroves are salt tolerant forest ecosystems of the topical subtropical inter tidal regions of the word. Mangrove ecosystem is a group of numerous plants and animal interacting with each other and their surroundings. In India, Vegetation formation also termed as ‘Tidal forests’. Macnae (1968) coined a new term to the mangroves i.e.; “mangal” for mangrove community and “mangrove” for individual species. Mangroves are prominent component of coastal vegetation occupying flood plains, margins of bays and tidal river in addition of shores. Uniqueness of mangrove ecosystem is that the biota is constantly under physiological stress caused by extreme conditions, mangroves have been successfully colonized by developing morphological, reproductive and physiological adaptations like pneumatophores, prop roots still roots and viviparous germination which facilities their growth in aquatic environment (Tomlinson,1986) Arunprasath and Gomathinayagam(2014) reported the phenology , reproductive biology and storage studies of five true mangrove species of Pichavaram mangrove forests of Tamil nadu. A detailed phytosociological and floristic composition of two natural mangrove vegetation including the study site Ayiramthengu of Kollam district was reported (Sekaran *et al*., 2015)

The scope of the present study is to analyse the various phonological and reproductive characteristics of two mangrove species in Ayiramthengu mangrove forest of Kollam district for developing a data which could be of help the forest managers in planning to regenerate the species of the mangrove forest.

2. MATERIALS AND METHODS

The present study was carried out on phenological observations and reproductive characteristics including seed development, maturation and number of days taken for produce mature propagules/seeds by the selected two important mangrove species *Rhizophora mucronata*. Prior *Bruguiera cylindrical* (L) Blume of the family Rhizophoraceae.

2.1. Seed Development and Maturation studies

The flowers of the two species were tagged separately considering the time of anthesis as the main criteria for determination of physiological

maturity. The propagules/seeds were collected from the tagged flowers at weekly intervals to tag more number of flowers to overcome the problem of heavy flower/ fruit shedding with most care. The results were expressed as weeks (1^{st}, 2^{nd}, 3^{rd}, 4^{th} ….etc) the physical characters namely length, dry weight fresh weight of seed were measured during every sampling time. Physiological characters such as percent germination were studied in the above seeds periodically.

2.2. Seed germination test

To obtain germination percentage five replicates of 20 seeds were germinated in sandy media in a plastic germination cover placed under the tidal condition. The number of seeds germinated and the germination percentage was calculated.

2.3. Root and Shoot length

Ten seedlings were taken 30 da after sowing for both *Rhizophora* and *Brugueira* random from the standard germination test. The seedlings were removed from the germination cover without damaging the root and shoot, washed thoroughly to remove the adhering soil particles. The length of root and shoot was measured individually for the entire seedling selected. The shoot length was measured from collar region to the tip of the leaf and root length from collar region to the tip of the primary root and their means were expressed in centimeters. (cm).

2.4. Vigour index

The vigour index was calculated adopting the formula proposed by Abdul Baki and Anderson (1973) and expressed in number.

Vigour index = Germination percent x (Root length + Shoot length cm)

2.5. Selection of Water Media for growth of seedlings.

To find out suitable water media for better growth of seedlings of both species. The following media were attempted in salt water and fresh water.

From this, the seedling height shoot height, basal diameter, node number, leaf number root biomass, stem biomass, leaf biomass and total biomass were recorded.

2.6. *Selection of suitable media for viability Test*

To find out suitable seed testing media for viability the following media were attempted in Sand, Sand+Humus +Soil and Clay soil

The observations on germination, Root length, Shoot length, collar diameter and number of leaves were carried out.

3. RESULTS AND DISCUSSIONS

3.1. Seed development and maturation studies

For the present study the changes in physical characters and germination of seeds/ Propagules of *Rhizophora mucronata* over a period of time from the date of anthesis to propagule / seed maturation at weekly intervals are given in tbale.1. The seed characters of *Rhizophora mucronata* is noted that the various physical characters such as are steadily increased during the process of seeds/ propagules maturation. The length, fresh weight and dry weight increased stadily in *R. mucronata* upto 12^{th} week after a thesis. On the other hand the moisture content of the seed was decreased during the propagules maturation i.e., (90% to 48.42%). The maximum moisture content were recorded in the initial stages of seed development.

The propagules / seeds maturity of *R. mucronata* can be identify on the basis of their seed colour (greenish to brownish in colour). In *R. Mucronata* the propagule maturity attain at the period of 13^{th} week. The in physical characters and germination of seeds of *B. cylindrical* over a period of time from the date of anthesis to seed maturation at weekly intervals are given in table. 3, The physical characters noted that such as fresh weight, dry weight and length were increased steadily up to 11^{th} week after anthesis. On the other hand, the seed moisture content was decreased rapidly throughout the study period i.e., 88.88% to 41.4%. the maximum moisture content were recorded in the initial stages of seed formation.

Table 3 clearly shows that the seed formation starts at 4^{th} week and the physical characters like fresh weight and dry weight of seed increases and the moisture content of the propagules / seeds decreased throughout the maturation period. The seeds / propagules of *B. cylindrical* were started to germinate at 6^{th} week after anthesis. The propagules of *B. cylindrical* attained physiological maturity at 12^{th} week.

The accumulation of maximum dry weight of seeds at 12^{th} week indicate the physiological maturity in *R. Murcronata* and *B. Cylindirca*. The physiological maturity denotes the attainment of maximum dry weight. Such increase in day weight of seed during development and maturation was reported by Hocking *et al*, (1980) in Nuytisa, Floribunda, (Husin *et al*. 1981) in *Hevea brassiliensis*.

Table 1. Seed development and maturation studies on *Rhizophora mucronata*.

Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Fresh Weight	0.30 (±0.08)	0.5 (±0.09)	2.2 (±0.15)	3.1 (±0.2)	5.28 (±0.26)	10.5 (±0.3)	14.92 (±0.5)	18.88 (±0.7)	22.86 (±0.8)	26.5 (±0.5)	28.92 (±0.3)	30.1 (±0.2)	34.67 (±0.22)	34.90 (±2.3)	34.90 (±2.3)
Dry Weight (gm)	0.03 (±0.0003)	0.07 (±0.0002)	0.3 (±0.1)	0.6 (±0.03)	1.1 (±0.04)	2.59 (±0.05)	4.1 (±0.29)	6.2 (±0.12)	7.5 (±0.35)	9.5 (±0.1)	11.1 (±0.26)	13.26 (±0.29)	17.2 (±0.17)	28.6 (0)	28.6 (±2.3)
Length (cm)	0.32 (±0.1)	0.5 (±0.05)	1.22 (±0.07)	2.3 (±0.1)	4.0 (±0.23)	6.9 (±0.29)	8.5 (±0.23)	11.25 (±0.25)	13.33 (±0.2)	14.56 (±0.11)	16.70 (±0.23)	17.9 (±0.24)	18.36 (±0.25)	19.85 (0)	19.85 (±2.3)
Moisture Content	90 (±2.5)	86 (±3.4)	86 (±3.4)	80 (±2.3)	79 (±2.3)	75 (±1.6)	72.5 (±1.3)	67.16 (±2.6)	67.10 (±2.1)	64 (±1.3)	61.61 (±1.9)	55.9 (±1.1)	50.38 (±0.5)	48.42 (±2.3)	48.42 (±2.3)
Germination (%)	-	-	-	-	-	-	-	5 (±0.01)	12 (±0.03)	15 (±0.03)	17 (±0.02)	25 (±0.04)	45 (±0.01)	50 (±0.01)	60 (±0.04)

Table 2. Seed development and maturation studies on *Bruguiera cylindrica*.

Week	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Fresh weight (gm)	0.09 (±0.002)	0.18 (±0.06)	0.25 (±0.07)	0.33 (±0.57)	0.57 (±0.15)	0.75 (±0.16)	1.12 (±0.19)	1.85 (±0.2)	2.18 (±0.23)	3.4 (±0.2)	4.09 (±0.2)	4.1 (±1.1)	4.1 (±1.2)	4.1 (±1.1)
Dry weight (gm)	0.01 (±0.001)	0.03 (±0.06)	0.05 (±0.003)	0.08 (±0.03)	0.15 (±0.01)	0.24 (±0.03)	0.43 (±0.05)	0.95 (±0.1)	1.10 (±0.19)	1.9 (±0.19)	2.4 (±0.05)	2.4 (±0.03)	2.4 (±0.04)	2.4 (±0.05)
Length (cm)	0.2 (±0.09)	0.5 (±0.1)	0.8 (±0.08)	1.2 (±0.1)	2.35 (±4.67)	4.67 (±0.23)	6.30 (±0.24)	7.92 (±0.3)	10.68 (±1.32)	12.32 (±0.2)	13.2 (±0.3)	13.2 (±1.2)	13.2 (±0.2)	13.2 (±0.3)
Moisture content (%)	88.88 (±2.2)	83.33 (±2.6)	80 (±2.5)	75.75 (±2.5)	73.36 (±1.7)	68 (±2.0)	61.60 (±1.9)	51 (±2.3)	49.54 (±2.1)	44.11 (±2.1)	41.32 (±1.1)	41.4 (±0.5)	41.4 (±1.1)	41.4 (±1.2)
Germination (%)	-	-	-	-	-	-	-	5 (±0.01)	12 (±0.03)	15 (±0.03)	17 (±0.02)	25 (±0.04)	45 (±0.01)	50 (±0.01)

± : Standard Deviation

Table 3. Effect of different seed testing media on germination percentage, shoot length and root length and Vigour index of *Rhizophora mucronata* and *Bruguiera Cylindrica*

Seed testing media	R.mucronata				B.cylindrica			
	Germination %	Shoot length (cm)	Root length (cm)	Vigour Index	Germination %	Shoot length (cm)	Root length (cm)	Vigour Index
Sand	44	18.3	10.3	1258	40	7.9	12.8	828
Sand + humus + Red soil	61	23.9	12.9	2245	56	10.1	14.5	1378
Clay soil	88	32.6	16.6	5210	75	12.3	18.9	2340

Table 4. Effect of water condition on growth parameters and biomass of mangrove seedling

Species	Period	Tidal water dipping seedlings					Land keeping seedlings				
		Shoot Length	Root length	Collar Diameter	Number of leaves	Biomass of seedling	Shoot length	Root length	Collar diameter	Number of leaves	Biomass Of seedling
R.mucronata	30 days	20.5	7.9	4.5	2	23.5	22	8	3.9	2	22
	60 days	29	10.5	6.8	6	28.9	28	10	6	4	26
	90 days	32.8	15.1	9.3	8	35.1	31	12.6	8	6	30
B.cylindrica	30 days	14.5	6.5	3.3	2	6.6	12.2	6.1	3.4	2	5.3
	60 days	19.1	8.6	5.7	4	9.1	16.1	7.3	5.2	4	7
	90 days	22.8	11.4	7.6	6	13.6	20	10.1	7	6	11.3

Rhizophora mucronata

Bruguiera cylindrica

Seed development stages of *R.mucronta*

Seed development stages of *B.cylindrica*

Growth Rate in Nursery Stages of *Rhizophora mucronata*

Growth Rate In Nursery Stages of *Bruguiera cylindrical*

Effect of growth of *Rhizophora mucronata* and *Bruguiera cylindrical* in tidal and land keeping seedlings

The increase in dry weight of seed might be due to decrease in the moisture content coupled with increased accumulation of food reserve material. however , the change in seed dry weight were not related to change in seed dry weight were not related to change in seed quality of marrow (Demir and Ellis,1993).

3.2. Effect of seed testing medium on germination, shoot length and root length

The seeds of Rhizophora mucronata were sown on different testing media such as sandy soil, sand + humus + redsoil, clay soil, conditions for the observation on germination, root-length, shoot length were recorded (Table.3). The freshly collected seeds of *R. mucronata* shows maximum germiability in clay soil ie., 88% and les germination in sandy soil i.e., 43%.

The maximum root length, shoot length and germination percentage of seeds were recorded in muddly soil present in that area. The average shoot length and root length of seeds are 33.6cm and 16.6cm, and it is decreased in other mediums.

The seeds of Bruguiera cylindrical were also sown in three different testing media such as muddy soil, sandy soil and sand soil + humus + Red soil, Conditions for observation on germination, root length and shoot length of seeds were recorded (Table.5). The freshly collected seeds of *B. Cylindrica* shows maximum germiability in muddy soil, i.e. 75% and less germination in sandy soil. The maximum root length, shoot length and germination percentage of seeds were recorded in muddy soil. The average root and shoot length of seeds are 12.3cm and 18.9cm.

3.3. Effect of growth in tidal and land keeping seedlings of R. mucronata and B.cylindrica

The seeds of *R.mucronata* and *B.cylindrica* are placed in tidal condition and in land, the effect of seeds of both species are different in these conditions. To observe the seedling growth of *R.mucronata* and *B.cylindrica* after 30 days, 60 days and 90 days, the tidal water dipping seedlings show s higher growth than land keeping seedlings. The characters of seedlings-shoot length, root length, collar diameter, number of leaves and dry weight of seedlings is higher in tidal water dipping seedlings and lesser in land keeping seedlings. (Table.4)

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PRELIMINARY PHYTOCHEMICAL SCREENING AND GC-MS ANALYSIS OF METHANOLIC LEAF EXTRACT OF *DRYPETES SEPIARIA* (WIGHT & ARN.) PAX. & HOFFIM. FROM SILAMBUR SACRED GROVE, TAMILNADU

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ABSTRACT

Drypetes sepiaria (Wight & Arn.) Pax. & Hoffim a medium sized tree member of Euphorbiaceae was investigated to determine the phytochemical constituents present in various extracts of the leaves through GC-MS analysis. Powdered leaf plant materials were subjected to successive extraction with organic solvents such as methanol by Soxhlet extraction method. In the present study, GC-MS analysis revealed that a total of 23 different compounds identified by using methanol extract and all the identified compounds were medicinally valuable for the treatment of various human ailments. In addition, all the phytochemical compounds were needed for further investigations on toxicological aspects for the development of new lead of therapeutic interest.

Keywords: *Drypetes sepiaria* preliminary screening, GC-MS analysis.

1. INTRODUCTION

Plants have been a rich source for drug discovery (Mishra and Tiwari, 2011). Plants and plant parts have been provide a good source of pharmaceutical active compounds, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids, saponin and some other secondary metabolites, which are rich in valuable bioactivities of antioxidant, anti-inflammatory, antitumor, antimutagenic, anti-carcinogenic (Maridass *et al.,* 2008). The genus *Drypetes* (Putranjivaceae (Euphorbiaceae) comprises nearly 160 species which has been used in the folk medicine of many cultures for many years (Nganga *et al.,* 2008). Even though the species was different, they used to treat similar disorders. Among the members of the genus *Drypetes* earlier phytochemical studies on some species including *D. parrifolia, D. laciniata, D. inaequalis, D. armoracia, D. gossweileri, D. molunduana, D. roxburghii* have yielded flavonoids, chalcone glycosides, saponins, tripterpenoids, phenolics, alkaloids, etc.

Drypetes sepiaria (Wight & Arn.) Pax. & Hoffim. an ever green tree locally known as Kalvirai (Tamil) commonly grown in foothills and shrub jungles and some sacred groves of Tamil Nadu. *Drypetes sepiaria* is traditionally used to treat pain and inflammation and seeds are used as a wild edible food and their root paste can be used as an antidote for scorpion bite. Decoction of leaves and seeds is also noted for reducing rheumatic inflammation (Arinathan *et al.,* 2007; Bharath Kumar and Suryanarayana, 2011). As per earlier literature, there is no scientific investigations found in *D. sepiaria* on phytoconstituents present. In ethnomedicinal point of view as described above, the GC-MS analysis was carried out with methanolic leaf extract of *D. sepiaria* to investigate the chemical constituents present in it.

2. MATERIALS AND METHODS

2.1. Collection of plant materials and preparation of the extract

The fresh leaves of *D. sepiaria* was collected from the sacred grove of Silambur (Lat, 11.35 °N; Long, 79.31°E), Ariyalur District, Tamil Nadu, India. The specimen was botanically identified and confirmed by Rapinat Herbarium, St. Joseph's College, Tiruchirappalli. The preserved plant specimens were submitted to the Department of Botany, Annamalai University, Annamalainagar, Tamil Nadu for further reference. The leaves were chopped into small pieces, shade-dried and coarsely powdered by using a pulverizor. The powdered leaf were then subjected to successive extraction with organic solvents such as hexane chloroform and ethanol by Soxhlet method (Catherine *et al.,* 1997). The extracts were then collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed and stored at 4°C. They were used for GC-MS analysis.

2.2. Preparation of extract

The powdered leaf of *D. sepiaria* (500 g) was extracted with methanol (95%) and double distilled water separately in a soxhlet extractor. The extract was evaporated to dryness at 60°C under reduced pressure in a rotary evaporator and kept in refrigerator at 4°C till used. The extracts were

dissolved in dimethylsulphoxide to make the final concentrations at the time study.

2.3. Preliminary phytochemical screening

A small portion of the dry extracts were subjected to preliminary phytochemical screening to detect the presence of various phytoconstituents present in the leaves of *D. sepiaria* (Harborne, 1973; Evans, 2003).

2.4. Gas chromatography- mass spectrometry (GC-MS) analysis

GC-MS analysis was performed with GC-MS Clarus 500 Perkin Elmer Equipment. Compounds were separated on Elite-5 capillary column (Crossbond 5% Phenyl 95% dimethylpolysiloxane) Oven temperature was programmed as follows: isothermal temperature at 60°C then increased to 200°C at the rate of 10°C/min., then increased up to 280°C at the rate of 5°C/min. held for 9 min. Ionization of the sample components was performed in the Electron energy (70 eV). The helium was used as gas carrier (1ml/min.), and 1.0 µL of sample was injected. The detector was Mass detector Turbomass gold Perkin Elmer. The total running time for GC was 36 min. and software Turbomass 5.2.0 was used in this GC-MS study (Manjamalai *et al.,* 2010).

2.5. Identification of compounds

All the compounds were identified from methanol extracts based on direct comparison of the retention times and their mass spectra with the spectra of known compounds stored in the spectral database, NIST (Version year 2005).

3. RESULT AND DISCUSSION

Preliminary phytochemical analysis showed (Table 34) the presence of alkaloids, flavonoids, saponins, and phenols showed in petroleum ether leaf extract and presence of flavonoids, saponins, phenols, steroids in methanolic solvent. Steroids, flavonoids, saponins and phenol Hexane leaf extract showed terpenoids and glycosides only. In chloroform leaf extract showed steroids, tannins, and saponins only.

Table 1. Preliminary phytochemical screening of *Drypetes sepiaria* leaves.

S.No.	Phytochemicals	PE	Chl	AC	E
1.	Alkaloids	+	-	+	-
2.	Steroids	-	+	+	+
3.	Terpenoids	-	-	-	-
4.	Flavonoids	+	-	+	+
5.	Tannins	-	+	-	-
6.	Saponins	+	+	+	+
7.	Glycosides	-	-	-	-
8.	Total phenol	+	-	+	+

PE- Petroleum ether, Chl-Chloroform, AC-Acetone, E-Ethanol, + present, - absent

The chemical constituents identified by the GC-MS analysis on methanolic leaf extract of *D. sepiaria* were enumerated along with Molecular Formula (MF), Molecular Weight (MW), Retention Time (RT), and peak area and peak area (%) is presented in Table-2.

Table 2. Compounds identified in methanolic leaf extract of *Drypetes sepiaria.*

S.No.	Peak Name	Retention Time (min)	Peak Area	% Peak area
1	Name: Propanoic acid, 2-oxo-, methyl ester Formula: $C_4H_6O_3$ MW: 102	2.83	352644	2.8966
2	Name: 2-Furanmethanol Formula: $C_5H_6O_2$ MW: 98	3.69	526502	0.4325
3	Name: 2-Cyclopenten-1-one, 2-hydroxy- Formula: $C_5H_6O_2$ MW: 98	4.82	251654	2.0671
4	Name: Benzaldehyde Formula: C_7H_6O MW:106	5.46	253243	2.0801
5	Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: $C_6H_8O_4$ MW: 144	5.69	232379	1.9087
6	Name: 2-Hydroxy-gamma-butyrolactone Formula: $C_4H_6O_3$ MW: 102	6.25	174726	1.4352

7	Name: 3-Acetylthymine Formula: $C_7H_8N_2O_3$ MW: 168	8.2	341872	2.8081
8	Name: Pyrimidine-4,6-diol, 5-methyl- Formula: $C_5H_6N_2O_2$ MW: 126	8.73	621868	0.5108
9	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: $C_6H_8O_4$ MW: 144	9.53	284421	2.3362
10	Name: 2-Propenal, 3-(2-furanyl)- Formula: $C_7H_6O_2$ MW: 122	10.04	247028	2.0291
11	Name: 2(1H)-Pyrimidinethione, 4,6-diamino- Formula: $C_4H_6N_4S$ MW: 142	10.52	546851	0.4492
12	Name: 1,6;3,4-Dianhydro-2-O-acetyl-á-d-allopyranose Formula: $C_8H_{10}O_5$ MW: 186	10.9	96626	0.0794
13	Name: Dianhydromannitol Formula: $C_6H_{10}O_4$ MW: 146	11.01	318631	2.6172
14	Name: 2-Furancarboxaldehyde, 5-(hydroxymethyl)- Formula: $C_6H_6O_3$ MW: 126	11.59	284490	2.3368
15	Name: 2H-Pyran-5-carboxylic acid, 2-oxo-, methyl ester Formula: $C_7H_6O_4$ MW: 154	12.64	544630	0.4474
16	Name: : Ethyl N-hydroxyacetimidate Formula: $C_4H_9NO_2$ MW: 103	12.95	218384	0.1794
17	Name: 2-Methoxy-4-vinylphenol Formula: $C_9H_{10}O_2$ MW:150	13.32	950045	7.8036
18	Name: 5-Formylsalicylaldehyde Formula: C8H6O3 MW: 150	13.79	644626	0.5295
19	Name: Phenol, 3,4-dimethoxy- Formula: $C_8H_{10}O_3$ MW: 154	14.15	505296	0.415
20	Name: Benzoic acid, 4-formyl-, methyl ester Formula: $C_9H_8O_3$ MW: 164	14.45	513228	4.2156
21	Name: Benzeneethanol, 4-hydroxy- Formula: $C_8H_{10}O_2$ MW: 138	16.39	405797	3.3332
22	Name: Phenol, 2-methoxy-4-(1-propenyl)- Formula: $C_{10}H_{12}O_2$ MW: 138	16.7	346247	2.844
23	Name: Cyclohexane, 1-methylene-4-(1-methylethenyl)- Formula: $C_{10}H_{16}$ MW: 164	18.18	655066	5.3806

24	Name: 2-Propanone, 1-(4-hydroxy-3-methoxyphenyl)- Formula: $C_{10}H_{12}O_3$ MW: 180	18.68	689038	0.566
25	Name: 3',5'-Dimethoxyacetophenone Formula: $C_{10}H_{12}O_3$ MW: 180	19.6	116518	0.9571
26	Name: Benzeneacetic acid, 3,4-dihydroxy- Formula: $C_8H_8O_4$ MW: 168	23.49	151320	12.4293
27	Name: Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester Formula: $C_{10}H_{12}O_4$ MW: 196	24.19	120783	0.9921
28	Name: (R)-(-)-4,4a,5,6,7,8-Hexahydro-4a-methyl-2(3H)-naphthalenone Formula: $C_2H_6N_2O$ MW: 164	24.42	197221	1.62
29	Name: 4-((1E)-3-Hydroxy-1-propenyl)-2-methoxyphenol Formula: $C_3H_6N_2O_2$ MW: 180	24.62	432379	3.5515
30	Name: Benzoic acid, 3-formyl-4,6-dihydroxy-2,5-dimethyl-, methyl ester Formula: $C_2H_6N_2O$ MW:224	25.57	144845	1.1897
31	Name: 3,4-Dihydrocoumarin-7-ol Formula:$C_5H_6O_2$ MW: 164	25.82	940934	7.7287
32	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_4H_6O_3$ MW: 296	26.53	531514	4.3658
33	Name: Undecanoic acid, 2-methyl- Formula: $C_8H_{16}O$ MW: 200	28.79	111426	0.9152
34	Name: n-Hexadecanoic acid Formula: $C_7H_{14}O$ MW: 256	30.13	161403	13.2575
35	Name: 9,12-Octadecadienoic acid, methyl ester Formula: $C_6H_8O_3$ MW: 294	32.86	154532	1.2693
36	Name: 10-Octadecenoic acid, methyl ester Formula: C_8H_8O MW: 296	32.99	246271	2.0228

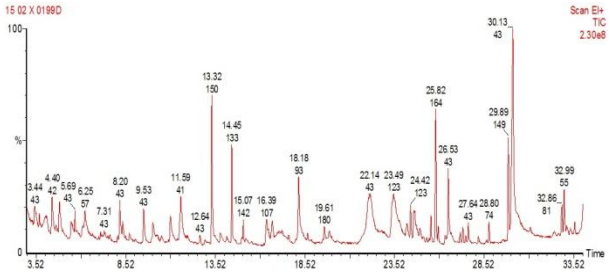

Fig. 1. GC-MS analysis of methanolic leaf extract of *Drypetes sepiaria*

In the methanolic leaf extract of *D. sepiaria,* a total of 36 compounds were identified, of which n-Hexadecanoic acid (13.25%), was found as major compound followed by other compounds namely, Benzeneacetic acid, 3,4-dihydroxy-(12.92%), 2-Methoxy-4-vinylphenol (7.80%), 3,4-Dihydrocoumarin-7-ol (7.72%), and Cyclohexane, 1-methylene-4-(1-methylethenyl)- (5.38%).

Phenolic compounds have antimicrobial properties. Phenol and phenolic compounds have

been extensively used in disinfections. Thus the reported antimicrobial properties of both plants may be attributed to phenolic compounds. Plants with tannins are used for healing of wounds, varicose ulcers and burns (Nafiu *et al.,* 2011). Among the identified phytochemicals, n-hexadecanoic acid has the property of antioxidant activity and it justifies with the earlier work in *Alstonea venenata* (Sutha, 2012).

4. CONCLUSION

The present investigation through the present study revealed that the species *D. sepiaria* is a reliable source of bioactive compounds like fatty acid esters, alcohols, hydrocarbons, alkanes, amines, terpenes, and sugars that justify the traditional usage of this species by the local healers in Tamil Nadu, India, for various ailments. As GC-MS is the first step towards understanding the nature of active principles. Further investigation in this species is suggested for the development of novel drugs.

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COMPARATIVE PHYTOCHEMICAL PROFILES OF TWO ACCESSIONS OF *MEMECYLON EDULE* ROXB. (MELASTOMATACEAE) BY GC-MS ANALYSIS

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ABSTRACT

Memecylon edule Roxb. a member of Melastomataceae and a valuable Indian ethnomedicinal plant and there are two accessions of this species was investigated to determine the phytochemical constituents present in various extracts of the leaves through GC-MS analysis. Powdered leaf plant materials were subjected to successive extraction with organic solvents such as methanol by Soxhlet extraction method. In the present study, a total of phytocompounds, twenty eight from Acc.1 and twenty five from Acc.2 were identified by GC-MS analysis using methanolic leaf extract, all the identified compounds were medicinally valuable for the treatment of various human ailments. In addition, all the phytochemical compounds were needed for further investigations on toxicological aspects for the development of new lead of therapeutic interest.

Keywords: Phytochemical profile, *Memecylon edule*, GC-MS analysis.

1. INTRODUCTION

The genus *Memecylon* L., belonging to the family Melastomataceae, is represented world over by around 250 species of shrubs and trees in the paleotropical region. Of which 30 species has been reported from India (Henry *et al.,* 1989; Santapau and Henry, 1973) and 16 species from Tamil Nadu state (Nair and Henry, 1983). Also the genus *Memecylon* is represented by 39 species of which 21 are endemic to the country and the Western Ghats is reported to host 29 species (Viswanathan and Manikandan, 2001; Santhosh Kumar *et al.,* 2003; Rajendraprasad *et al.,* 2006; Murugan and Gopalan, 2006; Manickam *et al.,* 2007). They are distributed in all types of habitats (Sivu *et al.,* 2013). *Memecylon* species are utilized worldwide as timbers, ornamentals, source of edible fruits and yellow dye in addition to their medicinal properties (Mabberley, 2005).

The leaves of *M. edule* is used to heal the burning wounds without scar. The anti-inflammatory, analgesic and antioxidant activities of the leaves used in traditional medicine in reliving inflammation and pain (Nualkew *et al.,* 2009). Decoction of stem has also been relief fever symptoms of common diseases such as common cold, measles and chicken box (Karuppawamy, 2007). The antibacterial activity of seeds were evaluated (Elavazhagan and Arunachalam, 2010). After pursuit of published literature, so far meager work has been done regarding the phyto-chemical evaluation on this selected plant. Hence, in the present study GC-MS analysis was carried out with methanol extracts of the leaves of two accessions of *Memecylon edule* Roxb. to examine the chemical constituents present in it.

2. MATERIALS AND METHODS

2.1. Collection of plant materials and preparation of the extract

The fresh leaves of *Memecylon edule* were collected from Acc.1.Authukurichi (Lat, 11.35 °N; Long, 79.31°E), Ariyalur District and Acc.2. Puthupattu, (12°05'74"N, 79°86'93"E) Villupuram District, Tamil Nadu, India. The specimen was botanically identified and confirmed by Rapinat Herbarium, St. Joseph's College, Tiruchirappalli. The preserved plant specimens were submitted to the Department of Botany, Annamalai University, Annamalainagar, Tamil Nadu for further reference. The leaves were chopped into small pieces, shade-dried and coarsely powdered by using a pulverizor. The powdered leaf were subjected to successive extraction with organic solvents such as hexane chloroform and ethanol by Soxhlet method (Catherine *et al.,* 1997). The extracts were then collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvents was removed in vacuo and stored at 4˚C. They were used for GC-MS analysis.

2.2. Gas chromatography- mass spectrometry (GC-MS) analysis

GC-MS analysis was performed with GC-MS Clarus 500 Perkin Elmer Equipment. Compounds were separated on Elite-5 capillary column (Crossbond 5% Phenyl 95% dimethylpolysiloxane) Oven temperature was programmed as follows:

isothermal temperature at 60°C then increased to 200°C at the rate of 10°C/min., then increased up to 280°C at the rate of 5°C/min. held for 9 min. Ionization of the sample components was performed in the Electron energy (70 eV). The helium was used as gas carrier (1ml/min.), and 1.0µL of sample was injected. The detector was Mass detector Turbomass gold Perkin Elmer. The total running time for GC was 36 min. and software Turbomass 5.2.0 was used in this GC-MS study (Manjamalai *et al.,* 2010).

2.3. Identification of compounds

All the compounds were identified from methanol extracts based on direct comparison of the retention times and their mass spectra with the spectra of known compounds stored in the spectral database, National Institute Standard and technology (NIST) (Version year 2005).

3. RESULT AND DISCUSSION

The chemical constituents identified by the GC-MS analysis on methanolic leaf extract of two accessions of *Memecylon edule* were enumerated along with Molecular Formula (MF), Molecular Weight (MW), Retention Time (RT), and Peak area and Peak area (%) is presented in Table-1. Of which nine compounds present in both the accessions of *M. edule* and are Furfural, Levoglucosenone, 1-Deoxy-d-altritol, 4H-Pyran-4-one, 2,3-dihydro-3,5-,1,4,3,6-Dianhydro-à-d-glucopyranose,1,2,3-Benzenetriol, 3, 7,11,15-Tetramethyl-2-hexadecen-1-ol and n-Hexadecanoic acid. Comparatively 1,2,3-Benzenetriol show higher percentage in both accessions.

Table 1. Phytocompounds identified from the methanolic leaf extract of *M. edule.* (Acc.1. Authukurichi, Acc.2. Puthupattu)

Sl.No.	Compound name	Formula	Mol. weight	Acc.1.	Acc.2
				% of peak area	% of peak area
1	Furfural	$C_5H_4O_2$	96	5.5959	1.5145
2	2-Cyclopenten-1-one, 2-hydroxy-	$C_5H_6O_2$	98	0.3292	-
3	1-Benzoyl-3-amino-4-cyano-3-pyrroline	$C_{12}H_{11}N_3O$	213	0.8871	-
4	2(3H)-Furanone, 3-acetyldihydro-	$C_6H_8O_3$	128	0.2274	-
5	Phentermin-propionyl	$C_{13}H_{19}NO$	205	0.6349	-
6	cis-1,2-Dihydrocatechol	$C_6H_8O_2$	112	0.392	-
7	1,2-Butanediol, 1-phenyl-	$C_{10}H_{14}O_2$	166	4.2074	-
8	Hydrouracil, 1-methyl-	$C_5H_8N_2O_2$	128	0.9593	-
9	Methyl 2-furoate	$C_6H_6O_3$	126	0.8868	-
10	Levoglucosenone	$C_6H_6O_3$	126	2.2612	0.179
11	1-Deoxy-d-altritol	$C_6H_{14}O_5$	166	0.224	0.7361
12	4H-Pyran-4-one, 2,3-dihydro-3,5-	$C_6H_8O_4$	144	2.6392	1.457
13	Benzoic acid, 2-hydroxy-, methyl ester	$C_8H_8O_3$	152	0.1067	-
14	1,4:3,6-Dianhydro-à-d-glucopyranose	$C_6H_8O_4$	144	1.4487	0.5732
15	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	13.488	-
16	2-Methoxy-4-vinylphenol	$C9H_{10}O_2$	150	0.3241	-
17	Hydroquinone	$C_6H_6O_2$	110	7.3113	-
18	Methyl-à-d-ribofuranoside	$C_6H_{12}O_5$	164	0.6927	-
19	1,2,3-Benzenetriol	$C_6H_6O_3$	126	29.278	17.066
20	1,3-Cyclohexanediol, 4,6-dimethyl-2-nitro-, diacetate (ester),	$C_{12}H_{19}NO_6$	273	0.3964	-
21	Dodecanoic acid	$C_{12}H_{24}O_2$	200	0.2861	-
22	D-Allose	$C_6H_{12}O_6$	180	15.256	16.808
23	Benzeneacetic acid, 4-hydroxy-3-methoxy-	$C_9H_{10}O_4$	182	2.7237	-
24	2-Cyclohexen-1-one, 4-(3-hydroxybutyl)-3,5,5-trimethyl-	$C_{13}H_{22}O_2$	210	1.9662	-
25	3,7,11,15-Tetramethyl-2-hexadecen-1-ol	$C_{20}H_40_O$	296	0.6571	0.1904
26	3,5-Dimethoxy-4-hydroxyphenylacetic acid	$C_{10}H_{12}O_5$	212	0.6745	-
27	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	5.168	9.6491
28	cis-9-Hexadecenal	$C_{16}H_{30}O$	238	0.9771	-
29	2,10-Dodecadien-1-ol, 3,7,11-trimethyl-,	$C_{15}H_{28}O$	224	-	3.2083

30	2H-1-Benzopyran-2-one, 7-methoxy-6-(3-methyl-2-oxobutyl)-	$C_{15}H_{16}O_4$	260	-	0.2698
31	Octadecanoic acid	$C_{18}H_{36}O_2$	284	-	1.0947
32	E-9-Tetradecenoic acid	$C_{14}H_{26}O_2$	226	-	4.1895
33	2H,8H-Benzo[1,2-b:5,4-b']dipyran-2-one, 8,8-dimethyl-	$C_{14}H_{12}O_3$	228	-	0.2698
34	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	-	0.3379
35	Benzeneacetic acid, 4-hydroxy-3-methoxy-, methyl ester	$C_{10}H_{12}O_4$	196	-	0.1605
36	1,6-Anhydro-à-d-Galactofuranose	$C_6H_{10}O_5$	162	-	10.409
37	7-Oxabicyclo[4.1.0]heptane, 1-(1,3-dimethyl-1,3-butadienyl)-2,2,6-trimethyl-, (E)-	$C_{15}H_{24}O$	220	-	1.7445
38	1-Hydroxy-6-(3-isopropenyl-cycloprop-1-enyl)-6-methyl-heptan-2-one	$C_{14}H_{22}O_2$	222	-	0.14
39	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	150	-	0.1694
40	2-Furancarboxaldehyde, 5-(hydroxymethyl)-	$C_6H_6O_3$	126	-	9.3267
41	Sucrose	$C_{12}H_{22}O_{11}$	342	-	2.1644
42	5H-1,4-Dioxepin,2,3-dihydro-2,5-dimethyl-	$C_7H_{12}O_2$	128	-	1.5161
43	2(1H)-Pyridinone, 6-hydroxy-	$C_5H_5NO_2$	111	-	0.2702
44	2-Ethylacrolein	C_5H_8O	84	-	0.1516

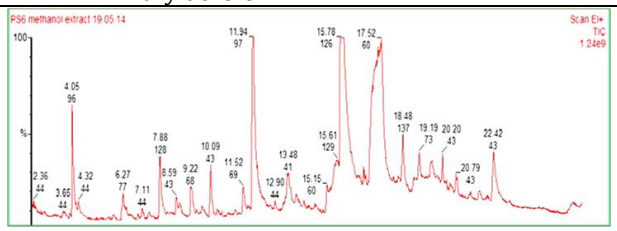

Fig. 1. GC-MS Chromatogram of methanolic leaf extract of *M. edule* (Acc.1. Authukurichi).

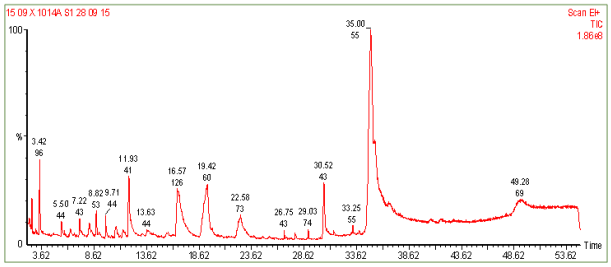

Fig. 2. GC-MS Chromatogram of methanolic leaf extract of *M. edule* (Acc.2. Puthupattu).

Plants serve as vast source for varied phytoconstituents exhibiting varied pharmacological property. Identifying such potential plants is of significance in medicine. In this connection, in the present study the methanolic leaf extract of two accessions of *M. edule* contains various phytocompounds. Secondary metabolites have proven to be medicinal in nature. They have various protective and therapeutic effects, which prevent diseases and maintain a state of well-being (Oyetayo, 2007).

These compounds are known to be biologically active. Tannins have been found to form irreversible complexes with proline-rich proteins (Hagerman and Butler, 1981) resulting in the inhibition of the cell protein synthesis. Tannins have important roles such as stable and potent antioxidants (Trease and Evans, 1983). Herbs that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery (Subhuti Dharmananda, 2003). Presence of Hexadecanoic acid, showing Antioxidant, Antiandrogenic, Hypocholesterolemic activities and used as nematicide, pesticide, lubricant, also it is an hemolytic 5-Alpha reductase inhibitor. Flavonoids have been referred to as nature's biological response modifiers because of strong experimental evidence of their inherent ability to modify the body's reaction to allergen, virus and carcinogens. They show anti-allergic, anti-inflammatory, anti- microbial and anti-cancer activity (Cushnie and Lamb, 2005; De Sousa *et al.,* 2007).

Tannins are known to possess general antimicrobial and antioxidant activities (Rievere *et al.,* 2009). Recent reports show that tannins may have potential value as cytotoxic and antineoplastic agents (Aguinaldo *et al.,* 2005). Other compounds like saponins also have anti-fungal properties (Mohanta *et al.,* 2007). Saponins are a mild detergent used in intracellular histochemistry staining to allow antibody access to intracellular proteins. In medicine, it is used in hyper cholestrolaemia, hyperglycemia, antioxidant, anticancer, anti-

inflammatory and weight loss, etc. It is also known to have anti-fungal properties (De-Lucca *et al.,* 2005). Saponins have been implicated as bioactive antibacterial agents of plants (Mandal *et al.,* 2005; Manjunatha, 2006). Plant steroids are known to be important for their cardiotonic activities, possess insecticidal and anti- microbial properties. Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity. Phenolic phytochemicals have antioxidative, antidiabetic anticarcinogenic, antimicrobial, antiallergic, antimutagenic and anti-inflammatory (Arts and Hollman, 2005; Scalbert *et al.,* 2005). The present report correlates along with the above bioactivities and phytocompounds by the earlier reports in the leaf extracts of *Memecylon umbellatum* (Murugesan *et al.,* 2011; Bharathi *et al.,* 2015).

4. CONCLUSION

The presence of various bioactive compounds present in the leaves of *M. edule* justifies the use of for various ailments by traditional practitioners. However, isolation of individual phytochemical compound will subjecting it to biological activity will definitely give fruitful results. It could be concluded that *Memcylon edule* contains various bioactive compounds. However, further studies will need to be undertaken to ascertain fully its bioactivity, toxicity profile, effect on the ecosystem and agricultural products.

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QUALITATIVE AND GC-MS ANALYSIS OF PHYTOCHEMICAL CONSTITUENTS OF TICK WEED (*CLEOME VISCOSA* L.)

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ABSTRACT

The potential of an allelopathic plant to exert direct and indirect effects depends in large part on the chemistry of the plant and whether putative allelochemicals reach meaningful levels in the environment surrounding the plant. *Cleome viscosa* L. (Capparidaceae) (synonym: *C. icosandra* L.) is a weed distributed throughout the tropics of the world and the plains of India Results on the qualitative analysis on the root, stem and leaves of *C. viscosa* showed that the presence of saponins and flavonoids in all their three organs. The presence of alkaloids was noticed only in Wagner's test not in the Mayor's and Dragendorff's test. GC-MS results of whole plant of *C.viscosa* showed the presence 3-O-Methyl-d-glucose (73%), followed by Benzofuran, 2,3-dihydro (9.844%) and : n-Hexadecanoic acid (4.707%) of the total 32 compounds.

Keywords: *Cleome viscosa*, GC-MS analysis, phytochemical.

1. INTRODUCTION

Allelochemicals are the small molecular weight compounds excreted from plants during the process of secondary metabolism (Rice, 1984). These chemicals usually accumulated in plants, soils, and other surrounding organisms. These compounds also vary in chemical composition, concentration and localization in plant tissues and from plant to-plant with changes in both biotic and abiotic conditions. (Waller and Einhellig, 1999). Allelochemicals produced in the tissues of such plants may enter soils as leaf leachates or root exudates or during tissue decomposition (Inderjit and Duke, 2003).There is even evidence for air borne allelopathy mediated by volatile allelochemicals (Matsuyama *et al.,* 2000).Impacts of putative allelochemicals produced by plants on other organisms can be direct, mediated through their acute or chronic toxicity to physiological processes in target organisms (Bais *et al*., 2003). Impacts can also be indirect, where putative allelochemicals modify the environment for other organisms in some way, such as through alterations in soil microbial communities, nutrient availability, or pH (Blum *et al.,* 1993).

The potential of an allelopathic plant to exert direct and indirect effects depends in large part on the chemistry of the plant and whether putative allelochemicals reach meaningful levels in the environment surrounding the plant. The suite of compounds possessed by each species has the potential to have both direct and indirect allelopathic effects, assuming that they can reach bioactive levels in the environment outside of the plant. For example, phenolic acids are noted for their direct toxicity to some organisms (Chon and Kim, 2002) and are also capable of interacting with nutrients in soils (Blum *et al.,* 1993), altering their availability to target organisms. The importance of such indirect effects probably varies across environmental gradients in the field.

Recently, allelopathy is getting more and more important. One reason is that this concept helps in the organic or natural farming without or less use of synthetic agrochemicals (herbicide, insecticide, fungicides, etc.). Other reason is the understanding of allelopathy in natural ecosystems. Allelochemicals belong to “Secondary metabolites”. Secondary metabolites mean not indispensable constituents in plants and exist only in plant kingdom. In the past, the meaning of these chemicals in plants seemed to be a pool of energy or reducing agents, or simple wastes. But recently, the Allelopathy hypothesis describes the real meaning of these secondary metabolites as a tool of immobile plants to protect themselves from surrounding plants or other life that might attack them, or a tool to communicate each other or to communicate with other life for their survival. It has been commonly assumed that there are more than 500,000 plant species and more than 30,000 secondary natural chemicals in this world. However, we are sure that there are still many natural chemicals unknown to us. Then the third importance of allelochemicals is their use as a source of new agrochemicals (Yoshiharu Fujii, 2009). Chemical components known to exert pharmaceutical effects may also be effective in allelopathy against other plants. On the other hand, chemical screening for allelopathy may lead us to the discovery of new biologically

functional compound. “Interdisciplinary information sharing” must, thus, be desired for a broader aspect. Hence the present work has been aimed to investigate the phytochemical analysis of Tick weed (*Cleome viscosa* L.) using GC-MS techniques.

2. MATERIALS AND METHODS

2.1. Qualitative phytochemical analysis

Qualitative phytochemical analysis (Jigna and Sumitra, 2007) of the crude powder of Tick weed (Cleome viscosa L.) was as follows:). Tannins (200 mg plant material in 10 ml distilled water, filtered); a 2 ml filtrate + 2 ml FeCl3, blue-black precipitate indicated the presence of Tannins. Alkaloids (200 mg plant material in 10 ml methanol, filtered); a 2 ml filtrate + 1% HCl + steam, 1 ml filtrate + 6 drops of MayorÕs reagents/WagnerÕs reagent/Dragendroff reagent, creamish precipitate/brownish-red precipitate/orange precipitate indicated the presence of respective alkaloids. Saponins (frothing test: 0.5 ml filtrate + 5 ml distilled water); frothing persistence indicated presence of saponins. Cardiac glycosides (Keller-Kiliani test: 2 ml filtrate + 1 ml glacial acetic acid + FeCl3 + conc. H2SO4); green-blue color indicated the presence of cardiac glycosides. Steroids (Liebermann-Burchard reaction: 200 mg plant material in 10 ml chloroform, filtered); a 2 ml filtrate + 2 ml acetic anhydride + conc. H2SO4. blue-green ring indicated the presence of terpenoids. Flavonoids (200 mg plant material in 10 ml ethanol, filtered); a 2 ml filtrate + conc. HCl + magnesium ribbon pink-tomato red color indicated the presence of flavonoids (Oguyemi ,1979).

2.2. GC-MS analysis

GC-MS analysis was carried out in SASTRA University, Thanjavur, Tamil Nadu. GC Clarus 500 Perkin Elmer system interfaced to a mass spectrometer (GC-MS) instrument employing the following conditions: column Elite-5ms fused silica capillary column (30 x 0.25 mm ID x 0.25µm film thickness, composed of 5% phenyl 95% Dimethyl polysiloxane), operating in electron impact mode at 70eV; helium (99.999%) was used as carrier gas at a constant flow of 1 ml /min and an injection volume of 1.0 µI was employed (split ratio of 10:1) injector temperature 290 ºC; ion-source temperature 200ºC. The oven temperature was programmed from 50ºC, with an increase of 87 ºC/min, to 220ºC hold for 5min, then 8ºC /min to 280ºC hold for 10 min. Mass spectra were taken at 70eV; a scan interval of 0.2 seconds and fragments from 40 to 600 Da.

2.3. Identification of Components

Interpretation on mass spectrum GC-MS was conducted using the database of National Institute of Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the separated components was compared with the spectrum of NIST library database. The identity of the spectra above 95% was needed for the identification of components.

3. RESULTS AND DISCUSSION

Cleome viscosa L. (Capparidaceae) (synonym: *C. icosandra* L.) is a weed distributed throughout the tropics of the world and the plains of India (Nadkarni, 1982). The plant is an annual, sticky herb with a strong penetrating odour, and is clothed with glandular and simple hairs. It grows about 30–90 cm high and is branched. The leaves are 3–5 foliate, obovate, and obtuse, gradually becoming shorter upward. The flowers are yellow, axillary, growing out into a lax raceme. The fruits are capsules, compressed and hairy throughout, while the seeds are finely transversely striate, sub globose, and become brownish-black when ripe (Vaidyaratnam, 1994). *C.viscosa* is known by various names such as wild mustard, dog mustard, and sticky cleome. In India, the plant is known by various vernacular names such as Hul-Hul, Pashugandha, Pivala tilvana, Kanphuti, Talwani,Naikkadugu etc.

Results on the qualitative analysis on the root, stem and leaves of *C.viscosa* showed (Table-1) that the presence of saponins and flavonoids in all their three organs. The presence of alkaloids was noticed only in Wagner's test not in the Mayor's and Dragendorff's test. GC-MS results of whole plant of *C.viscosa* showed (Fig-1 and Table-2) the presence 3-O-Methyl-d-glucose (73%), followed by Benzofuran, 2,3-dihydro (9.844%) and : n-Hexadecanoic acid (4.707%) of the total 32 compounds.

The seeds of *C. viscosa* are reported to have nutritive value, and have been found safe as edible material for human beings. The seeds are reported to contain 18.3% oil, a mixture of five fatty acids, seven amino acids, and sugar sucrose (Rukmini and Deosthale, 1979). The oil obtained from the seeds is rich in linoleic acid and other fatty acids such as palmitic, stearic, oleic, and linolinic (Rukmini, 1978; Afaq *et al.,* 1984; Deora *et al*., 2003). Gupta and Dutt (1938) reported two chemical constituents, viscocic and viscosin (a monomethoxy trihydroxyfavone), from the seeds. A novel umbelliferone derivative, designated as cleosandrin, has been isolated from the ethanol extract of the seeds (Ramchandran,

1979). The seeds are also reported to contain cleomiscosin- A, a coumarinolignoid (Ray *et al.,* 1980); cleomiscosin B (Ray *et al*., 1982); and cleomiscosin- C (Ray *et al*., 1985) and its regioisomer cleomiscosin D, a minor coumarino-lignan (Kumar *et al.,* 1988). Chattopadhyay *et al.* (2007) have developed a simple, accurate, and reproducible reverse-phase high performance liquid chromatography (HPLC) method for identifcation and quantifcation of two isomeric coumarino-lignoids, cleomiscosin A and B, in different extracts of the seeds using photodiode array detection at 326 nm.

Table 1. Qualitative phytochemical analysis of *C. viscosa*.

Weed Parts	Alkaloids			Saponins	Flavonoids	Steroids	Cardiac glycosides
	Mayor's test	Wagner's test	Dragendorff's test				
Root	-	+	-	+	+	-	-
Stem	-	+	-	+	+	-	-
Leaf	-	++	-	++	++	-	-

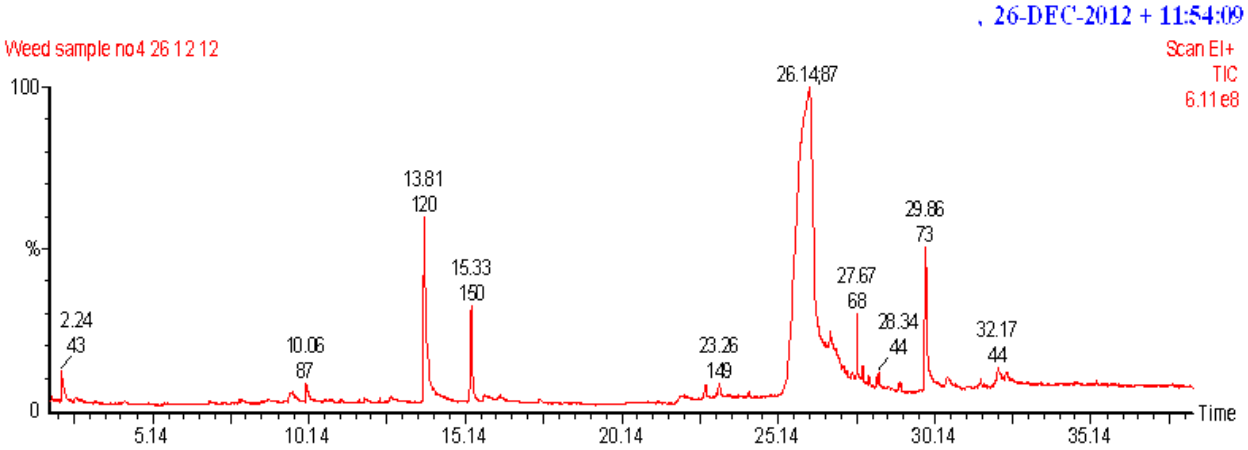

Fig.1. GC-MS spectrum of *C. viscosa.*

Table 2. Results of Phytochemical constituents of *C. viscosa* using GC-MS.

S.No.	Peak Name	Retention time	Peak area	%Peak area
1.	Name: 1,2-Ethanediol, monoacetate Formula: $C_4H_8O_3$ MW: 104	2.68	566902	0.1447
2.	Name: Acetic acid, 1-methylethyl ester Formula: $C_5H_{10}O_2$ MW: 102	3.33	191896	0.0490
3.	Name: 5-Hexen-2-one Formula: $C_6H_{10}O$ MW: 98	5.61	313432	0.0800
4.	Name: 2-Cyclopenten-1-one, 2-hydroxy- Formula: $C_5H_6O_2$ MW: 98	6.99	368597	0.0941
5.	Name: 2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan-3-one Formula: $C_6H_8O_4$ MW: 144	7.90	100411	0.0256
6.	Name: Alpha-monopropionin Formula: $C_6H_{12}O_4$ MW: 148	8.83	392761	0.1003
7.	Name: 5-Hexen-3-ol, 2,2,4-trimethyl- Formula: $C_9H_{18}O$	10.06	2105867	0.5375

	MW: 142			
8.	Name: 3-Penten-2-one, 3-ethyl-4-methyl- Formula: $C_8H_{14}O$	10.67	200057	0.0511
	MW: 126			
9.	Name: Cyclohexanol, 4-[(trimethylsilyl)oxy]-, cis- Formula: $C_9H_{20}O_2Si$	11.74	496708	0.1268
	MW: 188			
10.	Name: 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- Formula: $C_6H_8O_4$	11.93	880701	0.2248
	MW: 144			
11.	Name: cis-á-Terpineol Formula: $C_{10}H_{18}O$	12.41	336950	0.0860
	MW: 154			
12.	Name: 2,4,6-Octatriene, 2,6-dimethyl-, (E,Z)- Formula: $C_{10}H_{16}$	12.75	413609	0.1056
	MW: 136			
13.	Name: Benzofuran, 2,3-dihydro- Formula: C_8H_8O	13.81	38569260	9.8447
	MW: 120			
14.	Name: 2-Methoxy-4-vinylphenol Formula: $C_9H_{10}O_2$	15.32	12808473	3.2693
	MW: 150			
15.	Name: Hydroquinone Formula: $C_6H_6O_2$	15.76	3461378	0.8835
	MW: 110			
16.	Name: Phenol, 2,6-dimethoxy- Formula: $C_8H_{10}O_3$	16.25	3979017	1.0156
	MW: 154			
17.	Name: Benzaldehyde, 3-hydroxy-4-methoxy- Formula: $C_8H_8O_3$	18.03	117271	0.0299
	MW: 152			
18.	Name: Caryophyllene Formula: $C_{15}H_{24}$	18.75	130861	0.0334
	MW: 204			
19.	Name: 2-Butenoic acid, 4,4-dimethoxy-, methyl ester Formula: $C_7H_{12}O_4$	21.29	228778	0.0584
	MW: 160			
20.	Name: 3',5'-Dimethoxyacetophenone Formula: $C_{10}H_{12}O_3$	22.82	2371573	0.6053
	MW: 180			
21.	Name: Benzoic acid, 2-(1-oxopropyl)- Formula: $C_{10}H_{10}O_3$	23.26	1320971	0.3372
	MW: 178			
22.	Name: Megastigmatrienone Formula: $C_{13}H_{18}O$	24.20	581219	0.1484
	MW: 190			
23.	Name: 3-O-Methyl-d-glucose Formula: $C_7H_{14}O_6$	26.14	288638752	73.6740
	MW: 194			
24.	Name: Myo-Inositol, 4-C-methyl- Formula: $C_7H_{14}O_6$	26.71	1695484	0.4328
	MW: 194			
25.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$	27.67	4107243	1.0484

	MW: 296			
26.	Name: 2-Pentadecanone, 6,10,14-trimethyl- Formula: $C_{18}H_{36}O$	27.83	969780	0.2475
	MW: 268			
27.	Name: Methyl 6-methyl heptanoate Formula: $C_9H_{18}O_2$	29.06	1177290	0.3005
	MW: 158			
28.	Name: n-Hexadecanoic acid Formula: $C_{16}H_{32}O_2$	29.86	18441238	4.7071
	MW: 256			
29.	Name: 7-Hydroxy-6-methoxy-2H-1-benzopyran-2-one Formula: $C_{10}H_8O_4$	30.55	1856237	0.4738
	MW: 192			
30.	Name: 13-Octadecenal, (Z)- Formula: $C_{18}H_{34}O$	32.17	3461713	0.8836
	MW: 266			
31.	Name: Octadecanoic acid Formula: $C_{18}H_{36}O_2$	32.45	1015500	0.2592
	MW: 284			
32.	Name: 2H-Pyran-2-one, tetrahydro-6-nonyl- Formula: $C_{14}H_{26}O_2$MW: 226	35.30	478497	0.1221

Most tissues of plant, such as leaf, flower, fluid, stem, root and seed, even litter, can release a certain amount of allelochemicals into the surrounding environments. These allelochemicals can be very different as different parts or tissues of plants have different physiological functions. The extracts from the roots and stems were reported (Mo and Fan, 2001) that have autotoxicity and inhibit the root- ing and germination processes of *Braguiera gymnorrhiza,* yet other parts of the plants can stimulate its germination. Wu *et al.* (2001) examined the changes in allelopathic con tent 2,4-dihydroxy-7-methoxy 1,4-benzoxazin 3-one (DIMBOA) in different parts of wheat, and found that DIMBOA level in the root tissues is the highest followed by the stems. Ben-Hammouda *et al.* (2002) studied barley autotoxicity from the roots, stems and leaves extraction of barley, and the result showed that the leaves were the most important source of allelopathic substances, and the rootswere the last. Ben-Hammouda *et al.* (2001) also investi- gated the phytotoxicity of Hordeum vulgare on Triticum durum and *T. aestivum,* and showed that the allelopathic potential increased with physiological maturity, and leaves and roots were the most phytotoxic plant pats in *H. vulgare* plant parts.

Rice (1984) has classified these allelochemicals into 14 categories based on their diversiform chemical structures. Alleolochemicals, which can inhibit the growth of weeds,become the most favorable choice for natural pesticides (Nagabhushana *et al.,* 2001; Reigosa *et al.,* 2001). There have already been many identified allelochemicals that can be used to produce natural weedicides or pesticides (Xuan *et al.,* 2002). Some studies (Olofsdotter *et al.,* 2002) also show how to use allelochemicals as additives. In short, high diversity of allelochemicals means that they can be used in multiple purposes (Eng *et al.,* 2004). Extracting or synthesizing these compounds has great important ecological significance and economic potentials. The allelochemicals will become an important impetus for eco-agricultural development. On the other hand, studies on allelopathy can help explain the inhibitory effects or toxicity in the processes of rotation, intercrop and mulch and such studies can also help avoid wasting billions of dollars in worldwide agricultural practices.

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ALLELOPATHIC INFLUENCE OF SOME WEED RESIDUES ON GROWTH AND DEVELOPMENTAL CHANGES OF GREEN GRAM (*VIGNA RADIATA* (L.) WILCZEK)

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ABSTRACT

The term allelopathy refers to the detrimental effects of higher plants of one species (the donor) on the germination, growth, or development of another species (the recipient). In the present studyallelopathic influence of weed species, *Echinochloa colona* (L.) Link, (Poaceae), *Cleome viscosa* L. (Capparidaceae) and *Ammania baccifera* L. (Lythraceae) on green gram (*Vigna radiata* (L.) Wilczek were investigated. The individual and combined residues of three weeds wereincorporated to the soil at the quantities of 0,1,2,3 and 4% (w/w) in the plots and the germination, seedling growth dry weight of green gram were assessed. The results showed that all the concentrations of combined weed residues exhibited higher degree of inhibitory effects than the individual weed residues in all the parameters employed in the study except at 1% of *C. dactylon*, where insignificant growth promotion observed. The percentage of inhibitory effects of weed residues increases with increasing the magnitude of the residues. The degree of reduction percentage of all the growth parameters was concentration dependent.Among the three weeds, *A. baccifera* had more retarding effects on the growth of green gram and the order of inhibitory effect of three weed was *A. baccifera*, *C. viscosa* and *E. colona.*

Keywords: Allelopathy*, A. baccifera*, *C. viscosa*, *E. colona,* green gram, Weed residues.

1. INTRODUCTION

Allelopathy is recognized as an important ecological mechanism which influences plant dominance, succession and formation of plant communities, vegetation and crop productivity. It has been related to the problems with weed: crop interference. Weeds cause greater losses in crop yields than either insects or plant diseases. The weeds reduce the crop yields through (a) allelopathy, i.e., release of inhibitors from seeds, living plants and plant residues, (b) competition for growth resources (light, nutrients, water and space) with crops and (c) acting as an alternate host for insects and disease organisms. The decomposition of plant residues adds the largest quantity of allelochemicals to the soil. At plant death, materials compartmentalized in cells are released into the environment. The nature of the plant residues, the soil type are important pre requisite for decomposition. As the roots grow through the soil, at some points they may get in touch with decaying plant residues and are impacted by allelochemicals.The decomposition of plant residues potentially provides the largest quantity of allelochemicals that may be added to the rhizosphere. Patrick *et al*. (1964) reported that depending on the decomposing conditions, substances highly toxic, non-toxic or stimulatory to plants might be formed during the decomposition of similar plant residues.Different weed species differ widely in their ability to produce allelopathic effects (Hamayun *et al.,* 2005). Different parts of same weed also differ in their ability to produce allelopathic effects on germination and growth of crop plants. Some parts are inhibitorier than others (Tanveer *et al*., 2008). A number of studies have shown that allelochemicals release into the soil from residues of weeds, thus affecting the growth of crop plants (Kumar *et al.,* 2009). Furthermore, many allelopathic plants incorporated in soil are known to inhibit the growth of other plants (Rajashekhara *et al.,* 2007).

Green gram (*Vigna radiata* (L.) Wilczek), is one of the important pulse crop cultivated as intercrop along with rice and in the follow field of rice crop in Cuddalore District of Tamil Nadu India. Therefore, the present investigation, three common dominant weed species of paddy fields, namely, *Echinochloa colona* (L.) Link, (Poaceae), *Cleome viscose* L. and *Ammania baccifera* L. (Lythraceae) were selected to evaluate their allelopathic potential on germination and seedling growth of green gram (*Vigna radiata* (L.) Wilczek).

2. MATERIALS AND METHODS

Weed species were collected from post harvest paddy fields of Cuddalore District, Tamil Nadu and various quantities their residues were prepared from shade dried whole plant of the weeds

and also the equal quantity of three weed residues were mixed together for combined weed residues. Field experiments were conducted in the split plots (0.75 x0.75m) on the basis of Randomized Complete Block Design with three replicates. The weed residues were incorporated to the soil at the quantities of 0,1,2,3 and 4%(w/w)in the plots. The residue incorporated soil was allowed for three weeks afterwards the green gram cv.ADT-3 seeds were sown.

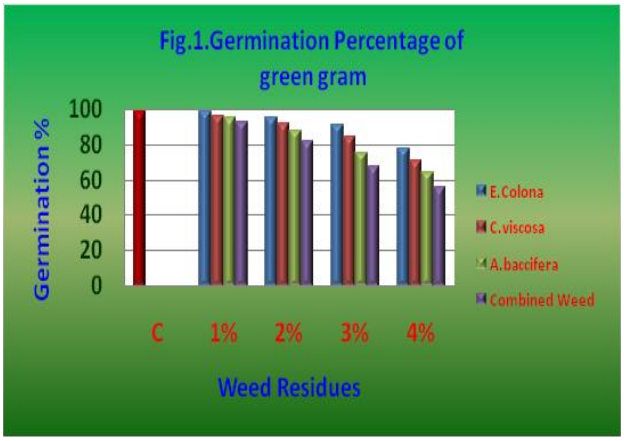
The parameters employed in the present studies are germination percentage,seedling length, root, stem and leaf biomass production of green gram were recorded at 15, 30, 45 and 60day old seedlings. Obtained data were analysed by ANOVA followed by Tuke'sMultiple Range Test at 5% probability level.

3. RESULTS AND DISCUSSION

Allelopathic influence of weed residues showed adverse effects on the seed germination (Fig.1), growth and pigment contents of green gram. The individual and combined residues of all the three weed species destructively influenced on the germination and growth of green gram except at 1% of residue of *E. colona,* where a slight non-significant stimulatory effects observed.Similar results were noticed by number of researchers. Quayyam *et al*. (2000) reported that the C*yperus rotundus* aqueous extracts and leachate of leaves and tubers significantly reduced the germination and seedling growth of rice. The leachate of *Echinochloa colonum* inhibited the germination and seedling growth of onion, radish and knolkhol (Challa and Ravindra,1998), *Cynodon dactyton*, *Cyperus rotundus* and other four weeds are adversely inhibited the seed germination of and growth of tomato (Sannigrahi and Chakrabortty, 2005). The root and shoot extracts of *Cyperus rotundus* decreased the seedling growth of rice with the reduction in root and shoot length, fading of leaves and curling of leaf tips (Bartariya *et al*, 2005). At higher concentrations of three weed residues showed maximum adverse effect on germination of green gram.The inhibition on seed germination may be due to the presence of high amount of allelochemicals in the weed residues.

Morphometric measurements *viz.,* seedling length, root, stem and leaf dry biomass of green gram crop were determined at four stages. A glimpse at tables-1-5 also reflected the inhibitory effects on germination. Apart from affecting germination, the root development, which plays a contributory role in plant growth, was also depressed. Understandably, a poorly development root system is disadvantageous to the emerging seedlings. This is exactly the trend emerging from the data where 16.8 to 65.6% of inhibition was evident. Allelochemicals decreased elongation, expansion and division of cells which are growth prerequisite (Qasem and Hill, 1989) Also, allelochemicals inhibit absorption of ions (Dos Santosh *et al.,* 2004) and therefore, resulted in arrested growth (Venkateshwarlu, 2001). One of the suggested explanation for disruption of seedling growth and development during allelopathy stress is modification in mitochondrial respiration leading to decreased supply of ATP for all energy demanding processes .The reduction of plant growth in the presence of allelochemicals is associated with the strong inhibition of mitosis or/and disruption of the structure of organelles e.g. nuclei and mitochondria (Gniazdowska and Bogatek, 2005).

All the concentrations of combined weed residues exhibited higher inhibitory effects than the individual weed residues in all the parameters employed in the study in all the four growth stages of the test crop. The percentage of inhibitory effects of weed residues increases with increasing the magnitude of the residues. The degree of reduction percentage was concentration dependent. The statistical analysis of all the parameters tested in the present investigation showed that significance effect of individual and combined extracts of three weed species on germination, growth, biochemical constituents and productivity of green gram.



Among the three weeds, *A. baccifera* had more retarding effect on the growth of green gram and the order of inhibitory effect of weed was *A.baccifera*, *C.viscosa* and *E.colona* .Hence it can be concluded that all the three weed species strongly exerted their negative allelopathic potential on the germination, growth and dry biomass of green gram (*Vigna radiata* (L.) Wilczek) cv-ADT-3.

Table 1. Root length (cm/plant) of Green gram plants treated with various concentrations of individual and combined weed residues.

Weed Residues (%)	*E. colona*				*C. viscosa*				*A. baccifera*				Combined weeds			
	DAS				DAS				DAS				DAS			
	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
C	7.5a	8.9b	16.0b	21.4a	7.5a	8.9a	16a	21.4a	7.5a	8.9a	16a	21.4a	7.5a	8.9a	16a	21.4a
1	7.8a	9.4a	17.1a	22.2a	7.2a	8.6a	15.6a	20.2b	6.8b	8.4b	15.3b	20.6b	6.7b	8.2b	15.5a	19.9b
2	7.2b	8.6b	15.2b	19.4b	6.7b	7.9b	14.7b	18.8b	6.4b	7.6c	14.3c	18.3c	6.2b	7.6b	13.7b	17.6c
3	6.4c	7.9c	13.7c	18.5c	6.1c	7.4c	13.0c	17.6c	5.8c	7.1d	12.7d	17.0d	5.4c	6.9c	11.8c	16.3d
4	6.2c	7.6d	13.0c	17.4d	5.7c	7.0c	11.6d	16.5e	5.4c	6.8d	11.2c	15.6e	5.0c	6.2d	10.2d	13.6e

Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) ($P < 0.05$).

Table 2. Shoot length (cm/plant) of Green gram plants treated with various concentrations of individual and combined weed residues.

Weed Residues (%)	*E. colona*				*C. viscosa*				*A. baccifera*				Combined weeds			
	DAS				DAS				DAS				DAS			
	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
C	15.3b	20.3b	23.8b	27.2b	15.3a	20.3a	23.8a	27.2a	15.3a	20.3a	23.8a	27.2a	15.3a	20.3a	23.8a	27.2a
1	16.2a	21.7a	25.3a	28.4a	14.8a	19.2b	22.7b	25.4b	14.5a	18.0b	21.8b	24.2b	13.5b	17.5b	21.3b	23.9b
2	14.1c	19.5b	22.8c	26.2c	13.9b	18.1c	19.8c	22.5c	12.0c	17.2b	18.3c	21.2c	10.8c	16.1c	16.1c	20.7c
3	13.5c	18.7c	21.1d	24.2d	11.5c	17.2d	19.0d	20.4d	10.4d	15.1c	15.8d	19.7d	9.3d	13.2d	14.3d	17.5d
4	12.0d	16.7d	19.19e	21.7e	10.1d	15.3e	16.9e	18.7d	8.3e	13.0d	14.3e	17.6e	6.8e	11.5c	12.5e	14.7e

Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) ($P < 0.05$).

Table 3. Root Dry weight (g/plant) of Green gram plants treated with various concentrations of individual and combined weed residues.

Weed Residues (%)	*E. colona*				*C. viscosa*				*A. baccifera*				Combined weeds			
	DAS				DAS				DAS				DAS			
	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
C	0.050b	0.232b	0.623a	0.664a	0.050a	0.232a	0.623a	0.664a	0.050a	0.232a	0.623a	0.664a	0.050a	0.232a	0.623a	0.664a
1	0.065a	0.248a	0.636a	0.673a	0.046b	0.215b	0.614a	0.637b	0.043b	0.206b	0.059b	0.605b	0.041b	0.187b	0.567b	0.584b
2	0.049b	0.225c	0.611b	0.626b	0.041c	0.197c	0.568b	0.603c	0.039c	0.179c	0.537c	0.575c	0.037c	0.165c	0.494c	0.553c
3	0.045b	0.209d	0.549c	0.604c	0.037d	0.178d	0.494c	0.577d	0.035d	0.016d	0.467d	0.534d	0.033d	0.146d	0.424d	0.503d
4	0.042c	0.193e	0.495d	0.578d	0.033e	0.155e	0.447d	0.505e	0.029e	0.141e	0.414e	0.465e	0.027e	0.124e	0.364e	0.427e

Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) ($P < 0.05$).

Table 4. Stem Dry weight (g/plant) of Green gram plants treated with various concentrations of individual and combined weed residues.

Weed Residues (%)	*E. colona*				*C. viscosa*				*A. baccifera*				Combined weeds			
	DAS				DAS				DAS				DAS			
	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
C	0.035a	0.333b	0.907b	1.531b	0.035b	0.333a	0.907a	1.531a	0.035a	0.333a	0.907a	1.531a	0.035a	0.333a	0.907a	1.531a
1	0.038a	0.357a	1.013a	1.731a	0.039a	0.325b	0.875b	1.366b	0.031b	0.294b	0.844b	1.194b	0.003b	0.248b	0.805b	1.102b
2	0.039a	0.314c	0.881c	1.227c	0.031c	0.283c	0.825c	1.093c	0.003b	0.267c	0.772c	1.053c	0.028b	0.204c	0.717c	0.944c
3	0.033b	0.282d	0.760d	1.102d	0.028d	0.253d	0.733d	1.002d	0.027c	0.214d	0.705d	0.993d	0.025c	0.184d	0.599d	0.893d
4	0.029c	0.263e	0.618e	0.962e	0.026d	0.224e	0.576e	0.896e	0.025d	0.197e	0.544e	0.866e	0.022d	0.167e	0.474e	0.736e

Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) ($P < 0.05$).

Table 5. Leaf Dry weight (g/plant) of Green gram plants treated with various concentrations of individual and combined weed residues.

Weed Residues (%)	*E. colona*				*C. viscosa*				*A. baccifera*				Combined weeds			
	DAS				DAS				DAS				DAS			
	15	30	45	60	15	30	45	60	15	30	45	60	15	30	45	60
C	0.083a	1.073a	4.027a	4.429a	0.083a	1.073a	4.027a	4.429a	0.083a	1.073a	3.937a	4.429a	0.083a	1.073a	4.027a	4.429a
1	0.090a	1.184a	4.145a	4.633a	0.080a	1.044a	3.826a	4.222a	0.077a	1.030a	3.528b	4.058a	0.075b	1.001a	3.326b	3.888b
2	0.079b	1.039b	3.698b	4.200c	0.076b	1.013b	3.548b	3.888b	0.074b	0.957b	3.382c	3.669b	0.069c	0.941b	3.063c	3.273c
3	0.072c	1.010c	3.325c	3.822d	0.069c	0.871c	3.133c	3.450c	0.067c	0.793c	2.863c	3.212c	0.057d	0.713c	2.326d	3.050d
4	0.070d	0.893d	3.078d	3.656e	0.062d	0.813d	2.848d	3.150d	0.059d	0.717d	2.427d	2.662e	0.052e	0.574d	2.027e	2.519e

Mean with different alphabets in a column differed significantly as per Tukey's Multiple Range Test (TMRT) ($P < 0.05$).

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ALLELOPATHIC INFLUENCE OF *CASUARINA EQUISETIFOLIA* L. ON GROWTH AND DEVELOPMENT OF RICE (*ORYZA SATIVA* L.)

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ABSTRACT

Leaf extracts of *Casuarina. equisetifolia* L. was evaluated for its allelopathic influenced on rice cultivars *viz*. IR- 20 and TKM – 9. Leaf extracts was tested at 2.5, 5, 10, 15 and 20% concentration. Seed germination, shoot length, biomass, chl - a , chl- b, carotenoids, starch, protein and amino acid contents were significantly reduced by leaf extracts and highest inhibition was observed in 20% concentration. But at lower concentration (2.5%), the seedling growth was slightly enhanced than control. The higher degree of germination and growth inhibition was observed in cultivar TKM-9 than IR-20.

Keywords: Allelopathy, *Casuarina equisetifolia*, rice cultivars.

1. INTRODUCTION

Agroforestry is the integration of Agriculture and forestry to increase the farm productivity and sustainability of farming system. *Casuarina equisetifolia* L. is a commonly grown in coastal regions of Tamil Nadu. It is an evergreen tree fixing atmospheric nitrogen and sheds the needles at maturity. Plants compete with each other for light, water and nutrients. Whenever two or more plants occupy the same niche in nature, they compete with each other for various life support requirements (Caton *et al*., 1999). Production of secondary metabolites, accumulation and release of these compounds is one of several complex defense strategies that have evolved by plants (Rice, 1984; Swain,1977). There are hundreds of secondary metabolites in the plant kingdom and many are known to be phytotoxic (EINHELLING, 2002). The interaction of plants through release of chemical is called allelopathy and it includes both positive and negative effects of one plant on the other.

In agro ecosystems several weeds, crops, agro forestry trees and fruit trees have been shown to exact allelopathic influence on the crops, thus, affecting their germination and growth adversely (Kohli *et al.,* 1998). The chemical interaction of plants through chemical signals or allelopathy has many possible agricultural applications and decline in crop yields in cropping and agro-forestry decades systems in recent years has been attributed to allelopathic effects. Allelochemicals were reported to be highest in the foliage of many plants; these chemicals were found to be released into the soil system through volatilization, root exudation and leaching from the foliage (Fisher, 1980). Systematic research approach in allelopathy was started only recently in the last two decades and allelopathic influence of multipurpose tree species on crops are being investigated under different agro-eco systems. Hence, this investigation was carried out to study the allelopathic potential of *Casuarina equisetifolia* leaf extracts on two rice cultivars viz. IR-20 and TKM- 9.

2. MATERIALS AND METHODS

The two varieties of Rice seeds such as IR-20 and TKM-9 were obtained from Tamil Nadu Agricultural University, Coimbatore, Tamil nadu. To prepare aqueous extracts, 200g dried powdered needles of *C. equisetifolia* were soaked in 1000ml water in a flask for 48h at room temperature (30±2°C) .Then filtered through Whatman No.1 filter paper. These extracts were of 20% concentration and further diluted to 2.5, 5, 15 and 20% concentrations. 25 seeds of IR-20, TKM-9 were treated with 0.1% mercuric chloride, washed thrice with distilled water and dried on an absorbent to eliminate fungal attack then sown in 20cm diameter pot containing normal garden soil. Tap water used as control. Different concentrations of extracts or tap water were added as per the treatment in alternative days up to 12 DAS. Germination percentage, plumule length, radical length and seedling dry weight and fresh weight, Pigment contents Chl- a, chl-b, (Arnon,1949), carotenoids (Kirk and Allen, 1965), starch (Clegg, 1956), protein (Lowry *et al*., 1951) and amino acid (Moore and Stein, 1948) contents were estimated on 12 days old seedlings of rice cultivars. The data was statistically analysed by ANOVA followed by Tuke's Multiple Range Test (TMRT) at $P<0.5\%$ level.

3. RESULTS AND DISCUSSION

C. equisetifolia leaf extracts decreased the germination (%) of rice cultivars IR-20 and TKM-9 (Table 1 and Fig. 1). The per cent reduction over control in the rice cultivars with different concentration of leaf extracts was maximum in TKM-9 (58.3%) than IR- 20 (46.9%). Among the concentration, maximum inhibition was observed in 20% followed by 15, 10, 5 and 2.5% (Table 1). The different leaf extracts of *C. equisetifolia* significantly decreased the germination. The inhibition of germination is dependent on the concentration of the extracts; perhaps it may be due to the entry of water soluble allelochemicals from the leaf of *C. equisetifolia.* Similar response of sorghum and sunflower exhibited to the extracts of *C. equisetifolia* leaf leachates (Singh, 1993; Suresh,and Vinaya Rai, 1987). The allelopathic compounds in soil come in contact with the roots of tested plant and may alter its absorption capacity for water and minerals, cell division and other physiological functions (Majeed *et al*., 2012).

Nandal *et al*. (2005) reported the aqueous extracts of poplar leaves adversely affected the germination and seedling growth of some wheat varieties at high extract concentration. The chemicals have harmful effects on the crop in the eco-system resulting in the reduction and delaying of germination, mortality of seedlings and reduction in growth and yield (Mcworthier, 1984; Herro and Callaway, 2003).

At lower concentration of (2.5%) *C.equisetifolia* leaf extracts enhanced the seedling growth of both IR -20 and TKM-9 but at higher levels (5, 10, 15 and 20%) inhibit the seedling growth (Table- 1). At 20% extract concentration the fresh weight decrease was in 53.6% in IR-20 and 57.7% in TKM-9. The variety, TKM-9 was found to be most sensitive than IR -20. Similar works on leaf leachates of *C. equisetifolia* significantly decreased the germination, plumule and radicle growth of rice and cowpea (Jadhav and Gaynar, 1995). The study of Umarani and Selvaraj (1996) showed that the stem and whole plant extract of *Trianthema portulacastrum* reduced the dry matter production on soybean. Beres and Kazinczi (2000) reported that the aqueous shoot extract of *Rumex obtusifolius* and *Asclepias syriaca* reduced the fresh and dry weight of corn. June (1976) reported the presence of phytotoxins, phenolics, terpenoides and organic cyamides in *Casuarina* extracts that cause allelopathic effect. Similarly, Jacob and Nair (1999) reported inhibitory effect of *Casuarina* leaf extracts on germination, plumule and radicle growth in rice and cowpea. This reduction in seedling growth and biomass may be due to imbalances in water uptake or osmotic balances of the tissues because of allelochemical toxicity (Blum *et al.,* 1999) and or root growth inhibition (Chon *et al*., 2002). Chon *et al*. (2002) mentioned that some plants root tip growth nearly inhibited to escape from allelochemicals absorption. Nevertheless, Sing *et al.,* (2003) found that aqueous leaf leachates of *Eucalyptus citriodora* inhibited seed germination and seedling growth of *Vigna* Species and elongation of plumule more suppressed than radicles. The aqueous extracts of seeds, leaf, root of *Ageratum conyzoides* decreased the root and shoot elongation in chickpea (Angiras *et al*., 1988). Aqueous extract of some plants inhibit seedling growth (Athanassova, 1996), root and shoot growth (Das and Bandyopadhyay, 2011).

Chlorophyll a, b and Carotenoids contents were increased at both test crops at lower concentration of (2.5%) of leaf extracts (Table 2). But at 20% chlorophyll a was reduced by 48.3% and 53.0% and Chlorophyll b was reduced by 54.3% and 56.4% respectively, in TKM-9 and IR-20. In the test crops, IR-20 and TKM-9 was showed a maximum decrease of chlorophyll b than chlorophyll a. The reduction in chlorophyll contents observed in all the concentrations might be due to the degradation of chlorophyll pigments or reduction in their synthesis and the action of flavonoids, terpenoids or other phytochemicals present in the leaf extracts (Tripathi *et al.,* 1999; 2000). The more reduction of chlorophyll b than chlorophyll a, indicated its susceptibility to stress (Djanaguiraman *et al.,* 2003). During stress situation, in tolerant species conversion of chlorophyll b to chlorophyll a may occur (Djanaguiraman *et al.,* 2003). Carotenoids may decrease the photosynthesis and thereby substantially decrease all the metabolities *viz.,* total sugars, proteins and soluble amino acids (Singh and Rao, 2003). Reduction in pigments was previously reported as a result of allelochemical stress (Ervin and Wetzel,2000; Moradshahi *et al*., 2003; Singh *et al*., 2009). A correlation between photosynthetic alternation and the action of some allelochemical compounds was shown in previous works (Einhellig, 1986; Heji *et al.,* 1993) being the disruption of electron transport chain one of the most usual ways for affecting photosyntheisis by allelochemical compounds (Nimbal *et al*., 1996 and Gonzelz *et al*., 1998).

Table 1. Effect of *Casuarina equisetifolia* leaf extracts on germination %(G.%), plumule length (P.L.), radicle length (R.L.), fresh weight (F.Wt.) and dry weight (D.Wt.) of two Rice cultivars.

Extract Conc. (%)	IR-20					TKM-9				
	G. %	P.L.	R.L.	F.Wt.	D.Wt.	G.%	P.L.	R.L.	F.Wt.	D.Wt.
C	98	6.63	3.58	98.60	32.19	96	6.55	3.60	99.60	33.12
2.5	100 (2.0)	6.79 (2.4)	3.69 (3.1)	98.90 (0.3)	32.40 (0.7)	98 (2.1)	6.78 (3.5)	3.74 (3.9)	99.85 (0.3)	33.34 (0.7)
5	96 (-2.0)	6.12 (-7.7)	3.36 (-6.1)	90.12 (-8.6)	30.14 (-6.4)	90 (-6.3)	5.83 (-11.0)	3.20 (-11.1)	87.12 (-12.5)	25.09 (-8.7)
10	82 (-16.9)	5.55 (-16.3)	2.85 (-20.4)	80.48 (-18.4)	25.85 (-19.7)	78 (-18.8)	5.10 (-22.1)	2.65 (-26.4)	76.12 (-23.6)	21.28 (-20.1)
15	69 (-29.6)	4.57 (-31.1)	2.21 (-38.3)	65.89 (-33.2)	20.61 (-36.0)	60 (-37.5)	4.30 (-34.4)	1.98 (45.0)	64.65 (-37.1)	18.85 (-39.4)
20	52 (-46.9)	3.37 (-49.2)	1.55 (-56.7)	45.76 (-53.6)	13.79 (-57.2)	40 (-58.3)	2.99 (-54.4)	1.27 (-64.7)	42.13 (-57.7)	2.96 (-60.3)

Data in parenthesis indicates % increase (+), decrease (-) over control.

Table 2. Allelopathic effect of *C.equisetifolia* leaf extracts on pigments (Chlorophyll a, b and Carotenoid contents (mg/g.fr.wt.)) of Rice cultivars

Extracts Concentrations (%)	IR - 20			TKM-9		
	Chl. a	Chl. b	Carotenoids	Chl. a	Chl. b	Carotenoids
Control	1.45	1.05	0.84	1.51	1.10	0.70
2.5	1.64 (13.1)	1.17 (11.4)	0.93 (10.7)	1.70 (12.6)	1.20 (9.1)	0.76 (8.6)
5	1.34 (-7.6)	0.95 (-9.5)	0.74 (-11.9)	1.37 (-9.3)	0.97 (-11.8)	0.61 (-12.9)
10	1.19 (-17.9)	0.85 (-19.0)	0.65 (-22.6)	1.18 (-21.9)	0.85 (-22.7)	0.53 (-24.3)
15	0.99 (-31.7)	0.69 (-34.3)	0.53 (-36.9)	0.99 (-34.4)	0.69 (-37.1)	0.43 (-38.6)
20	0.75 (-48.3)	0.48 (-54.3)	0.37 (-56.0)	0.71 (-53.0)	0.48 (-56.4)	0.29 (-58.6)

Data in parenthesis indicates % increase (+), decrease (-) over control.

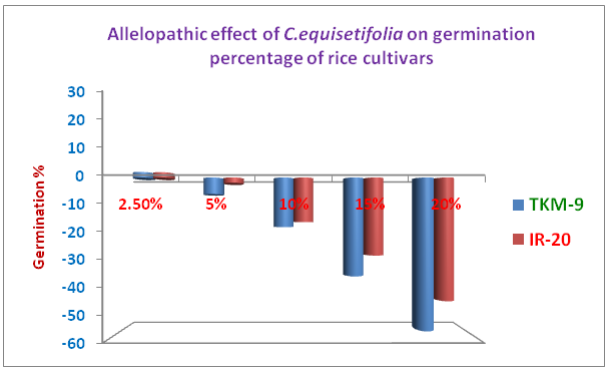
Table 3. Allelopathic effect of *C. equisetifolia* leaf extracts on Starch, protein and Amino acid contents (mg/g.fr.wt.) of Rice cultivars

Extracts Concentrations (%)	IR - 20			TKM-9		
	Starch	Protein	Amino acids	Starch	Protein	Amino acids
Control	5.20	2.28	1.54	5.35	2.47	1.58
2.5	5.49 (5.6)	2.40 (5.3)	1.61 (4.5)	5.61 (4.9)	2.57 (4.0)	1.64 (3.8)
5	4.96 (-4.6)	2.13 (-6.6)	1.40 (-9.1)	5.08 (-5.0)	2.26 (-8.5)	1.37 (-13.3)
10	4.30 (-17.3)	1.74 (-23.7)	1.16 (-24.7)	4.36 (-18.5)	1.87 (-24.3)	1.15 (-27.2)
15	3.47 (-33.3)	1.44 (-36.8)	0.94 (-39.0)	3.44 (-35.7)	1.47 (-40.5)	0.92 (-41.8)
20	2.60 (-50.0)	1.09 (-52.2)	0.63 (-59.1)	2.49 (-53.7)	0.99 (-59.9)	0.57 (-63.9)

Data in parenthesis indicates % increase (+), decrease (-) over control.

The biochemical constituents i.e. starch, protein and amino acid contents showed the same trend like pigments contents of rice seedlings (Table-3). The 20% of leaf exract concentration had more inhibitory effect on starch (50% and 53.7%), protein (52.2% and 59.9%) and amino acid (59.1% and 63.9%) contents respectively for IR-20 and TKM- 9 over control. The application of aerial or root biomass of *Rhamnus virgattus* tree significantly decreased the starch content in *Triticum aestivum, Eleusine coracana, Lens culinaris and Phaseolus mungo* as compared to control (Prasad *et al*., 1999). The leaf extract of *Populus deltoides* reduced protein content in three varieties of green gram (Mandal *et al*., 2005). Tripathi *et al.,* (1998) studied the allelopathic activity of *Tectona grandis, Albizia procera and Acacia nilotica* on starch, protein and amino acid contents of soy bean. Leaf extracts of all the three species at the lower concentration showed stimulated effect and at the higher concentration showed the inhibited effect on biochemical constituents (starch, protein and amino acid) in the soybean. These studies are in conformity with the present findings.

Fig 1. Germination % of two Rice cultivars against the *Casuarina equisetifolia* leaf extracts treatments.



Based on the results it can be concluded that allelopathy is a concentration dependent phenomenon, as the concentration of the *C. equisetifolia* leaf extracts increases, its effect also increases . But at lower concentration (2.5%) of leaf extracts showed promotory effects on all the studied parameters in both the test crops.. In general the inhibitory effect was observed more in TKM-9 than IR- 20. The negative allelopathic effects of *C. equisetifolia* on IR- 20 and TKM-9 may be due to the presence of allelo chemicals in the extracts, particularly phenolics and other secondary metabolites like growth regulators, alkaloids, terpenoids, organic cyanides and toxins which are reported by June, (1976). However, further studies required to identify the specific phytochemical of *C. equisetifolia* and their allelopathic actions on crop growth under field study.

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ALLELOPATHIC INFLUENCE OF *TRIANTHIMA PORTULACASTRUM* L. ON GROWTH AND DEVELOPMENTAL RESPONSES OF SESAME (*SESAMUM INDICUM* L.)

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ABSTRACT

An experiment was conducted in order to determine the allelopathic effects of the aqueous extract of *Trianthima portulacastrum* L.on the seed germination, seedling growth and chlorophyll content ofsesame (*Sesamum indicum* L.). Greenhouse experiment was carried out as RCBD (Randomized complete block design) with four replications. Treatments included 0, 1, 2, 3 and 4% (W/W) residues of whole plant of *T.portulacastrum* with normal field soil. Results showed that the low concentrations of *T.portulacastrum* had no significant effect on the germination percentage, seedling length, dry weight, total chlorophyll contents at lower concentration(1%) of weed residues. However, treatments with higher concentrations had negative effects on germination, growth and seedling dry weight of sesame.

Keywords: Allelopathy, Chlorophyll, Germination, *Sesamum indicum, Trianthima portulacastrum.*

1. INTRODUCTION

Allelopathy is a phenomenon of direct or indirect, beneficial or adverse effects of a plant on its own or another plant through the release of chemicals into the environment. It affects plant distribution, community formation, intercrop evolution and biodiversity conservation and is now arousing further international interest (Zhang *et al.,* 2004). Allelopathy occurs in every ecosystem, from forests andgrasslands to deserts. Plants produce numerous chemical compounds during the period of growth. These compounds become free in terms of leaching gas from shoots, root discharges, or by decomposing of plants remaining at the environment (Roa, 2000). Chemicals extracted from plant roots or shoots (allelochemicals) have been shown to directly inhibit or stimulate germination, growth, and development of other plants (Putnam and Weston, 1986). The aim of this research was to evaluate the possible effects of the weed species, *Trianthima portulacastrum* L. on the seed germination characteristics, seedling growth and chlorophyll content of sesame (*Sesamum indicum* L.). Sesame is a crop of greatantiquity which is widely grown in tropical and subtropical regions of Asia, Africa, South and North America and especially in Tamil Nadu of India for edible oil and for animal feed purposes.*T. portulacastrum* is an annual herb of flowering plant in the Aizoaceae known by the common names desert horse purslane, black pigweed, and giant pigweed. It is native to areas of several continents, including Africa and North and South America, and present as an introduced species in many other areas. It grows in a wide variety of habitat types and it can easily take hold in disturbed areas and cultivated land as a weed.

2. MATERIALS AND METHODS

This study was conducted to findout the allopathic influence of *Trianthima portulacastrum* L.on seed germination, initial growth properties and pigment changes of sesame (*Sesamum indicum* L.) cv TMV-1. *T. portulacastrum* (Fig.-1) were collected from crop fields located in Faculty of Agriculture, Annamalai University and the green house experiments were carried out at Botanical garden, Department of Botany, Annamalai University. The weeds were shade dried for12daysthenchopped to a fine pieces andwere mixed with soil at the proportion of 0%, 1%,2%,3% and 4% in 3kg of garden soil. Earthen pots (30 x 15cm) werefilledwith different rate of weed residues and soil for the germination studies. The viable seeds of sesame were surface sterilized for two minutes in 0.2% mercuric chloride ($HgCl_2$), washed thoroughly in running tap water and sown @15 seeds/pot^{-1}. Each pot was irrigated uniformly with normal tap water (pH-7.2) on alternate days up to the 15th day. Germination was recorded upto seventh day after seed sown (DAS). On the seventh and fifteenth day, growth characteristics such as seedling length, dry weight and total chlorophyll contents (Arnon, 1949) were recorded. The depth of significance between the treatments could be brought out clearly by multiple range testing programme.

3. RESULTS AND DISCUSSION

The green house studies showed that the weed residues of *T. portulacastrum* significantly

reduced germination of sesameover the control and the magnitude of reduction differed depending upon the concentration of the weed residues employed (Fig. 2-5). The 1% residues slightly inhibited germination while the residues of higher concentrations the seeds were drastically reduced their germination potential. Seed germination is considered to be the most critical stage especially under stress conditions. During germination, biochemical changes take place, which provides the basic framework for subsequent growth and development. The initial metabolic changes that occur immediately after the imbibitions of water are the increase in the hydrolytic enzymes, such as alpha-amylase and protease. Alphaamylase is an important starch degrading enzyme in the endosperm. The reaction products provide substrate and an energy source for the embryo during germination. The inhibition of seed germination is also due to disturbance in the activities of peroxides, alpha-amylase, and acid phosphates. Inhibition of seed germination of crop plants is also due to disturbance in the activities of peroxidase, alpha-amylase and acid phosphates (Alam and Islam, 2002) The results of present study revealed the marked allelopathic potential of *T. portulacastrum* on sesame. These results are in accordance with the findings of *Meihua et al.* (2006) who reported inhibitory effects of water extracts of *Lactariushatsudake* on seedling growth of rape (*Brassica campestris*) and radish (*Raphanus sativus*).

Fig.1. *Trianthima portulacastrum*

The weed *T. portulacastrum* contains water-soluble bases and potassium salts , punarnavine and a new alkaloid , trianthemine and ecdysterone are present in the aerial parts . They also contain oxalic acid. 5,2-dihydroxy-7-methoxy-6,8-dimethylflavone and 5,7- dihydroxy -6,8- dimethyl- chromone (leptorumol). Roots contain saponin glycoside (Ghani, 2003). Sherif and Gharieb (2011) reported the presence of P-Hydroxybenzoic acid, Caffeic acid, Vanillicacid,ferrulic acid, o-coumaric acid, Pyrogallic acid, Protocatechuic acid and trans-Cinnamicacidin the leaves andstem of *T. portulacastrum.* These allelochemicals play an important role in allelopathic interactions, and their biological activities on growth of some crop plants and weeds were studied using different bioassay tests (Chung *et al.,* 2002).

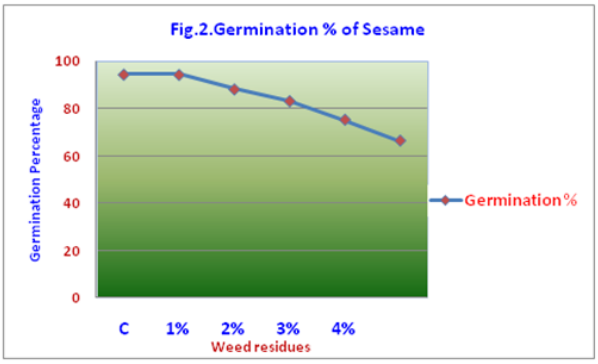
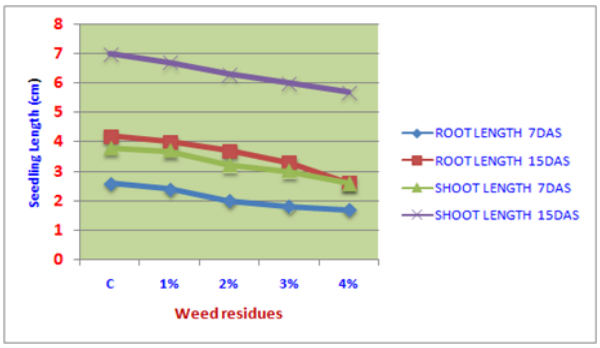


Fig.3. Seedling length (cm/plant) of sesame



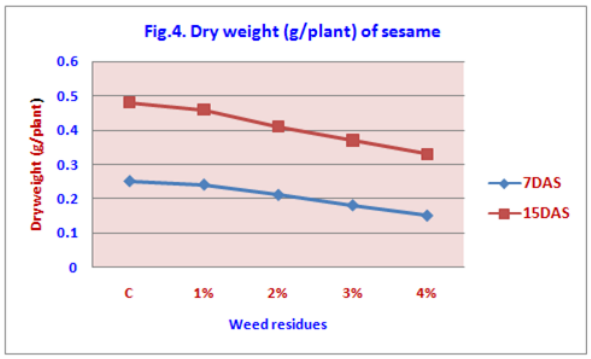
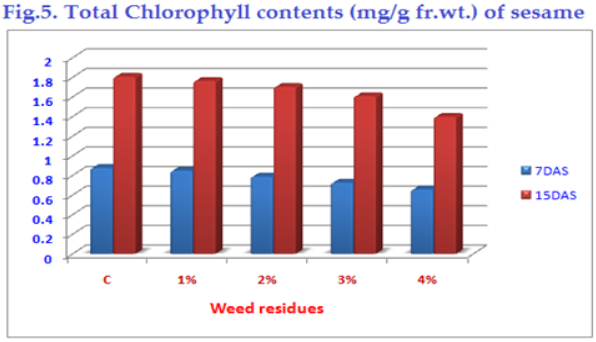


Fig.5. Total Chlorophyll contents (mg/g fr.wt.) of sesame



It seemsthat these compounds together with someother unknown compounds in *T. portulacastrum* are responsible for its allelopathic behaviors. Residues of T.*portulacastrum* significantly decreased and delayed the seed germination of target plants withincreasing residual concentration, that is, from control (0%) to 4%, the degree of inhibition increased. The allelopathic effects of *Scariola orientalis* and *Agropyron elongatum* were studied on *Onobrychis viciaefolia* germination propertiesby Rezaei and Khajeddin (2008). They found that theextracts of the two allelopathic species significantly decreased the seed germination, germination rate, andgermination speed of *O. viciaefolia*. Allelopathic extract could be confounded with osmoticeffects on rate of inhibition, delayed initiation of germination,and especially cell elongation (Black, 1989); the mainfactor that affects root growth before and after the tippenetrates the seed coat (Bewley and Black, 1978). Bhawmik and Doll (1982) stated that allelopathy influencedseed germination and seedling development bypreventing cell division and inhibiting cell elongation.Avers and Goodwin (1956) reported that phenolic compounds,as main parts of allelochemicals, prevented rootcell division. From studies using aqueous alfalfa leafextract by Chon *et al.* (2004), they concluded that delayedseed germination and, especially, reduced root elongationwere due mainly to toxic factors of the leaf extract

The results of seedling lengthand dry weight are presented in tables-1& 2. Aqueous extract of *T. portulacastrum* decreased the root length, shoot length and fresh weight of sesame in both the sampling days. The adverse effect gradually increased which resulted in the growth and biomass decreased in the sesame seedlings. The probable reason could be the inhibitory effect of allelochemicals in uptake of water by seedlings and reduction in other physiological processes of the crop. The reduction in dry weight per seedling was due to reduction in root length and root thickness.Water soluble inhibitors could be the reason of reducing the root and shoot length of rice significantly (Kil and Yun, 1992). Cell division might have been affected which reduced the root and shoot lengths of rice seedlings as allelopathic compounds are known to inhibit functioning of gibberellin and indole acetic acid (Tomaszewski and Thimann 1966).Several studies have noticed that many secondary metabolites are released into the environment, either as exudation from living plant tissues or by decomposition of plant material under certain conditions (Einhelling, 1995). These chemicals like phenolics, terpenoids and alkaloids and their derivatives are potential inhibitors of germination, seedling growth, fresh weights and dry weights (Herro and Callaway, (2003); Siddiqui and Zaman, (2004); Siddiqui and Zaman (2005).

The photosynthetic pigmentstotal chlorophyll contents were gradually decreased with increasing the quantity of *T. portulacastrum* residues (Fig. 2).The decrease in chlorophyll synthesis is a common response of plants toallelochemicals, and this might be a subsequent response of plant to these chemicals beside cellular damage. Allelochemicals adversely affect chlorophyll biosynthesis and accumulation by interfering in chlorophyll biosynthesis and/or destruction. The upcoming negative effects of these processes would be retarding of photosynthesis and poor plant growth (Tanveer *et al.,* 2008).

4. CONCLUSION

The present findings revealed that residues of *T. Portulacastrum* at various quantity levels inhibited the germination, growth and reduced dry weights of sesame seedlings. The germination and growth suppression of sesame seedlings indicate that the allelochemicals released into soil after decomposition residues of *T. portulacastrum.* Further, the detailed studies are required to explain the possible physiological mechanism related to allelopathic effects on soil health and other plants.

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