DOI:10.26524/krj.2021.13

*Kong. Res. J.* 8 (2): 1-7, 2021
**Publisher**: Kongunadu Arts and Science College, Coimbatore.

https://www.krjournal.com

**RESEARCH ARTICLE**

# SACRED GROVES – AN ANCIENT TRADITION OF NATURE CONSERVATION OF STHALAVRIKSHAS IN COIMBATORE, TAMIL NADU, INDIA

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**ABSTRACT**

In India sthalavrikshas worship in temples was a religrous practice. In Tamil Nadu almost every temple is associated with a plant or tree connected to the history and mythology of the temple and or deity. The worshipper who comes to the temple attains a healty spiritua; enlighten. Sthalavrikshas is a natural tree found in the temple site brfore construction of the temple. The present investigation was carried out in coimbatore district to enumerate the sthalavrikshas associate with temple by field observation method. During the field visit temples were frequently visited and surveyed for the sthalavrikshas from the month of December 2019 - February 2020. The survey was conduced at 46 ancient temples of the coimbatore district and revelated the occurrence of 19 sthalavrikshas species were observed in different area of coimbatore district. These sacred plants are worshiped by the local people for getting the blessing of health and wealth by positive powers of nature. Sthalavrikshas are germplasm reservers and an indicator of socio-cultural conservation strategy. It is concluded that the Sthalavriksha worship is an age old practice, myths, beliefs and floklore play a major role in the exsistance of Sthalavrikshas worship and this customms help in plant conservation.

**Keywords:** Myths, Temple, Worship, Sthalavrikshas

## 1. INTRODUCTION

In ancient periods, The India people of India considered some of the trees as sacred and worship in Indian mythology and by folklore is called Sthalavrikshas. Sthalavriksha or sthalavruksham are monumental trees that are indigenous to every historical Hindu temples [1]. Sthalavriksha refers to the plant of which 'Sthala' means Place and 'Vriksha' means tree. Sthalavriksha are valued for their botanical, medicinal, environmental, religious and mythical importance [2]. Sthalavriksha are an integral part of worship and their practice is still in vogue in Tamil Nadu and its bordering states of Kerala, Karnataka, Andhrapradesh and the neighbouring island nation Sri Lanka [3].

Sthalavrikshas are single plant and mostly in the form of a tree or in some places occurring as an herb, shrub, grass or climber. Many trees have been very important in Indian Mythology, such as *Emblica officinalis, Ficus religiosa, Anthocephalus chinensis, Mangifera indica Azadirachta indica* , *Prosopis cineraria*, *Stychnos-nux-vomica, Ficus glomerata, Eugenia jambolana, Acacia catchu, Vepris bilocularis, Bambusa arundinaceae, Mesua ferrca, Butea monosperma, Elaeodendron glaucum, Jasminum auriculatum, Aegle marmelos, Terminalia arjuna, Musa ferrea, Calamus sp., Atrocarpus heterophyllus, Calotropis gigantean and Madhuca longifolia* [4].

The Sthalavrikshas of Tamil Nadu constitute a part of genetic resources for the conservation of species diversity and forms an important biological heritage of our nation [5]. The social, economical, medicinal and environmental importance of these trees was recognised and the *Sthalavriksha* evolved as a means of conserving the land's rich genetic plant diversity. They are symbolic of a single genetic resource and play an important role in the conservation of biodiversity. Hence, the present was designed to enumeration



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and catalogue the stharavrilshas species and their associated details in the temples of Coimbatore.

## 2. MATERIALS AND METHODS

2.1. *Study area and Study period*

In Tamil Nadu state, Coimbatore has many temples and customary practice follows with religious faith and culture. So that Coimbatore district is selected as a study area. It is lies in the western part of Tamil Nadu state. It is bounded by the Nilgiris district in the northwest, Erode district in the East and Northeast, Dindigul district in the Southeast and Palakad district in Kerala in the west. It is located between 10°58' and 11°03' North latitude and between76°54' and 77°03' East longitude. Temples were frequently visited and surveyed for the Sthalavrikshas from the month of December 2019-February 2020.

2.2. *Taxonomic identification of Sthalavrikshas and its associated plants*

Sthalavrikshas were collected, photographed and characters were noted for the identification purpose. Plants were identified by Flora of the Presidency Madras [6] and Flora of the Carnatic Tamil Nadu [7]. Documentation of the associated plants of the Sthalavrikshas was recorded during observation. The Medicinal value information of the sthalavrikshas has collected from the available literature.

2.3. *Phenology and Conservation status of Sthalavrikshas*

The phenology of sthalavrikshas was documented by interviewing the priest and direct observation of plants. Threatened status of sthalavrikshas was determined based on the red data book of Indian plants (IUCN redlist.org. 2014).

## 3. RESULTS

3.1. *Enumeration of Sthalavrikshas*

The present investigation was carried out in coimbatore district to enumerate the sthalavrikshas associate with temple by field observation method. During the field visit temples were frequently visited and surveyed for the sthalavrikshas from the month of December 2019 - February 2020. The field visited areas are Vadamadurai, Thudiyalur, Saibabakovil, Kavundampampalayam, Marudhamalai, Perur, Thoppampatti, N.S.N palayam, Townhall, VSK Nagar, G.N, Mills, Mettupalayam and karamadai.

**Table 1. list of Sthalavrikshas recorded in selected temples of Coimbatore**

| S. No. | Sthalavrikshas | No. of temples |
|---|---|---|
| 1. | *Aegle marmelos* Corr. | 1 |
| 2. | *Azardirachta indica* A.Juss | 12 |
| 3. | *Canthium coromandium* L. | 1 |
| 4. | *Careya arborea* Roxb. | 1 |
| 5. | *Couroupita guianensis* Aubl. | 1 |
| 6. | *Ficus benghalensis* L. | 1 |
| 7. | *Ficus racemosa* L. | 2 |
| 8. | *Ficus religiosa* L | 15 |
| 9. | *Guettarda speciosa* L. | 1 |
| 10. | *Hydnocarpus wightiana* Bl. | 1 |
| 11. | *Moringa oleifera* Lam. | 1 |
| 12. | *Nerium oleander* L. | 2 |
| 13. | *Nycthanthes arbor-tristis* L. | 1 |
| 14. | *Ocimum sanctum* L. | 1 |
| 15. | *Prosopis cineraria* (L) Druce | 2 |
| 16. | *Syzygium cumini* (L.) Skeels. | 1 |
| 17. | *Tamarindus indica* L. | 1 |
| 18. | *Terminalia arjuna* W. & A. | 1 |
| 19. | *Thespepsia populnea* Cav. | 1 |

A total number of 46 temples which include 10 Vinayagar and 18 Amman temple, 4 Siva temple,4 Perumal temple and 2 Ragukedhu temple, 1 Karuparayar temple, 3 Puthukannu kovil and 2 Murugar and 1 Saibaba temple. In usually most of the recorded Sivan temple sthalavriksha species was *Agle marmelous* but in perur Sivan temple 3 different Sthalavrikshas Species (*Guettarda speciosa, Borassus flabellifer and Tamarindus indica*) are observed. In our data collection totally 19 sthalavrikshas species belonging to 16 genera, encompassing 13 families were observed in different area of coimbatore

district. Karuparayar temple at V.S. K. Nagar *Careya arborea* sthalavriksha species, vanabhathakaliamman temple at mettupalayam *Hydnocarpus weightiana* sthalavrikshas species, Virundheeshwarar temple at vadamadurai *Moringa oleifera* sthalavrikshas temple, Lalithambigai temple and bala vinayagar temple sthalavrikshas is *Nerium oleander*, Narasima perumal at thoppampatti temple sthalavriksha is *Ocimum sanctum,* Aranganathar temple karamadi temple sthalavriksha is *Canthium coromandium,* Maruthamalai murugar temple sthalavriksha is *Terminalia arjuna.* In only one temple sthalavriksha was associate with other plant. In puthukannu at V.S.K. nagar the sthalavriksha *Azardirachta indica* is found associate with *Ficus religeosa* (Tables 1 and 2).

**Table 2. List of Sthalavrikshas recorded during a survey of temple trees in Coimbatore**

| S. No. | Botanical name | Local name | Temple | Family | Phenology |
|---|---|---|---|---|---|
| 1. | *Aegle marmelos* Corr. | Vilvam | Vilayatumariamman kovil, vadamadurai | Rutaceae | April-May |
| 2. | *Azardirachta indica* A.Juss | Veppa maram | Muthalamman kovil, N.S.N palayam | Meliaceae | Feb-Aug |
| | | | Vanjiamman kovil, periyanayakan palayam | | |
| | | | Magaliyamman kovil, periyanayakan palayam | | |
| | | | Puthukannu kovil,ukkadam | | |
| | | | Mriyamman kovil, periyanayakan palayam | | |
| | | | Uchimagali amman kovil, N.S.N palayam | | |
| | | | Batharakali amman, thopampatti | | |
| | | | Magaliamman kovil, thudiyalur | | |
| | | | Puthu kannu kovil, metupalayam | | |
| | | | Vragheamman kovil, periyanayakan palayam | | |
| | | | Magaliamman podanur | | |
| 3. | *Canthium coromandium* L. | Kaarai maram | Aranganathar kovil, karamadai | Rubiaceae | Jan-Mar |
| 4. | *Careya arborea* Roxb. | Keluva maram | Karuparayan thiru kovil, V.S.K nagar | Lecythidaceae | July-Sep |
| 5. | *Couroupita guianensis* Aubl. | Nagalingam maram | Sri Konni Amman kovil, town hall | Lecythidaceae | Nov-Jan |
| 6. | *Ficus benghalensis* L. | Aalamaram | Adhimulan vinayagar, mettupalayam | Moraceae | Mar-june |
| 7. | *Ficus racemosa* L. | Athi maram | Anuvavi Subramaniyan kovil | Moraceae | April-July |
| | | | Kanimar kovil, maruthamalai | | |
| 8. | *Ficus religiosa* L. | Arasamaram | Valampuri Vinayagar kovil, V.S.K nagar | Moraceae | Nov-Dec |
| | | | Mundhi viyagar, puliyakulam | | |

| | | | | | |
|---|---|---|---|---|---|
| | | | Sakthi vinayagar, Vadamadurai | | |
| | | | Puthukannu kovil, vadamadurai | | |
| | | | Sri Konni amman kovil, town hall | | |
| | | | Kannimar kovil, thudiyalur | | |
| | | | Attru vinayagar kovil, perur | | |
| | | | Ragu kethu kovil, thoppampatti | | |
| | | | Patti vinayagar kovil, perur | | |
| | | | Sakthi vinayagar kovil, metupalayam | | |
| | | | Bathirakali amman kovil, thudiyalur | | |
| | | | Vinayagar kovil, saibaba kovil | | |
| | | | Aranganathar kovil, palamalai | | |
| | | | Sri Magali amman kovil, thudiyalur | | |
| 9. | *Guettarda speciosa* L. | Panneermaram | Perur patteshwaras | Rubiaceae | April-May |
| 10. | *Hydnocarpus wightiana* Bl. | Thothimaram | Vanabadrakali amman kovil, metupalayam | Achariaceae | Jan-April |
| 11. | *Moringa oleifera* Lam. | Vana murungai | virundeeshwarar temple, vadamadurai | Moringaceae | April-June |
| 12. | *Nerium oleander* L. | Aralichedi | Lingammal kovil, vadamadurai | Apocynaceae | April-June |
| | | | Bala vinayagar kovil, N.S.N. palayam | | |
| 13. | *Nycthanthes arbor-tristis* L. | Pavazhamalli | Sivan kovil, kanuvai | Oleaceae | Aug-Nov |
| 14. | *Ocimum sanctum* L. | Thulasi | Narasima perumal, thoppampatti | Lamiaceae | Throughout the year |
| 15. | *Prosopis cineraria* (L.) Druce | Vannimaram | Ragukethu temple, vadamadurai | Fabaceae | April-May |
| | | | Konniyamman kovil, town hall | | |
| 16. | *Syzygium cumini* (L.) Skeels. | Naval | Lalithambigai kovil, anuvavi | Myrtaceae | March-May |
| 17. | *Tamarindus indica* L. | Pirava puli | Pateeshwarar temple perur | Fabaceae | July-Dec |
| 18. | *Terminalia arjuna* W. & A. | Marudhamaram | Murugar temple, Marudhamalai | Combretaceae | April-June |
| 19. | *Thespepsia populnea* Cav. | Puvvarasha | Balaganapathi, N.J puram | Malvaceae | Throughout the year |

**Table 3. Sthalavrikshas observed in coimbatore and their medicinal utility and IUCN category**

| S. No | Botanical name | Family | Plant part | Medicinal use | IUCN status |
|---|---|---|---|---|---|
| 1. | *Aegle marmelos* Corr. | Rutaceae | Ripe fruits and seeds | Laxative | Threatened |
| | | | Flowers | Antidiarrhoeal. | |
| 2. | *Azardirachta indica* A. Juss | Meliaceae | seed oil | Antiseptic | Not evaluated |
| | | | Kernel oil | Antifungal, antimicrobial, skin diseases. | |
| | | | Leaves | Antidiabetic | |
| | | | Leaf | Stop vomitting | |
| 3. | *Canthium coromandium* L. | Rubiaceae | Root | Antitode for snakebite | Vulnerable |
| | | | Bark | Head ache | |
| 4. | *Careya arborea* Roxb. | Lecythidaceae | Flower | Cold | Least concern |
| | | | Bark | Skin infection | |
| 5. | *Couroupita guianensis* Aubl. | Lecythidaceae | Bark | Skin infection | Least concern |
| 6. | *Ficus benghalensis* L. | Moraceae | Latex | Rheumatism, ulcers | Least concern |
| | | | Root | Long hair | |
| | | | Bark | Anti diabetic and dysentery | |
| 7. | *Ficus racemosa* L. | Moraceae | Leaf | Bronchitis | Least concern |
| | | | Fruit | Astringent, stomach ache | |
| | | | Root | Antidiabetes | |
| 8. | *Ficus religiosa*s L. | Moraceae | Bark | Astringent, wounds. | Least concern |
| | | | Leaf | Laxative | |
| | | | Fruit and seed | Laxative | |
| 9. | *Guettarda speciosa* L. | Rubiaceae | Bark | Wounds, chronic dysentry | Least concern |
| 10. | *Hydnocarpus wightiana* Bl. | Achariaceae | Seed oil | Leprosy | Not evaluated |
| 11. | *Moringa oleifera* Lam. | Moringaceae | Seed | Anti tubercular | Vulnerable |
| 12. | *Nerium oleander* L. | Apocynaceae | Leaf | anti inflammatory | Least concern |
| | | | Root | Ulcers | |
| 13. | *Nycthanthes arbor-tristis* L. | Oleaceae | Leaf | Fever, rheumantism | Not evaluated |
| | | | Seed | Anti dandruff | |
| 14. | *Ocimum sanctum* L. | Lamiaceae | Root | Malarial fever | Least concern |
| | | | Leaf | Cold, cough | |
| 15. | *Prosopis cinararia* (L.) Druce | Fabaceae | Flower | Safeguard miscarriage. | Threatened |
| | | | Bark | Rheumantism | |
| | | | Pod | Astringent | |
| 16. | *Syzygium cumini* (L.) Skeels. | Myrtaceae | Seed | Anti diabetes | Not evaluated |

| | | | | | |
|---|---|---|---|---|---|
| 17. | *Tamarindus indica* L. | Fabaceae | Fruit | Laxative | Least concern |
| | | | Leaf | Anti inflamatory | |
| 18. | *Terminalia arjuna* W. & A. | Combretaceae | Fruit oil | Rhemantism | Not evaluated |
| 19. | *Thespepsia populnea* Cav. | Malvaceae | Latex | Antifungal | Least concern |

Prabakaran and Sabarilakshmi [2] reported Balasubramaniya samy and Sakradevi temple at Othumalai temple sthalavriksha is *Syzgium cumini* and we are recorded Lalithambigai temple at annuvav temple sthalavriksha *Syzgium cumini*. In sri Iyamar swamy at sempulichipalayam temple sthalavriksha is *Nycthanthes arbortristis* [8] but in our findings kanuvai shivan temple sthalavriksha is *Nycthanthes arbortristis.* Periyaswamy and Saranya [9] reported Karuppanar temple at Bhavani temple Sthalavriksha is *Thespesia populnea* but in our findings Balaganapathi at N.J puram temple *sthalavriksha* is *Thespesia populnea.* In Athimulur Vinayagar temple *sthalavrikshas* species *Ficus bengalensis* was observed. Gunasekaran and Balasubramanium [3], Sharma Tarun *et.al.* [9] and Prabakaran and Sabarilakshmi [2] documented the muthumariamman temple sthalavriksha is *Azardirachta indica* and we also get the similar result in our data collection.

Sharma Tarun *et al.* [8] observed *Tamarindus indica* as sthalavrikhas species in Subramaniya swamy temple but in our observation Perur Shivan temple sthalavrikhas species *Tamarindus indica.* Shivalingam *et al*., [10] enlisted *Prosopis cineraria* temple sthalavrikshas associated with Vridhagireeswami temple, Kailasanathat temple and Karpooranathaeswarar temple. In our findings *Prosopis cineraria* was associated with Konniamman kovil and Vinayagar kovil. Shivalingam *et al*., [10] reported Shivan temple sthalavriksha is *Ficus religiosa* were alike to our findings. Gunasekaran and Balasubramanium [3] and Sharma Tarun *et.al.* [8] were found Natadreeswarar at Karunkalpalayam temple *Sthalavrikshas* is *Ficus racemosa* but in our observation *Ficus racemosa* is temple Sthalavriksha species in Annuvavi Subramaniyar kovil and Kannimar kovil. All the recorded 19 plant species are having medicinal properties. The flowering and fruiting stage of the sthalavriksha were recorded and present in the Table 2.

3.2. *Biocultural aspects of sthalavrikshas*

In Subramaniya swamy temple at maruthamalai and Konniamman temple at townhall the sthalavriksha *Ficus religiosa* and Uchi magaliamman temple at N.S.N. palayam the sthalavriksha *Azardirachta indica* were considered as the God. In those trees are placing turmeric and kumkum, lighting of camphor and also keep flowers, tie yellow rope and cradle on the tree, people trust these kinds of worship helps for quick marriage for virgin women and pregnant women pray for getting children.

3.3. *Conservation status of Sthalavrikshas*

According to IUCN Redlist category and criteria version 3.1 (IUCN ,2013). Four different types of species are found Vulnerable (Vu), Threaten (T), Least concen (Lc) and Not evalvates (NE) at local level. Out of this 2 species (*Aegle marmelos*, *Prosopis cinararia*) are threatened, 10 species are least concern and two are vulnerable (table 3). In India , many individual plants are considered sacred and worshipped as well as protected. Prominent among them are *Aegle marmelos, Ficus religiosa* and *Ocimum sanctum* in India, Mapple leaf tree in Canada, Red wood tree in America and *Ficus religiosa* as wellas *Ginko biloba* in China and Bhutan [11].

## 4. DISCUSSION

Sthalavrikshas are very common in India. They are found in villages, in the countryside and the heart of some temples. Gunasekaran and Balasubramanian (2012) enumerated the ethnomedicninal uses of sthalavrikshas occurring in the temples of Tamilnadu and observed 101 sthalavrikshas species. Sivalingam *et al*. (2016) access the conservation status of 383 temple sthalas in Tamil Nadu and recorded 16 plant species with their religious and medicinal uses. Prabakaran and sabarilakshmi (2017) surveyed the Sthalavriksha of 106 temples in Salem, Namakkal, and Kaurur district of Tamil Nadu.and recorded the 18 plant species with the medicinal uses. The survey of sthalavrikshas of temples was conducted (Periyasamy and Saranya 2018) in Erodes district of Tamil Nadu and total number of 25 sthalavriksha species belongs to 14 families and 25 genera were recorded. These sacred plants are worshiped by the local people for getting the

blessing of health and wealth by positive powers of nature.

## 5. CONCLUSION

These sacred plants are worshiped by the local people for getting the blessing of health and wealth by positive powers of nature. Sthalavrikshas are germplasm reservers and an indicator of socio-cultural conservation strategy. Proliferation of sthalavrikshas in temples devotes to the conservation of our floral diversity. So, it is the work of contemporary generation to preserve and promote these aesthetic treasures to conserve biodiversity and nature, which will surely play a part in progeression of human beings.


## ACKNOWLEDGEMENT

We are very much grateful to Dr. M. Aruchami, President and Dr. C. A. Vasuki, Secretary and Director**,** Kongunadu Arts and Science College, for their patronage and permission accorded to us doing this project work.


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DOI:10.26524/krj.2021.14

*Kong. Res. J.* 8 (2): 8-26, 2021
**Publisher**: Kongunadu Arts and Science College, Coimbatore.



**RESEARCH ARTICLE**

# DOCUMENTATION OF MEDICINAL PLANTS USED BY THE TRADITIONAL HEALERS, MAYANNUR FOREST, THRISSUR DISTRICT, KERALA, INDIA

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## ABSTRACT

The study was carried out to document the medicinal plants used by the traditional healers in Mayannur Forest, Thrissur District, Kerala, India. In the present study, 107 plant species belonging to 46 families were documented in the Mayannur forest, Kerala. The informants of an age group ranging from 50-80 were selected for collecting data of the plants in the forest. The medicinal plants such as herbs (36%), shrub (19%), trees (27%) and climbers (18%) were mainly used by traditional healers for the treatment of fever, wound healing, skin diseases and menstrual problems. *Saraca asoca* was mainly in the traditional medicines to cure diseases and for treating menstrual problems. Medicinal plants used by the traditional healers were documented along with their scientific name, common name and medicinal uses. The present study shows that, the plant material mainly used was leaves for the treatment of diseases. Among the 107 plant species Rare Endangered Threatened (RET) plant species were also documented. Documentation of knowledge and conservation of the endangered plants helped for the sustainable development.

**Keywords:** Mayannur Forest, Traditional healers, Medicinal plants, RET plants

## 1. INTRODUCTION

Biodiversity is the variety of life forms on earth. India is the richest country in biodiversity. Indian flora has 10.78% of the total global flora. Biodiversity facing an unpredictable destruction by humans and environmental changes which result in the expiration of many forest flora and fauna [1]. Forest have greater impact on life, as they derive food, fodder, medicine, housing material and it also has the largest demand for fuel and wood. Due to increasing demand of forest products and various anthropogenic activities many plant species have been disappearing. Forests are dominant terrestrial ecosystem of earth, having 75% of gross primary production [2]. It is the storehouse of flora and fauna. In the present century, civilization is dominating the forest involving self-destruction of forest. Floristic inventory is a taxonomic study of a major division of flora in a particular area [3]. They are vital prerequisite for fundamental research in the tropical community, ecology such as modelling patterns of species diversity and understanding the species distribution.

Floristic studies have promising importance in recent years, in developing and under developing countries to document their plant wealth. The forests are disappearing at alarming rates owing to deforestation for extraction of fire wood and other forest products. It facilitates, to know the basic aspects of evolution, isolation and endemism. Flora of an area is not a fixed one, it changes from time to time. Identification of local plants along with description of an area is essential, as it can provide information about particular species in local area, its growing season, species hardness of any new species establishing in a particular area and effect of climate conditions like over grazing and drought on vegetation [4]. The knowledge of floristic composition is essential to understand the ecosystem of an area. The study also reflects the variety of vegetation of a specific geographical area, which provides an occasion for proper identification and sustainable utilisation of plants. The data of flora of an area is necessary for the study of biodiversity and understanding the present environment [5]. This study is not only a good source of botanical information of a geographical area, but also provides a suitable



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starting point for further comprehensive studies. The present study was conceded to document the diversity of medicinal plants of the Mayannur Forest, in Mayannur village, Kerala.

## 2. MATERIALS AND METHODS

*1.1. Study Area*

The Study was carried out in Mayannur Forest, Mayannur village Thrissur district, Kerala. The Forest is 10°23'0" N 76°42'30" E and covers 285 square kilometre, and has tropical Climate with an average of minimum temperature varying from 8°- 14°c and an average of maximum temperature from 23°- 29°c. The hottest month and the coolest month are April-May and January-February. The annual average rainfall is from 2717 to 45 43 mm. The forest has red soil, blackish brown and reddish brown soil. There are small streams running inside the forest which add beauty to the forest and so far no systematic ethno botanical survey has been made in this area and this is the first report on the medicinal plants. Life styles of people living in the forest are poor and economically, they depend on cattle grazing, agriculture and use of natural resources from the forest for their livelihood.

*1.2. Data collection*

Extensive and repeated field survey was carried out during the month of May – December, 2020. A comprehensive checklist of plants was prepared to understand the range of distribution of species, ecological variations and type of adaptation. The plants are enumerated alphabetically with their botanical name with author citation, family name, habit form by referring to Flora of Presidency of Madras [6] and Flora of Presidency of Tamil Nadu Carnatic [7]. The botanical data's were collected through discussions among traditional healers and local people residing near the study area. Most of the information was gathered from elderly people of an age group ranging from 50-80. The informants are selected based on their sound knowledge of the medicinal plants. This information has transferred to successive generations.

*1.3. Interview with the Informants*

Medicinal plants information was collected from the informants with the age group ranging from 50 – 80, through interview and oral conversation. Questionnaires were used to obtain information on medicinal plants, their local names, parts used and their mode of administration etc.

*1.4. Preservation of plant specimens*

Standard method was followed for the collection of plant specimens, drying mounting, preparation and preservation of plant specimens. Identified plants were arranged alphabetically with their botanical name with author citation, family and habit referring to Flowering plants of Kerala [8] Flora of Presidency of Madras [6] and Flora of Presidency of Tamil Nadu [7]. The preserved Herbarium was deposited in the Department of Botany, Nirmala College for Women, Coimbatore.

## 3. RESULTS AND DISCUSSION

Traditional knowledge of indigenous plants and uses of native medicinal plants were studied and documented. The present study showed that 107 species were belonging to 42 families, and used traditionally by the local people residing near the forest. (Table 1). Among the families, Fabaceae (10 species) has the highest number of species followed by Asteraceae (9 species) and Amaranthaceae (6 species). Different habits of the medicinal plants such as herbs (39), shrubs (20), climber (19) and trees (29) were found in the study area. Different parts of the plants were used to prepare medicines by using local traditional healers, among them 49% of the medicines were prepared by using leaves followed by the whole plant (29%), bark (8%), fruit (8%) and seeds (6%). In Ayurveda, mostly leaves were used for preparing herbal formulation. Mayuri *et al.,* [9] reported that leaves are the most frequently used parts than the other parts.

The medicine prepared from the plants involve various processes such as pasting, decoction, juice, tea and also used to cure various diseases like menstrual disorders, fever, and arthritis. Before giving the treatment the condition of patients were observed and the herbal remedies were taken orally according to their age and weight [10]. More over a single plant is used for more than one disease for example *Abelmoschus moschatus* (urinary infection, sexual disorders) *Adathoda vasica* (respiratory disease and menstrual problems), *Aerva lanata* (body pain, control diabetes) *Azadirachta indica* (tooth pain, blood purification) *Clitoria ternetea* (Menstrual problems and wound healing) *Desmodium gangeticum* (heart disease and rheumatism). *Evolvulus alsinoides* (infertility and to improve

**Fig. 1. Some medicinal plants of Mayannur forest, Thrissur, Kerala.** a) *Naregamia alata* b) *Glycosmis pentaphylla* c) *Clerodendrum infortunatum* d) *Euphorbia hirta* e) *Ophiorrhiza roxburghiana* f) *Aerva lanata* g) *Biophytum sensitivum* h) *Saraca asoca* i) *Capsicum annum*

**Table 1. Documentation of medicinal plants used by the traditional healers of Mayannur forest, Mayannur Village, Thrissur, Kerala.**

| S. No | Binomial Name | Family | Common name | Habit | Parts used | Uses |
|---|---|---|---|---|---|---|
| 1 | *Abelmoschus moschatus* L. | Malvaceae | Kasturi venda | Shrub | Leaves, Fruits, Seeds | Tea made using the leaves are used to cure urinary infection. Seeds are made into paste and mix with honey taking this daily will cure mouth ulcers and sexual disorders. |
| 2 | *Abrus precatorius L.* | Fabaceae | Kunni kuru | Shrub | Leaves, Seeds | Paste of seed is used in skin disorders. Leaves of *Abrus* is mixed in and kept in the inflammated area. A tea made of using the leaves are used against cough and cold. |
| 3 | *Acacia pycnantha* Benth. | Fabaceae | Acacia | Tree | Bark | Decoction of bark is used for mouth ulcers. |
| 4 | *Achyranthes aspera* L. | Amaranthaceae | Kadaladi | Herb | Leaves | Leaves are made into paste and used against skin diseases. Seeds of this plant are used by tribal people as gruel. Leaves of *Achyranthus* mixed with honey is used for digestive problems. |
| 5 | *Adathoda vasica* L. | Acanthaceae | Adalodakam | Herb | Leaves | Leaves are made into paste and mixed with honey and taken in empty stomach for cough and bronchitis. Leaves of *Adathoda* mix with jaggery used against menstrual problems. |
| 6 | *Adenanthera pavonina* L. | Fabaceae | Manjadi | Tree | Leaves & Bark | In traditional medicine, a decoction of the young leaves and barks used to treat diarrhoea. Also, the ground seeds are used to treat inflammation. |
| 7 | *Aegle marmelos (L.)* | Rutaceae | Koovalam | Tree | Root , Leaves ,Fruits | Having Juice of leaves 10 mL daily will control diabetes, Root is made into juice and used against ear diseases, and also have healing properties. Leaves are made into paste and apply on the breast of feeding mother will prevent the child from diseases. |

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| 8 | *Aerva lanata* (L.) | Amaranthaceae | Cherula | Herb | Whole plant | Boil water using *Aerva lanata* and taking bath using that water will reduce body pain. Juice of leaves mixed with milk taken daily will prevent kidney stones. Leaves are made into paste and mixed with curd, taken daily prevent diabetes. |
| 9 | *Ageratum conyzoides* L. | Asteraceae | Kumminipacha | Herb | Leaves | Oil made using the leaf is used to cure Arthritis. Leaf juice is also applied for healing wounds. |
| 10 | *Albizia lebbeck* (L.) Benth | Miomosaceae | Kattuvaka | Tree | Bark | Decoction of bark is used medicinally to treat inflammation, jaundice and mouth ulcers. |
| 11 | *Alstonia scholaris* L. | Apocynaceae | Daivappala | Tree | Bark | Bark is made into paste used for skin diseases. |
| 12 | *Alternanthera brasiliana* L. | Amaranthaceae | | Herb | Leaves | Paste of leaf is used for wound healing. |
| 13 | *A.sessilis*(L) | Amaranthaceae | Ponnanganni | Herb | Leaves | Juice of leaf is used for curing eye diseases. Tribal people use the leaves for making kajal. The whole plant is used by tribes (Ulukupidutham; a mode of treatment by tribal people) in curing back pain. |
| 14 | *Amaranthus spinosus* L. | Amaranthaceae | Mullucheera | Herb | Leaves and stem | Leaf decoction with adding a pinch of salt and used for digestion. Juice made up of tender leaves is used to increase blood count in dengue patients. Tribes make curry using the leaves without adding oil it are used for anemia. Soup made of leaves and stem are used to control cholesterol and helps in digestion. |
| 15 | *Amorphophallus commutatus*(Schott) | Arecaceae | Kattuchena | Herb | Fruit | Including the fruit in diet will control obesity, in curing piles, control blood pressure and diabetes. |
| 16 | *Anacardium occidentale* L. | Anacardiaceae | Kasu mavu | Tree | Fruit and Leaves | Fruit has anticancer activity, it is also used for vitamin C deficiency. Decoction of fruit is used for vomiting. |

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| 17 | *Artocarpus hirsutus* Lam. | Moraceae | Anjili | Tree | Fruit | Fruits are used for digestive problems and it also increases sperm production. |
| 18 | *Artabotrys odoratissimus* R.Br. | Annonacea | Manoranjini | Climber | Leaves and flowers | Tea made up of leaves and root prevents cancer. Leaf decoction also used to treat cholera. |
| 19 | *Asystasia gangetica* L. | Acanthaceae | Chinese Violet | Herb | Leaves | Decoction of leaf is used for asthma. |
| 20 | *Azadirachta indica* A.Juss. | Meliaceae | Veppu | Tree | Leaves | 4-5cm long tender stem is caused in the form of tooth brush and bridging using that will cure tooth pain and cleanse the mouth. Dried leaves are made into power and mixing it with milk it is used for blood purification. Powered leaf mixed with turmeric is used for pimples and all skin diseases. Decoction of leaf is used to cleanse the scalp it cures dandruff problems. |
| 21 | *Bambusa arundinacea* (Retz.) Willd. | Poaceae | Mula | Tree | Bark, Root | An ointment from the root is said to be a folk remedy for cirrhosis and hard tumors, especially tumors of the abdomen, liver, spleen and stomach, Decoction of bark is mixed with honey is used for respiratory disease. Decoction of leaf is used for stimulating mensuration. Tribal people used stripes of bamboo for curing back pain. |
| 22 | *Biophytum sensitivum* DC. | Oxalidaceae | Mukkutti | Herb | Whole plant | 25g of whole plant is made into paste and mixing it in coconut oil and massaging it in head helps to cure nasal polyps (small out growth in nose). The whole plant is made into paste and mixing it with milk and having it daily will help to maintain youth. Leaves made into paste and mixing it with 1 teaspoon of honey having it will help to cure while discharge in women. |
| 23 | *Bixa orellana* L. | Bixaceae | Kurannumanna l | Shrub | Leaves and Fruits | The shrub is most well known as the source of the red-orange, annatto pigment. , the plant has anticancer activity. |
| 24 | *Blepharis maderaspatensis* (L.) | Acanthaceae | Elumbotti | Herb | Leaves | It is used to treat eye disease. Juice extracted from leaf is heated with gingelly oil and applied on |

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| | | | | | | affected places to heal wound. |
| 25 | *Blumea axillaris* (Lam).DC. | Asteraceae | Kukkura | Herb | Whole plant | Whole plant is made into juice and taken orally for diarrhea. |
| 26 | *Boerhaavia diffusa* L. *nom. cons* | Ncytaginaceae | Thazhuthama | Herb | Whole plant | The whole plant is added in boiling water and it is used daily in empty for weight loss. The leaves are made into curry and used for constipation and anemia. 15 ml of leaf juice is taken daily for bronchitis. Whole plant is made into paste and mixed with milk and it is given to people who are addicted to alcohol. A handful of leaves are made in to juice and mixed in mother's milk and used for eye disease. |
| 27 | *Calotropis gigantea* (L.) Dryand. | Apocynaceae | Erikku | Shrub | Whole plant | 2 or 3 leaves are heated and holding it tightly to the heel will cure heel pain. Leaf is made into paste and applying it to the ear to cure ear pain. A 4-5cm long stem is taken and its tip is crushed in the tooth brush and brushing using it cure tooth pain. |
| 28 | *Capsicum annum* L. | Solanaceae | Kanthari mulakku | Shrub | Fruits | Including fruits in the diet regularly will control cholesterol, heart diseases and diabetes. A drink is made using fruits, curry leaves and curd used for digestion. |
| 29 | *Cardiospermum halicacabum* L. | Sapindaceae | Karuttakunni | Herb | Whole plant | Decoction of the plant is used daily by pregnant women for normal delivery. Whole plant is made into paste and it is used by traditional people as an alternative for shampoo which helps in hair growth. A paste is made using leaf and coconut milk and applying it on hair once in a week helps in hair growth. |
| 30 | *Cassia fistula* L. | Caesalpiniaceae | Kanikkonna | Tree | Leaves, Bark | Paste of leaf is used for scorpion bite. A paste made using leaf and rice water is applied on skin to treat skin diseases. Decoction of bark is used for stomach pain. Oil made using flowers are used for skin diseases. |

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| 31 | *C. occidentalis* L. | Caesalpiniaceae | Kanikkonna | Shrub | Leaves | Decoction of leaf is used for curing fever in small children during the time of formation of milk teeth. Paste of leaf and castor oil used for skin diseases. Leaf is made into paste and mixing it with coconut oil and applying it daily on head and body before bath will cure headache and prevent pigmentation in skin. |
| 32 | *Carallia brachiata* Lour. | Rhizophoraceae | Varrungu | Tree | Bark | Decoction of bark is used for skin diseases. Paste of bark is used for wound healing. |
| 33 | *Cenchrus ciliaris* L. | Poaceae | Buffel grass | Herb | Leaves | Decoction of leaf is used for urinary tract infection. |
| 34 | *Centrosema pubescens* Benth. | Fabaceae | Butterfly pea | Climber | Whole plant | Decoction of whole plant is used for stomach discomfort |
| 35 | *Chromolaena odorata* (L.) | Asteraceae | Communist pacha | Shrub | Leaves ,Root | Malayalam name communist pacha is because it has healed the wounds of many comrades during the freedom fights. Root Juice mixed in milk is used for kidney stones. Taking bath in water boiled using the leaf will cure body pain in chikengunia patients. Having the decoction of leaf daily will help to maintain the pH of stomach. |
| 36 | *Cleome rutidospermum* DC. | Cleomaceae | spider flowers | Herb | Leaves | Leaf juice is used for skin diseases. |
| 37 | *Clerodendron infortunatum* L. | Verbenaceae | Perungulam | Shrub | Leaves | The tender leaves of the plant and make it into a paste and then applying it on the toe nail will cure migraine. Leaves are made into juice and mixing it with milk it's used as a medicine against snake bite. |
| 38 | *Chrozophora rotteleri* L. | Euphorbiaceae | Suryavarthi | Shrub | Leaves | A paste is made using leaves and mixed with turmeric used for wound healing. |
| 39 | *Clitoria ternetea* L. | Fabaceae | Shangupushpam | Climber | Whole plant | 3g of roots are made into paste and mixed with ghee having this daily will increase brain function. Decoction of whole plant is used to treat alcohol |

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| | | | | | | addiction. A paste made up of 1 g of leaves and honey is used to treat menstrual problems. Decoction of leaf is used for wound healing. A paste made up of flowers and the flowers of *Saraca asoca* are used for heavy bleeding during menstrual cycles. |
| 40 | *Coccinia indica* Wight & Arn. | Cucurbitaceae | Kooval | Leaves & Fruits | Fruits and leaves | Eating fruits will control cholesterol and blood pressure. Leaves are used for curing ulcers. |
| 41 | *Commenlia bengalensis* L. | Commelinaceae | Kanavazhai | Herb | leaves | Paste of leaves is used for swellings. Paste of tender leaves is applied on burns. |
| 42 | *Crotalaria pallida* Aiton. | Fabaceae | Kilukki | Shrub | Seeds and leaves | Seeds are used to make shampoo which cure dandruff. Decoction of leaves is used for urine infection. |
| 43 | *Canthium rheedii* DC. | Rubiaceae | Karamullu | Shrub | Leaves | Decoction of leaves is used to prevent cancer. |
| 44 | *Cynadon dactylon* L. | Poaceae | Karuka | Herb | Whole plant | Whole plant is made in to paste and holding this in to the wound will stop bleeding. |
| 45 | *Cyclea peltata* Arn. | Menispermaceae | Padathali | Climber | Leaves and tuber | Decoction of leaves and tuber are used for Kidney stones. Oil made using leaves is used for hair growth. Paste of leaves is used for snake bite. Decoction of leaves is used for fever. |
| 46 | *Datura stramonium* L. | Solanaceae | Ummam | Shrub | Leaves and Flowers | A paste of leaves mixed in Coconut oil is applied on scalp for treating dandruff. Decoction of leaves and flowers are used for bronchitis. Paste made up of fruit, flowers and turmeric is applied for any kinds of pain or inflammation in breast. Decoction of leaves is used for menstrual pain. |
| 47 | *Desmodium gingeticum* L. (DC). | Fabaceae | Oorila | Shrub | Root | Root juice mixed with curd us used to cure blood in stool. Root paste applied for scorpion bites. Root juice mixed with milk is used daily to prevent heart disease. Decoction of root used for rheumatism. Root juice mixed in honey is used to prevent white discharge in women. |

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| 48 | *Duranta erecta* L. | Verbenaceae | Chebazhukka | Shrub | Fruits | Decoction of fruits is used for malaria. |
| 49 | *Eclipta prostrata* (L.) | Asteraceae | Kannunni | Herb | Whole plant | Decoction of whole plant (Marcarasayanam) used for skin diseases. Decoction is also used for jaundice and liver disease. 10g of whole plant is made into paste and oil is made by mixing in 1 litre of coconut oil. Using this daily will prevent eye disease, headache and increase hair growth. |
| 50 | *Eleusine indica* (L.) | Poaceae | Kattuthina | Herb | Whole plant | Tea made by the leaves is used for ovarian cysts. Having the decoction of whole plant daily will increase the amount of water in body and expels salt as urine. Holding the heated leaves tightly to the inflammated part will cure inflammation. Applying the juice of leaves in wounds will stops bleeding from wounds. Drinking the boiled leaves cure urinary tract infection. |
| 51 | *Eucalyptus globulus* Labill. | Myrtaceae | Eucali | Tree | Leaves | *Eucalyptus* oil is made by crushing the leaves and mixing it in oil and keeping it under sun for 10 days. Inhaling thus oil prevent migraine, Stress, and anxiety. Applying this oil will also cure joint pain. |
| 52 | *Eranthemum pulchellum* L. | Acanthaceae | Neelamulla | Herb | Root | Decoction of the root is used for ulcers. |
| 53 | *Evolvulus alsinoides* L. | Convolvulaceae | Vishnukranti | Climber | Whole plant | Decoction of root is used for fever. Whole plant is mixed with milk and ghee used for infertility. Whole plant mixed in milk and having this for 48 days improves brain functioning. Decoction of whole plant is used to control diabetes. Having 2g of whole plant improved functioning of nervous system. Decoction of whole plant is used for stomach pain. |
| 54 | *Euphorbia hirta* L. | Euphorbiaceae | Murikooti | Herb | Whole plant | It is often used traditionally for female disorders. 5 g of leaves are made into juice and applying it on teeth using a cotton to cure tooth pain. Latex from the stem is used for pimples. Juice of leaves |

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| | | | | | | mixed in curd is used as face mask. Leaves juice are mixed with curd and having it daily will prevent white discharge in women. |
| 55 | *Ficus hispida* L.f. | Moraceae | Parakam | Tree | Leaves | Having a mixture of leaf juice and milk will increase milk in lactating women. Mixture of leaf and gum is used for leprosy. A paste is made using the leaf and leaf of Datura and it's mixed in rice water and used for Rabies. |
| 56 | *Glycyrrhiza glabara* (L). | Fabaceae | Irattimaduram | Tree | Root | Root extracts of mulethi aids in increasing the production of lymphocytes and macrophage thereby improving your defense mechanism & powder if root is used for curing dandruff. |
| 57 | *Gloriosa superba* | Liliaceae | Menthoni | Climber | Tuber | Paste of tuber is used for skin diseases. A paste made using tuber and leaves is applied on throat for itching in throat. |
| 58 | *Glycosmis pentaphylla* (Retz.) DC | Rutaceae | Kuttuppanal | Shrub | Root , leaves | Extract of root bark have been shown to exhibit significant activity in treatment of diarrhoea. Adding a handful of leaves to boiling water and taking bath with that water will reduce body pain. |
| 59 | *Gomphrena serrata* L. | Amaranthaceae | Globe Amaranth | Shrub | Leaves | More effective against diarrhea, heavy fever, pains, carminative, bronchial asthma, diabetes, and dermatitis. |
| 60 | *Hibiscus sabdariffa* L. | Malvaceae | Pulivenda | Shrub | Flowers | Consumption of tea made using petals daily reduce hypertension. |
| 61 | *Holigarana arnottiana* Wall. | Anacardiaceae | Karincher | Tree | Seeds | Seeds are used to treatment inflammation, arthritis, hemorrhoids, obesity, tumor, cancer, leaves and its latex cause allergy. |
| 62 | *Holarrhena pubescens* Wall. ex G.Don | Apocynaceae | Kutakappaala | Tree | Leaves | To check blood coming from stool, paste of leaves are given with caster oil. According to Ayurveda, the bark is useful in treatment of piles, skin diseases and biliousness. |
| 63 | *Holoptelea integrifolia* (Roxb.) Planch | Ulmaceae | Aaval | Tree | Bark | The bark is boiled and squeezed out and applied for rheumatic swellings. |

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| 64 | *Hyptis suaveolens* L. | Lamiaceae | Ganga tulasi | Herb | Root and leaves | A decoction of the roots is valued as an appetizer. A decoction of the root is said to be emmenagogic, and a stimulant if employed in rheumatism. Seeds are used to make a refreshing drink using lemon. |
| 65 | *Ichnocarpus frutescens* (L.) W.T.Aiton | Apocynaceae | Palvalli | Climber | Whole plant | Decoction of whole plant is used for Cough, dysentery. |
| 66 | *Ipomoea obscura* (L.) Ker Gawl. | Convolvulaceae | Thiruthali | Climber | Whole plant | Root bark is used as purgative, whole plant is used for snake bite. |
| 67 | *Kyllinga bulbosa* Beauv. | Cyperaceae | Velutta nirvasi | Herb | Whole plant | A decoction of the whole plant is used as a treatment against a variety of complaints including malaria; colds with fever; whooping cough; bronchitis; swelling pain in the throat. |
| 68 | *Lantana camara* L. | Verbenaceae | Arippu | Shrub | Root | Decoction of fresh root is used for dysentery. |
| 69 | *Leucas aspera* (Willd.) Link | Lamiaceae | Thumba | Herb | Whole plant | Decoction of whole plant cure malarial fever, Juice of leaves are applied to skin to treat skin diseases and swelling. |
| 70 | *Macaranga peltata* Roxb. | Euphorbiaceae | Vatta | Tree | Leaves | Leaves are used to make a food called "Ada" before rainy season which helps to prevent all the disease during the rainy season and also boosts immunity. Decoction made of bark is used to cure cough and fever. Chewing a tender leaf daily helps to increase immunity. Washing the wounds using the decoction of leaves helps to heal the wounds fast. |
| 71 | *Mallotus philippensis* (Lam.) | Euphorbiaceae | Cenkolli | Tree | Leaves | Leaf of juice mixed in cured is used to cure digestive problems. Leaf is made into paste and applied on skin to cure skin disease. |
| 72 | *Mimosa pudica* L. | Miomosaceae | Thottavadi | Herb | Whole plant | Paste of whole plant is used for wound healing. Having the decoction of leaves daily in the morning control diabetes. 5ml of juice mixed in |

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| | | | | | | tender coconut water is used to cute asthma in children. Whole plant is made into a paste and then mixed with honey is used for heavy bleeding during menstrual cycle. |
| 73 | *Mikania micrantha* Kunth | Asteraceae | Bitter vine | Climber | Leaves | Paste of leaf is used for insect bites and also for wound healing. |
| 74 | *Momordica charantia* | Cucurbitaceae | Pavakka | Climber | Leaves and Fruits | Handful of tender leaves are chopped and boiled in 2 glass of water and having this daily can control diabetes.D Decoction of leaves can also cure menstrual problems. Having the in diet also control diabetes. |
| 75 | *Mollugo nudicaulis* Lam. | Molluginaceae | Parpadakapullu | Herb | Whole plant | They are used for sprue and mouth infections. In India, the whole plant is used as a mild laxative medicine, also as stomachic, antiseptic and emmenagogue. In China, it is made into a soup to promote appetite, while a decoction of the roots is used to treat eye diseases. |
| 76 | *Morinda tinctoria* Roxb. | Rubiaceae | Manjapavitta | Tree | Leaves | Juice of leaves are applied externally to relieve pain. |
| 77 | *Mussaenda glabarata* L. | Rubiaceae | Vellila | Shrub | Leaves | Decoction of tender leaves and roots are used for Kidney disease. Decoction of white leaves is used for asthma. Paste made using green leaves is used for hair growth. |
| 78 | *Naregamia alata* Wight & Arn. | Rutaceae | Nilanarakam | Herb | Whole plant | Whole plant is made into juice and applied on head daily before bath can cure migraine. |
| 79 | *Oldenlandia corymbosa* L. | Rubiaceae | Parpadakapullu | Herb | Whole plant | Juice of whole plant is used to treat menstrual problems. Juice of whole plant mixed with turmeric is used for fever in children. |
| 80 | *Parietaria officinalis* L. | Urticaceae | Pellitory of wall | Herb | Whole plant | The whole plant is administered in the form of infusion as diuretic, cholagogue, emollient, healing and soothing. The herbal tea is recommended against cold, cough, sore throat and rheumatism. |

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| 81 | *Passiflora foetida* L. | Passifloraceae | Poochapalam | Climber | Leaves | Juice of leaves are applied in wounds, Decoction of leaves cure anxiety and sleep problems. |
| 82 | *Peperomia pellucida* Kunth . | Piperaceae | Mashithand | Herb | Whole plant | Whole plant is made into juice and having this daily in empty stomach will prevent kidney disorders. Juice made with the whole plant is used as a refreshing drink during summer. Leaves are made into paste and applied on inflammated area and also as a pain killer. Leaf decoction also used for cholesterol. |
| 83 | *Phyllanthus emblica* L. | Phyllanthaceae | Nelli | Tree | Fruits | A juice is made using fruits and it is mixed with turmeric and having this daily will control diabetes. Fruit is dried under sun and it is made into a powder, washing hair using this powder will prevent hair fall and increase hair growth. Fruit juice mixed in jaggery used for joint pain. A paste made using fruit and honey applied in eyes to cure eye diseases. Boiled water made using fruit and ginger and this daily in empty stomach will help in weight loss. Having a fruit daily will cure mouth ulcers and gives pink colour to lips. |
| 84 | *P. niruri* L. | Phyllanthaceae | Kizharnelli | Herb | Whole plant | Whole plant is washed and made into pieces and adding it to boiling sesamum oil and massaging the head with that oil can cure migraine. Having the decoction of plant daily will help to control diabetes. Handful of leaves is made into paste and mixing it with ghee and having it during the time of periods will reduce heavy bleeding. Paste of plant mixed with turmeric is used to cure soreasis. |
| 85 | *P. urinaria* L. | Phyllanthaceae | Chirukizhukan elli | Herb | Fruit | Decoction of fruits is used in folk medicine to treat jaundice, diabetes, malaria, and liver diseases. |
| 86 | *Poa pratensis* L. | Poaceae | Blue grass | Herb | Leaves | Decoction of leaves are used for urinary tract infection. |

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| 87 | *Pongamia pinnata* L. | Fabaceae | Ungu | Tree | Flowers | Decoction of flower is used for blood pressure. Water is made by adding two teaspoons of power of dried flowers, turmeric powder, fenugreek powder boiling it for 5-7 minutes and having this daily in empty stomach can control diabetes. |
| 88 | *Psidium guajava* L. | Myrtaceae | Pera | Tree | Leaves and Fruits | Having a fruit daily provides glowing skin, increase hair growth, strong teeth, boost immunity. Tea is made using tender leaves and having this daily will control diabetes. Water boiled using the tender leaves and steaming using that water cure tooth pain. Water boiled using tender leaves is used for diarrhea. |
| 89 | *Premna integrifolia* | Verbenaceae | Munja | Shrub | Leaves | Leaves are boiled in water and taking bath in this water cure fever in children. Having 5 leaves daily will cure ulcer. |
| 90 | *Pulicaria vulgaris* Gaertn. | Asteraceae | False fleabane | Shrub | Whole plant | Decoction of root is used for dysentery . Paste of the plant applied externally to wounds. |
| 91 | *Quisqualis indica* L. | Combretaceae | Thookuchethi | Climber | Root | Decoction of the root is used to treat rheumatism and a concentrated decoction of the fruit is used as a gargle effective against toothache. |
| 92 | *Rauwolfia serpentina* L. | Apocynaceae | Sarpaganthi | Shrub | Whole plant | 1g of root decoction is used for snake bites. Chewing the roots prevent heart disease. Leaves are made into paste and mixed in vinegar applied for skin diseases. Root juice is also used for nervous problem. |
| 93 | *Ruellia prostrata* Poir. | Acanthaceae | Thuppalpotty | Herb | Whole plant | Decoction of whole plant is used for diabetes. |
| 94 | *Santalum album* L. | Santhalaceae | Chadanam | Tree | Whole plant | It is used for treating the common cold, cough, bronchitis, fever, and sore mouth and throat. It is also used to treat urinary tract infections (UTIs), liver disease, gallbladder problems, heatstroke, gonorrhea, headache, and conditions of the heart and blood vessels (cardiovascular disease). |

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| 95 | *Saraca asoca* (Roxb). Wild. | Fabaceae | Ashokam | Tree | Whole plant | According to traditional medicine even seeing the tree will reduces stress and sitting under the shelter of the tree will reduce our sorrows. A paste is made using flowers rice powder and jaggery it's used for menstrual problems and it also purifies the blood. Paste if flowers are also used for skin diseases. Decoction of flowers and bark is used fromm1-4 days if periods to cure all the pain during periods. Seeds are powdered and mixed in tender coconut water for urine infections. |
| 96 | *Scoparia* dulcis L. | Scopariaceae | Kallurikki | Herb | Whole plant | Paste of whole plant mixed in tender coconut water and having this daily for 1 week will cure Kidney stones. Paste of leaves is also used for wound healing. |
| 97 | *Sida acuta* Burm.f.. | Malvaceae | Kurumthotti | Herb | Whole plant | Whole plant is boiled in 2 L of water it's used for rheumatism and body pain. Juice of whole plant mixed with milk is used to control blood pressure. |
| 98 | *Stachytarpheta indica* (L).Vahl. | Verbenaceae | Seemakogini | Herb | Whole plant | Decoction of whole plant is used for diabetes. Tea made by using leaves is used for fever. Paste of whole plant is used for wound healing. |
| 99 | *Strychnos nux vomica* L. | Loganiaceae | Kajiram | Tree | Seeds | Seeds are used only after purification; it is done by keeping the seeds in milk for 7 days and then driving it under sun for 7 days. Seeds are powdered and used for diabetes, piles, arthritis and headache. Leaves are made into paste and mixed in ghee and used for skin diseases. |
| 100 | *Stereospermum colais* DC. | Bignoniaceae | Puupathiri | Tree | Whole plant | Decoction of whole plant is used for fever. |
| 101 | *Synedrella nodiflora* (L.) Gaertn | | Mudiyendrapacha | Herb | whole plant | The plant is traditionally used by some Ghanaian communities to treat epilepsy. It has anticonvulsant and other neuropharmacological effects of a hydro-ethanolic extract of the whole plant using murine models. |

| | | | | | | |
|---|---|---|---|---|---|---|
| 102 | *Syzygium cuminii* L. | Myrtaceae | Njaval | Tree | Bark | Decoction of bark is used to control diabetes. Seeds are powdered and used for diabetes. Having the fruits daily cure digestive problems urinary tract infection, control blood pressure, provide strong teeth prevent heart disease and increase haemoglobin in blood. |
| 103 | *Tecoma stans* (L.) Juss. Kunth | Bignoniaceae | Vishnukiridam | Tree | Leaves | Tea made using leaves are used for treatment of diabetes, digestive problems. |
| 104 | *T. grandis* L.f . | Lamiaceae | Teak | Tree | Flowers and leaves | Decoction of flowers is used for bronchitis and menstrual problems. Water boiled using the leaves are used throat infection. Decoction of whole plant is used for diabetes. |
| 105 | *Tinospora cordifolia* Thunb. | Menispermaceae | Chittamruth | Climber | Bark | Bark is made into juice and it is mixed with honey used for fever. 15ml of decoction of whole plant is used for Kidney disorder. A paste is made using whole plant, leaves of *Phyllanthus niruri*, root of *Boerhaavia diffusa* are used for rheumatic fever. |
| 106 | *Trianthema portulacastrum* L. | Aizoaceae | Pigweed | Herb | whole plant | Decoction of whole plant is used for anemia and stomach diseases. |
| 107 | *Vernonia cinerea* (L). H. Rob. | Asteraceae | Puvankurunal | Herb | Whole plant | Decoction of whole plant is used for blood purification, malaria and eye diseases. Traditional people make a kajal by making a juice if whole plant and dipping a cotton cloth in it then burning the cloth and collecting that smoke using a clay pot and then mixing that ash in coconut oil applying the kajal will cure eye diseases. Oil made using whole plant is used for hair growth. |

functioning of nervous system). *Macaranga peltata* (fever and wound healing) *Phyllanthus emblica* (control diabetes, eye diseases, mouth ulcers). *Rauwolfia Serpentina* (Snake bite and nervous problems). *Saraca asoca* (reduce stress and for menstrual problems). *Tinospora cordifolia* (Kidney disorders and fever). *Vernonia cinerea* (hair growth and malaria). *Abrus precatorious*, *Ichnocarpus frutescens*, *Adathoda vasica*, *Parietaria officinalis* were used for treating common cold. The plants used to treat menstrual problems were *Adathoda vasica*, *Clitoria ternetea*, *Datura stramonium*, *Mimosa pudica*, *Oldenlandia corymbosa*, *Saraca asoca*. Most of the medicinal formulations were documented for the first time. The traditional healers used herbal remedies to treat common minor diseases and even some major diseases like malarial fever, cancer, kidney disorders, skin diseases and wound healing.

Plants are mainly used in the form of decoction and paste. Some plants like *Strychnos nux vomica* seeds were used only after a long process of purification for seven days. (Morvin Yabesh *et al.,* [11], Savithramma *et al.,* [12] reported that the herbal formulations include decoction (48%) paste (26%), juice (18%), and tea (8%), whereas decoction is obtained more when compared to other formulations. *Saraca asoca* Roxb De Wilde., which is an endangered tree according to IUCN (2011) has identified to have many medicinal uses like reducing stress and for menstrual disorders. Sandeep Rout *et al*., [13] studied and reported that, owing to its greater demand for the bark, and for its shortage, it is adulterer with bark of *polyalthia longifolia*. Among the 107 plant species many plants were divided into various categories of RET (Rare Endangered Threatened) were identified. Those which need urgent conservation were noted and collected for herbarium preservation. The medicinal use of plants in Mayannur forest has not been documented until the performance of present study.

## 4. CONCLUSION

The present study was carried out in Mayannur Forest, Kerala and documented 107 plant species belonging to 46 families. In recent days there is a hindrance in the transfer of traditional knowledge from generation to generation. So the knowledge about medicinal plants, traditional healers and their uses were highly important. This documentation study helped for the conservation of endangered plant species and for the identification of medicinal plants.

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DOI:10.26524/krj.2021.15

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**Publisher**: Kongunadu Arts and Science College, Coimbatore. https://www.krjournal.com

**RESEARCH ARTICLE**

# TAXONOMIC IDENTITY AND ECOLOGICAL STATUS OF TWO RARE ORCHIDS FROM SOUTHERN WESTERN GHATS, INDIA

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**ABSTRACT**

An assessment has been made to identify the rare, endemic and threatened species in the Velliangiri hills of Southern Western Ghats, India. During field explorations, two rare species of terrestrial saprophytic orchids were collected. On critical appraisal and authentication of herbarium specimens, they were identified as *Aphyllorchis montana* Rchb. f. and *Epipogium roseum* (D. Don) Lindl. The present paper deals with their correct taxonomic identity, distribution and ecological status.

**Keywords:** Saprophytic orchids, *Aphyllorchis montana*, *Epipogium roseum*, Western Ghats

## 1. INTRODUCTION

Orchidaceae is one of the most ecologically and morphologically diverse families of flowering plants. It is the second largest family of flowering plants in the world, comprising of about 779 genera and 22,500 species [1]. They have diverse habits with variously modified vegetative and floral structures. Based on their varying habits, orchids are classified as holomycotrophic or saprophytic (growing on dead and decaying matter), terrestrials (growing on ground) and epiphytic (growing on trees or shrubs). They are very sensitive to habitat degradation and fragmentation.

The Indian subcontinent has diverse climatic regimes, forest types and habitat conditions that provide a favourable environment for accommodating diverse life forms and species [2]. Geologically, the drifting of the Indian subcontinent from the Gondwanaland through various latitudes lead to immigration and extinction of species which are engraved in the present day floristic composition [3]. The orchid diversity is represented by 1,331 species belonging to 186 genera in India [4].

The endemism in the flora of a country or geographical region provides an important insight into the biogeography of that region and also to the centers of diversity and adaptive evolution of the floristic components of that region. A large concentration of endemic species is found in the tropical moist deciduous and tropical semi-evergreen patches of Western Ghats and to a much lesser degree in Eastern Ghats [5].

The Western Ghats possess a high percentage of endemic species, about 48% of 4000 species occur in this region [6]. It is on the brink of endemic plant collapse, about 1500 species have a highly fragmented population and at least 50 endemic species have not be relocated after repeated surveys [7]. The present study is an attempt made to identify the rare, endemic and threatened species in the Velliangiri hills of Southern Western Ghats, India.

## 2. STUDY SPECIES

### *2.1. Aphyllorchis montana* Reichb. f. (Fig. 1)

*Aphyllorchis* Blume is a leafless terrestrial orchid genus with total of 30- species is known to exist in various parts of the world [8]. Of which three species *viz*., *A. alpina* King & Pantl., *A. gollanii* Duthie and *A. montana* Rchb. f. have been reported from India. *Aphyllorchis montana* Rchb. f. is a terrestrial mycoheterotrophic orchid species [9] which grows in low and midland broadleaved forests of India, Sri Lanka, Malaysia, Borneo, the Philippines, southern Japan, southern China, Vietnam and Taiwan [10].

On an authentication of the specimen in the Madras Herbarium (MH), a collection of M.H.



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Lawson from Nilgiris (South India) and of C.A. Barber from Cadamonay, Mysore (South India), both of which have misidentified as *Pogonia carinata* Lindl. are actually *Aphyllorchis prainii* Hook. f. Similarly a collections of E. Vajravelu from silent valley (Kerala), A.N. Henry from Kanyakumari (Tamil Nadu) and S.R. Srinivasan from Ramanathapuram (Tamil Nadu), all of which have wrongly been identified as *A. prainii* Hook. f. The species *A. prainii* Hook. f. is very much allied to *A. montana* of Sikkim and Ceylon (which are possibly different species), differing in the winged claw of the lip [11].

The species *A. montana* Rchb. f. was also reported by P.C. Radhakrishnan from Ramanathapuram (Tamil Nadu), G.R. Rao from Karnataka and A. Nageswara Rao from Andhra Pradesh. The present collection revealed that the species was recollected from the Coimbatore district after 1921 which forms its extending distribution. The species is categorized as a *Data deficient* orchid of conservation concern in India and is enlisted in the RET plant list of India [12,13, 14].


**Fig. 1. *Aphyllorchis montana* Reichb. f.**

*2.2. Epipogium roseum* (D. Don) Lindl. (Fig. 2)

The genus *Epipogium* S. Gmelin *ex* Bork. is a curious leafless mycotrophs orchid widely distributed in temperate regions of Europe and Asia and also in tropical Africa [15]. It is represented by 2 – 5 species preponderance in tropical regions of the world [16]. On critical examination and authentication of herbarium at MH, Botanical Survey of India, Coimbatore, the collected species were identified as *Epipogium roseum* (D. Don) Lindl. and is poorly represented in MH. The locality from where this saprophytic orchid has been collected of dense semi-evergreen forest and only five individuals could be spotted.

A scrutiny of literature and specimens in the herbarium revealed that the species *E. roseum* (D. Don) Lindl. hitherto not recollected/ reported after the collection of C.E.C. Fischer in the year 1927 from the Bolampatti hills, Coimbatore district. About a century, the present collection from the Velliangiri hills in the Southern Western Ghats of Coimbatore district form its rediscovery. Hajra [17] reported it to be threatened and Henry et al [18] stated as rare plant.

**Fig. 2. *Epipogium roseum* (D. Don) Lindl.**

## 3. TAXONOMIC IDENTITY, DISTRIBUTION AND ECOLOGICAL STATUS

***3.1. Aphyllorchis montana*** Reichb. f. in Linnea 41: 57. 1876; Hook. f., Fl. Brit. India 6: 116. 1890; King & Pantl. in Ann. Roy. Bot. Gard. (Calcutta) 8: t. 349. 1898; C.E.C. Fischer in Gamble, Fl. Madras 3: 1019. 1957 (repr. ed.). *A. prainii* Hook. f., Fl. Brit. India 6: 117. 1890 & in Hooker's Icon. Pl. t. 2192. 1894; Vajravelu & Rathakrishnan in Bull. Bot. Surv. India 10 (1): 99. 1968; Joseph, Orchids Nilgiris 15. 1982.

Saprophytic, leafless, achlorophyllous, erect herb of 40-60 cm long. Rhizome short,

creeping; roots spreading, stout, tuberous, branched, 3-8 cm long. Stem with many membranous sheaths; proximal sheaths tubular, 0.5-2 cm; sterile bracts 1-1.5 cm. Inflorescence terminal, in lax raceme, elongate, 20-30 cm long. Bracts 1.3-1.5 cm long, much shorter than pedicel and ovary, linear-lanceolate, acuminate, recurved. Flowers spreading, yellow with pinkish margin, pedicellate, bracteates, 3-3.5 cm long. Sepals ± 1 cm long, linear-oblong, acute, 3-nerved; dorsal one concave. Petals slightly shorter but broader than sepals, oblong, rounded at apex, 3-nerved. Lip shorter but broader than sepals and petals, ovate, concave, narrowed towards the obtuse apex; side lobes rounded; claw with 2 short, erect, parallel, triangular, acute wings facing towards the column. Column about 6 mm long. Ovary with the short pedicel, 2.4 cm long, slightly curved. Fruits not known.

*Flowering & Fruiting*: June – September

*Habitat*: Found in grasslands and semi-evergreen forests.

*Ecological status*: Rare in habitat at an altitude of 1600 m.

*Distribution*: India, Sri Lanka, Malaysia, Borneo, the Philippines, Japan, and Taiwan.

*Specimen examined*: Tamil Nadu: Velliangiri hills, V. Aravindhan 8213 (23.06.2012); Ramanathapuram, S.R. Srinivasan 65990 (22.07.1980); Kanyakumari, A.N. Henry 100446 (03.08.1977); Ramanathapuram, P.C. Radhakrishnan 50720 (18.08.1967). Andhra Pradesh: A. Nageswara Rao 65078 (28.06.1986).

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Saprophytic, unbranched leafless, tuberous herb, 50-60 cm high; tubers oblong-ovoid, globose or ellipsoid, 5-6 cm, brownish-black. Stems straw-coloured, hollow, often sheathed; sheath 5-7, inflated, 0.8-1 × 0.5-0.6 cm, truncate. Inflorescence a raceme, 20-25 cm long. Flowers 30-45, in terminal, usually white often flushed with pink, pendulous with prominent ovary; bracts large, oblong-lanceolate, ca.1.5×0.5, membranous, acute at apex. Sepals and petals sub-equal, free, narrow, 1-1.3 cm long, adhering to linear; lip entire, 1-1.5 × 0.5-0.6 cm, ovate, trilobed, concave with erect side margins and short apiculum having a small dorsal oblong swelling on it, margin irregularly erose beyond the base, 2- crested lamellae one on each side of the median nerve. Spur seroliform, 0.2-0.5 cm long, adhering to ovary, disk with two glandular ridges; anther thick, dorsally 2-celled. Ovary 1-1.2 cm long, swollen. Pollinia 2, each with a long and filiform caudicle.

*Flowering & Fruiting*: April – July.

*Habitat*: Found in semi-evergreen forest and growing in dead and decaying, shady places.

*Ecological status*: Rare in habitat at an altitude of 1400 m.

*Distribution*: West Africa, Indo-Malaysia.

*Specimen Examined*: India: Anamalai Hills, Coimbatore district (C.A. Barber – 29.04.1903 – MH: 50727); Bolampatti Hills, Coimbatore district (C.E.C. Fischer – 09.01.1927 – MH: 50724); Yanaikundhi Shola, Coimbatore district (J. Joseph – 29.01.1962 – MH: 51044); Ouchterlong Valley, Nilgiri district (J.L. Ellis – 29.01.1971 MH: 51056); Nilgiri West slopes (V. Chitra – 02.03.1976 – MH: 51068). Velliangiri Hills, Coimbatore district, (V. Aravindhan, 21.07.2012 – MH: 173550).

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DOI:10.26524/krj.2021.16

*Kong. Res. J.* 8 (2): 31-38, 2021
**Publisher**: Kongunadu Arts and Science College, Coimbatore.

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**RESEARCH ARTICLE**

# ANTIMICROBIAL ACTIVITIES OF CERTAIN SEAWEEDS FROM MUDDY SHORE PLACES OF KERALA

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## ABSTRACT

A study was carried out to reveal the growth inhibitory effect of methanol crude extract (MCE) and methanol supernatant extract (MSE) of sea weeds: 1) *Gracillaria corticata,* 2) *Hypnea musciforms,* 3) *Gelidium micropterum* and 4) *Hypnea valentiae* against six bacterial pathogens, 1) *Pseudomonas aeruginosa,* 2) *Bacillus licheniformis,* 3) *Serratia marcescens,* 4*) Aeromonas hydrophila,* 5) *Acinetobacter baumanii,* 6) *Escherichia coli* and two fungal strains, 1) *Aspergillus niger* and 2) *Candida albicans* respectively. Well diffusion method using zone of inhibition as indicator for growth inhibition was adopted. The results showed that methanol extracts of seaweeds *viz.*, *Gracillaria corticata, Hypnea musciforms,* and *Hypnea valentiae* prevented the growth of pathogenic bacteria and fungi. The effect on growth was observed as zone of inhibition, the diameter of which was indicated in the units of a millimeter. The growth of the bacterium, *Serratia marcescens* was affected by methanol supernatant extract of the three types of seaweeds, *Gracillaria corticata, Hypnea musciforms,* and *Hypnea valentiae,* and by the methanolic crude extract of *Hypnea musciforms* and *Hypnea valentiae.* However, the growths of other species of bacteria were not controlled by either of the extracts of the seaweeds except, *Bacillus licheniformis* which was controlled by only *Gracillaria corticata.* The growth of fungi: *Aspergillus niger* and *Candida albicans* were inhibited by the methanol extracts of *Gracillaria corticata.* Between the two forms of methanolic extracts i.e., supernatant and crude, the efficiency of the supernatant extract was greater than that of crude one. Further, among the three types of seaweeds which showed an effect on the growth of microbes, the level of the zone of inhibition caused by *Gracillaria corticata* was statistically higher than that of the other two, *Hypnea musciforms,* and *Hypnea valentiae.*

**Keywords:** *Gracillaria corticata, Hypnea musciforms, Gelidium micropterum, Hypnea valentiae,* Antimicrobial activity**.**

## 1. INTRODUCTION

Various natural antimicrobial compounds have been recorded in a marine environment more than those in the terrestrial one. Marine organisms such as marine algae are source materials for structurally unique natural products with pharmacological and biological activities. Among the marine organisms, the macroalgae (seaweeds) occupy a special site as a source of biomedical compounds [1]. Seaweeds have been recognized as potential sources of antibiotic substances. Synthesis of different metabolites from seaweeds is an indicator of the presence of antimicrobial active compounds [2].

Algae are part of a heterogeneous group of photosynthetic organisms and the division includes multicellular organisms, macroalgae or seaweed (reaching sizes of up to 60 m in length), and unicellular organisms, also known as microalgae (measuring from 1 mm to several cm). One way to classify macroalgae is on the basis of their pigmentation: (i) brown seaweed- Phaeophyceae, (ii) red seaweed-Rhodophyceae and (iii) green seaweed-Chlorophyceae [3]. Global utilization of macro-algae is a multi-billion dollar industry. In recent years pharmaceutical firms have started looking towards marine organisms, including seaweeds, in their search for new drugs from natural products [4]. Compounds with biological activities or pharmacological properties (bioactivities) have been discovered in marine bacteria, invertebrates, and algae [5]. Seaweeds contain many different secondary metabolites which have a wide spectrum of biological activities. It was observed, the presence



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of cytostatic, antiviral, anthelmintic, antifungal, and antibacterial activities compounds in green, brown, and red algae with cytostatic, antiviral, anthelmintic, antifungal, and antibacterial activities [6]. Seaweeds are considered to be the main source of bioactive compounds with a wide range of biological activities, such as antibiotics, antioxidants, and anti-inflammatory [7]. Some macroalgae have bio-active components which affected the germination of some pathogenic bacteria, it contains different substances which incorporated medicine and pharmacotherapy, whereas some of the isolated substances have bacteriostatic and bactericidal properties. Different diseases were treated with antibiotics, extracted from terrestrial sources that were used as therapeutic agents; new compounds were present in oceans and have commercial value [8].

Several researchers attempted to identify organisms that produce bioactive substances, antioxidant and antimicrobial activity of seaweeds have been reported previously [9]. A good number of reports show that seaweeds are also a rich source of antioxidant compounds and screened extensively to isolate lifesaving drugs or biologically active substances all over the world. Marine algae are a potential source of new secondary metabolites [9]. Numerous chemical compounds were identified as antimicrobial agents including phenolic compounds, phlorotannins, terpenoids, fatty acids, chlorellin, and steroids [10].

However, the quantitative ranges of these bioactive molecules can fluctuate widely according to season, environmental conditions, geographical location, and reproductive stage [11]. It has been reported that among the algae groups, Phaeophyceae exhibited the highest antibacterial activity and 75% of the most secondary metabolites were derived from brown algae [12]. Currently, a large number of structurally unique bioactive agents derived from seaweeds have been identified and some of them are under experimentation or are being developed as new pharmaceutical drugs [13]. Most of the compounds of the marine algae show anti-bacterial activities and also metabolites isolated from marine algae have been shown to possess bioactive effects [14]. Seaweed *Ulva fasciata* have shown antimicrobial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa* that are commonly found among human infections [15].

In recent years there has been found an increase in the resistance of microorganisms to antibiotics that are usually used in the treatment of some diseases. To overcome this problem, new therapeutic drugs from natural products have been explored [16]. Marine algae have been extensively documented for their capacity to provide a rich source of primary and secondary metabolites [17]. There are several substances obtained from algae that are already in use in traditional medicine for a long time [18].

Although there are numerous publications on antimicrobial activity of algal extracts, they usually report results of algae collected from their natural habitats. In fact, to the best of my knowledge, there is no data concerning the antimicrobial potential of algae from aquaculture systems. The objectives of the present study are therefore to evaluate the antimicrobial activity of methanol extracts of four seaweeds *viz*., 1) *Gracillaria corticata,* 2) *Hypnea musciforms*, 3) *Gelidium micropterum* and 4) *Hypnea valentiae* against six bacterial pathogens, *viz.*, 1) *Pseudomonas aeruginos,* 2) *Bacillus licheniformis,* 3) *Serratia marcescens,* 4) *Aeromonas hydrophila* 5) *Acinetobacter baumanii,* 6) *Escherichia coli* and two fungal strains *viz*., 1) *Aspergillus niger* and 2) *Candida albicans.* In the present study, we report the efficacy of seaweeds collected from the West coast of India against multi resistant pathogens.

## 2. MATERIALS AND METHODS

### *2.1. Sample collection*

The seaweeds, *Gracillaria corticata, Hypnea musciforms, Gelidium micropterum* and *Hypnea valentiae* were collected from Manjeshwar coast, Kerala. The collected samples were thoroughly washed with seawater to remove all the extraneous matter such as epiphytes, sand particles, pebbles, and shells, and samples were brought to the laboratory species-wise in separate plastic bags. The samples were again cleaned with fresh water and distilled water in the laboratory. The samples were then air-dried under the shadow. Each type was separately powdered and stored at room temperature until extraction.

### *2.2. Preparation of seaweed methanol extract*

The powdered sample (10 g) was extracted in Soxhlet apparatus using methanol (250 ml) as a solvent for 8h at 60˚C. Extracts are filtered using a muslin cloth, followed by Whatman No.1 filter paper and the solvents were allowed to evaporate in Hot air oven under 40˚C. The extracts obtained were stored in a refrigerator. The extracts were carried out following the standard procedure dried extracts are used for assay [19]. Each concentrated filtrate

was made into different concentrations (20 µg/ml, 40 µg/ml, 60 µg/ml and 80 µg/ml) using methanol.

*2.3. Test pathogens*

The pathogens used in the present study viz. *Pseudomonas aeruginosa, Bacillus licheniformis, Serratia marcescens, Aeromonas hydrophila, Acinetobacter baumanii, Escherichia coli, Aspergillus niger,* and *Candida albicans* were obtained from the Research Department of Biotechnology, Kongunadu Arts and Science College, Coimbatore, Tamil Nadu, India.

*2.4. Assay of antimicrobial activity*

All the bacterial and fungal cultures were subjected to test their susceptibility/ resistance pattern to the methanol extract of the seaweeds by well diffusion method as described by Bauer *et al*. [20] using Mueller Hinton agar (HiMedia, India). Sterilized media were dispensed into sterile petri dishes aseptically. Broth cultures of the test organisms were swabbed over the surface of the separate agar plate using sterile cotton swab aseptically. In each of these plates, wells (10mm) were cut out using a sterile cork borer.

Different concentrations (20, 40, 60, and 80 µg/ml) of dried seaweed extracts were prepared in methanol and loaded into the respective labelled wells using sterile pipettes. In each plate, one of the wells was used as a control (solvent alone). The plates were incubated at 37$^{o}$C for 24hrs for bacteria and 5 days for fungi.

After the incubation, the diameters of inhibition zones were measured using a ruler and expressed in millimeters. Based on the level of inhibition zone the response of microbes to the extract was compared and classified as resistant, intermediate, and sensitive according to Johnson and case [21]. If the diameter of the zone of inhibition was 10 mm or less, the microbe concerned was considered resistant to the seaweed extract. If the diameter was 16mm and above, the microbe was marked susceptible. If it was in the range between 11and 5mm, the microbe was noted as intermediate in response to the type of seaweed used.

## 3. RESULTS

*3.1. Antimicrobial activity of the seaweed, Gracillaria corticata*

The control well showed no zone of inhibition and the bacterial, as well as fungal growth was found around the well which was incubated with solvent, methanol only. There was a zone of inhibition in the medium of *Bacillus licheniformis* of 11 mm around the well provided with crude methanol extract at a concentration of 80 µg/ml. In the experiment with methanol extract (supernatant) the zone of inhibition for *B. licheniformis* extended to the level of 15mm (Figures 3 and 4). The growth of bacterium *Serratia marcescens* was well controlled by this species of seaweed as evidenced by a gradual increase in the diameter of zone of inhibition from 13 mm to 20 mm for the concentrations from 20 µg/ml to 80 µg/ml of methanol extracts supernatant (Figure 5).

The species *Gracillaria corticata* the seaweed also showed its effect on the growth of fungus, *Aspergillus niger* as indicated from the zone of inhibition as 10mm, 12 mm, and 30 mm for concentrations 40 µg/ml, 60 µg/ml, and 80 µg/ml, respectively (Figure 6). The fungus, *C. albicans* showed a susceptible level of the zone of inhibition (16 mm) to the crude methanol extract of the seaweed, *G. coticata*. Thus, both the bacterial species and the fungal species were found as susceptible to the methanol extract supernatant of this seaweed.

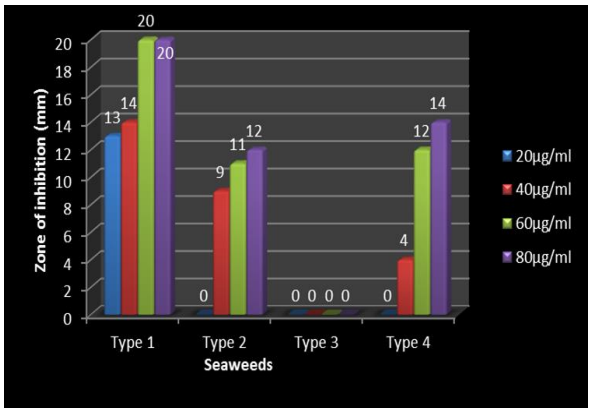


**Fig. 1.** Diameter of Zone of inhibition (mm) as observed in culture media of pathogenic bacteria, *Serratia marcescens* treated with methanol extract supernatant of seaweeds, 1) *Gracillaria corticata, 2) Hypnea musciforms, 3)Gelidium micropterum* and 4) *Hypnea valentiae*

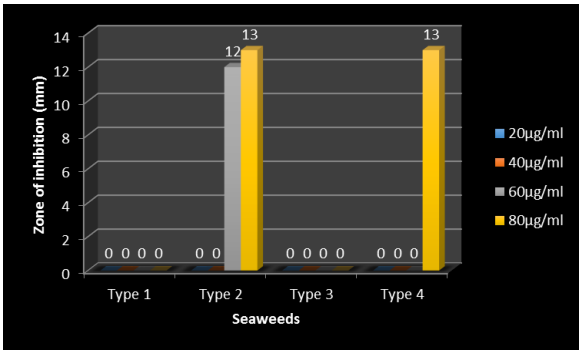


**Fig. 2.** Diameter of Zone of inhibition (mm) as observed in culture media of pathogenic bacteria, *Serratia marcescens* treated with crude methanol extract of seaweeds, 1) *Gracillaria corticata, 2) Hypnea musciforms, 3) Gelidium micropterum* and 4) *Hypnea valentiae*

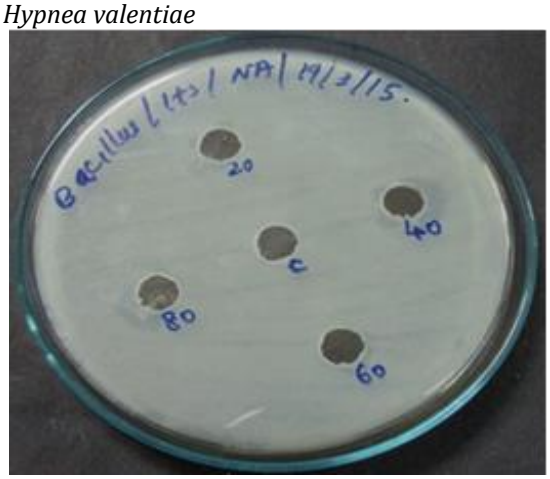


**Fig. 3.** The photograph shows zone of inhibition on well diffusion medium between *Bacillus licheniformis* and *the* methanol extract (supernatant) of seaweed, *Gracillaria corticata* at concentrations, 20 µg/ml, 40 µg/ml, 80 µg/ml and control.

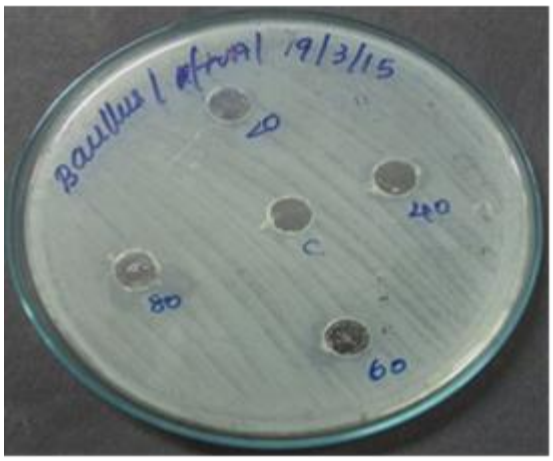


**Fig. 4.** Photograph shows zone of inhibition on medium between *Bacillus licheniformis* and methanol extract (crude) of seaweed, *Gracillaria corticata* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

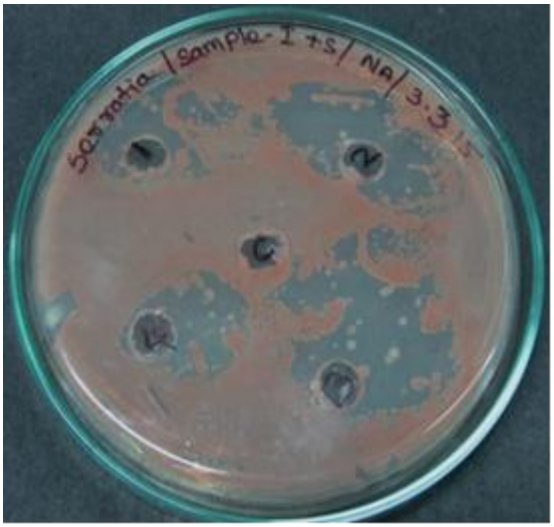


**Fig. 5.** Photograph shows zone of inhibition on medium between *Serratia marcescens* and methanol extract (Supernatant) of seaweed, *Gracillaria corticata* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

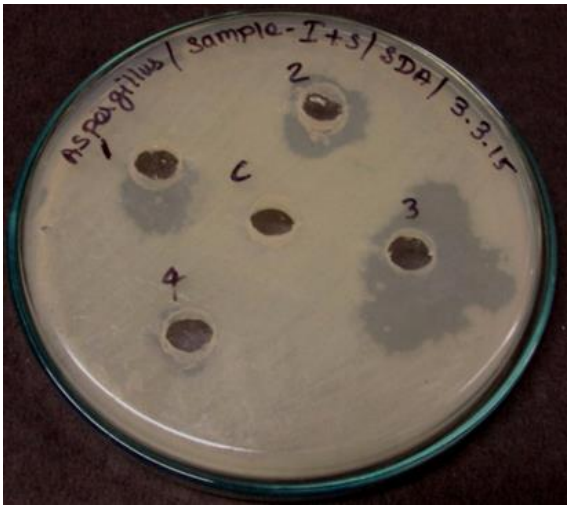


**Fig. 6.** Photograph shows zone of inhibition on medium between *Aspergillus niger* and methanol extract (Supernatant) of seaweed, *Gracillaria corticata* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

*3.2. Antimicrobial activity of the seaweed, Hypnea musciforms*

The controlling effect was observed for both crude methanol extract as well as methanol extract supernatant of this seaweed over the bacterium, *Serratia marcescens*. The crude extract at concentrations 60 µg/ml and 80 µg/ml prevented the growth of this species of the bacterium as evidenced from the diameter of zone of inhibition, 12mm and 13mm, respectively. The zone of inhibition of 9 mm, 11 mm, and 12mm was recorded for methanol extract supernatant concentrations 40 µg/ml, 60 µg/ml and 80 µg/ml, respectively. The growth of other bacterial and fungal types *Pseudomonas aeruginos, Bacillus licheniformis, Aeromonas hydrophila, Acinetobacter baumanii, Escherichia coli, Aspergillus niger* and *Candida albicans* were not affected by this seaweed.

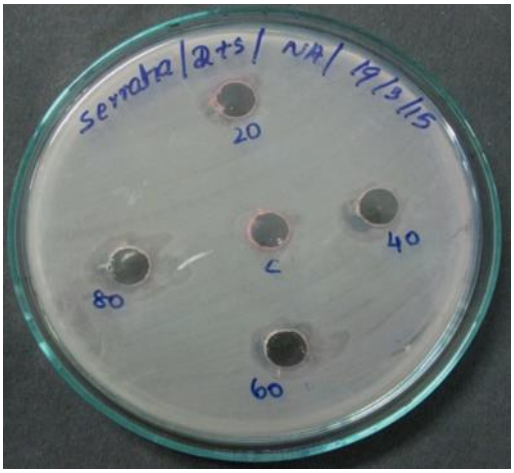


**Fig. 7.** Photograph shows zone of inhibition on medium between *Serratia marcescens* and methanol extract (supernatant) of seaweed, *Hypnea musciforms* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

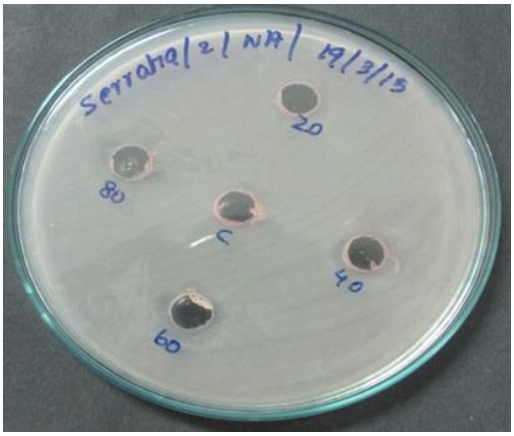


**Fig. 8.** Photograph shows zone of inhibition on medium between *Serratia marcescens* and methanol extract (crude) of seaweed, *Hypnea musciforms* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

*3.3. Antimicrobial activity of the seaweed, Gelidium micropterum*

Either crude methanol extracts or methanol extracts supernatant of this seaweed showed no effect on the growth of any of the bacterial and fungal types used in the present study. The culture medium inoculated with pathogens, *Pseudomonas aeruginos, Bacillus licheniformis, Serratia marcescens, Aeromonas hydrophila, Acinetobacter baumanii, Escherichia coli, Aspergillus niger,* and *Candida albicans* and different concentrations of the seaweed, *G. micropterum* was identical to that of the control in appearance. The growth of microbes was uniform throughout the area of culture irrespective of the wells supplied with different concentrations of methanol extracts of this seaweed.

*3.4. Antimicrobial activity of the seaweed, Hypnea valentiae*

The seaweed *H. valentiae* showed its effect on the growth of bacterium *Serratia marcescens* by crude methanolic concentration, 80 µg/ml as evidence that from a zone of inhibition of diameter 13mm. The extract supernatant was highly effective even at its low concentrations. The zone of inhibition at concentration 40 µg/ml was 4mm and at 60 µg/ml it was 12mm (Figures 9 and 10). The highest inhibition of growth was found around well provided with a concentration of 80µg/ml where the diameter of the zone of inhibition was 14mm. The gradual increase in the diameter of the zone of inhibition with methanol extract concentration of the seaweed indicates its antimicrobial activity against the bacterium. However, in either form of the extract, this seaweed did not show the effect on the growth of any other microbes selected for this study.


**Fig. 9.** Photograph shows zone of inhibition on medium between *Serratia marcescens* and methanol extract (Supernatant) of seaweed, *Hypnea valentiae* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

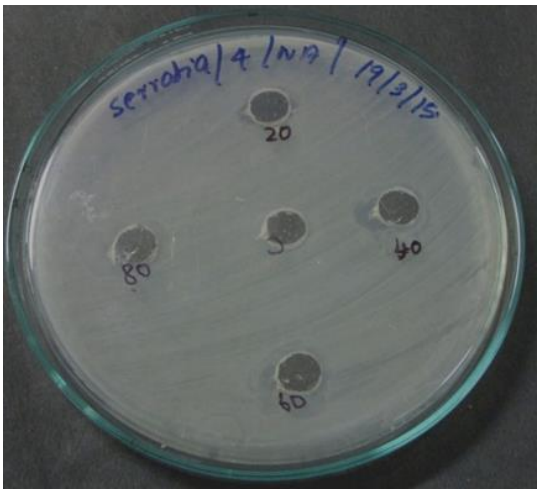


**Fig. 10.** Photograph shows zone of inhibition on medium between *Serratia marcescens* and methanol extract (Crude) of seaweed, *Hypnea valentiae* at concentrations, 20 µg/ml, 40 µg/ml, 60 µg/ml, 80 µg/ml and control.

*3.5. Comparative account of the effect of seaweeds on microbes*

Among the four types of seaweeds, *Gracillaria corticata, Hypnea musciforms, Gelidium micropterum* and *Hypnea val entiae* used in the present study no preventive effect was shown by *G. micropterum* on the growth of all the types of microbes selected, *Pseudomonas aeruginos, Bacillus licheniformis, Serratia marcescens, Aeromonas hydrophila, Acinetobacter baumanii, Escherichia coli (*Bacteria) *Aspergillus niger* and *Candida albicans* (Fungi).

The growth of a bacterium, *Serratia marcescens* was affected by methanol extract supernatant of the three types of seaweeds, *Gracillaria corticata, Hypnea musciforms,* and *Hypnea valentiae,* and by the crude methanolic extract of *Hypnea musciforms* and *Hypnea valentiae* (Fig. 1&2). However, the growths of other species of bacteria were not controlled by either of the extracts of the seaweeds except, *Bacillus licheniformis* which was controlled by only *Gracillaria corticata.* The growth of fungus, *Aspergillus niger,* and *Candida albicans* was inhibited by the methanol extracts of *Gracillaria corticata.*

Between the two forms of methanolic extracts i.e., supernatant and crude, the efficiency of extract supernatant was found greater than that of crude one. Further, among the three types of seaweeds, significant effect on the growth of microbes, the level of zone of inhibition caused by *Gracillaria corticata* was statistically higher than that of the other two, *Hypnea musciforms,* and *Hypnea valentiae.*

**4. DISCUSSION**

Many researchers have reported on the antioxidant and antimicrobial activity of seaweeds [22,23]. The extraction of antimicrobials from different species of seaweeds was solvent dependent. Methanol was a good solvent for the extraction of antimicrobials from brown seaweeds whereas acetone was better for red and green species [24]. Extracts of marine algae were reported to exhibit antibacterial activity [25]. Several workers have reported that the seaweed extracts exhibit inhibitory activity against a number of gram positive and gram negative bacterial pathogens. A number of seaweeds have been studied for their antibacterial activity. Padma Sridhar *et al*. [26] screened the antibacterial activity of extracts of marine algae representing Chlorophyta and Rhododphyta collected from Vishakapatnam Coast against two pathogens and also tested their ability to inactivate the enzyme penicillinase under *in vitro*. Padmakumar and Ayyakkannu [27] revealed the antimicrobial activity of marine algae collected from Porto Novo and Pondicherry waters, against 6 bacterial and 2 fungal pathogens.

Rao and Parekh [28] showed that crude extracts of seaweeds are active only against gram positive bacteria. Vanitha *et al*. [29] reported the antibacterial action of nine seaweeds collected

from the Kanyakumari coast against human upper respiratory tract pathogens which include both Gram positive and Gram negative bacteria. Kandhasamy and Arunachalam [30] found out the *in vitro* antibacterial property of seaweeds viz. *Caulerpa racemosa, Ulva lactuca, Gracilaria folifera, Hypnea musciformis, Sargassum teneerimum, S. myriocystem,* and *Padina tetrastomatica* collected from Koodankullam village, Tirunelveli, Tamilnadu against gram negative and gram positive pathogenic bacteria. Some commonly occurring marine algae *Caulerpa scalpelliformis, Ulva lactuca, Pandina tetrastromatica, Stoecchospermum marginatum* and *Acanthophora spicifera* have been collected from the coast of Tuticorin, Tamilnadu and evaluated for antifungal and antibacterial activity by using four solvents such as petroleum ether, chloroform, methonal, and benzene [31]. Arul Senthil *et al*. [32] found out the antibacterial activity of the methanol, diethyl ether, acetone, and dichloromethane extracts of *Padina boergesnii* collected from Tuticorin Coast against 10 human pathogenic bacteria.

## 5. CONCLUSION

To conclude, among the extracts of seaweeds tested in the present study, *Gracillaria corticata* has been found as highly effective against bacterial and fungal pathogenic organisms and hence recommended to be used as antimicrobial agent. Further, the effect of methanol extract supernatant has shown greater effect rather than its counterpart crude methanol extract. Hence, suggested that further analysis is required to identify the active component which would be relatively showing higher effect in methanol extract supernatant.


## ACKNOWLEDGEMENT

Authors are grateful to the Department of Zoology and Biotechnology of Kongunadu Arts and Science College for providing laboratory facilities.


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DOI:10.26524/krj.2021.17

*Kong. Res. J.* 8 (2): 39-43, 2021
**Publisher**: Kongunadu Arts and Science College, Coimbatore.

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**RESEARCH ARTICLE**

# SEQUEL OUTCOME OF AUTOMOBILE SECTOR ON ACCOUNT OF COVID – 19 THROUGH TECHNICAL ANALYSIS

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**ABSTRACT**

The COVID – 19 pandemic has deteriorated multiple facets of the stable functioning of economies of most countries. Social restrictions associated with the immediate response to the pandemic has curtailed dynamic functioning of many industries that buttress the economic development of countries. Performance of automotive industries was expected to nosedive following the travel restrictions. One of the major sources of profit for the automotive industries in India is their consumer base in countries like U. K, Germany, and China etc. Severity of the pandemic in these countries entailed trade regulations that propelled a negative trend in the market growth of Indian automotive industries. But the economy of automotive sector of India was saved from a free fall by the countering effect of the domestic demand in private transportation. This paper presents the technical analysis on the Maruti Suzuki Private Limited to measure the stock movement of the Automobile sector in the Indian Stock Market.

**Keywords:** COVID – 19 pandemic, Indian automotive industries, Maruti Suzuki Private Limited, NSE, Nifty Index, NIFTY 50.

## 1. INTRODUCTION

Stock Exchange is a place where stock brokers and traders trade stocks, bonds and other securities and often functions as continuous auction. It also facilitates issue and redemption of securities and other capital events including the payment of income and dividends.

Securities traded in a stock exchange include stocks issued by listed companies, unit trusts, derivate, pooled investment products and bonds. National Stock Exchange (NSE) of India is a fast growing stock exchange.

India is the fourth largest automobile market in the world after China, US and Japan. The automobile sector in India contributes to 7.1% of the National GDP. The two wheeler segment with 81% market share is the leader of the Indian Automobile market owing to a growing demand from the financial middle class and young populace. Moreover, the growing interest of companies in exploring the rural demand further aids the growth of the sector. The Passenger Vehicle (PV) segment has 13% market share. India is also a prominent auto exporter and has strong projections for growth in export. In April-March 2016, overall automobile exports grew by 1.91%. Passenger Vehicles, Commercial Vehicles and Two Wheelers registered a growth of 5.24%, 16.97% & 0.97% respectively in April - March 2016. Government of India and Automobile Companies have taken various initiatives to transform India to be a leader in the Two Wheeler & Four Wheeler market of the world by 2020. But Covid-19 has reached India with a different plan, which scattered the hopes and dreams of Automobile Sector. Still the fear factor of the public towards Covid-19 and more caution towards social distancing kept away them from public transportation, which in turn gradually pushed the Automobile Sector upward after the liberalisation of lockdown. Hopefully, Indian Automobile sector may emerge as one of the leading segments in the stock market as they constitute lucrative and regularly traded stocks. In this paper, we perform technical analysis on the Maruti Suzuki Private Limited to measure the stock movement of the Automobile sector in the Indian Stock Market.

## 2. PRELIMINARIES

### *2.1. Intrinsic Value*

Intrinsic value is the true value of an asset and is not similar to the current market price. It is the key of fundamental analysis for the investors to assess the stocks.



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*2.2. Simple Moving Average (SMA)*

The Simple Moving Average is simply the average price of stocks over the specified period. A simple moving average (**SMA**) calculates the average of a selected range of prices, usually closing prices, by the number of periods in that range.

$$\textbf{SMA} = \frac{\sum N}{N}$$

*2.3. Exponential Moving Average (EMA)*

The Exponential Moving Average is a technical chart indicator that tracks the price of an investment (like a stock or commodity) over time. EMA is calculated as

**EMA = (Close price – Previous day EMA) *(WMA+ Previous day EMA)**

*2.4. Moving Average Convergence and Divergence (MACD)*

MACD is about the convergence and divergence of the two moving averages, they converge as they move towards each other and diverge as they move apart. Moving average convergence divergence (**MACD**) is a trend-following momentum indicator that shows the relationship between two moving averages of a security's price. The **MACD** is calculated by subtracting the 12-period exponential moving average (EMA) from the 26-period EMA.

**MACD = 12D EMA – 26D EMA**

## 3. TECHNICAL ANALYSIS

Technical analysis seeks to interpret the outcomes in price of stocks examining historical data. It helps traders and investors navigate the gap between intrinsic value and market price by leveraging techniques like statistical analysis and behavioural economics. Technical analysis helps to guide traders to actually find the future prediction of the stocks.

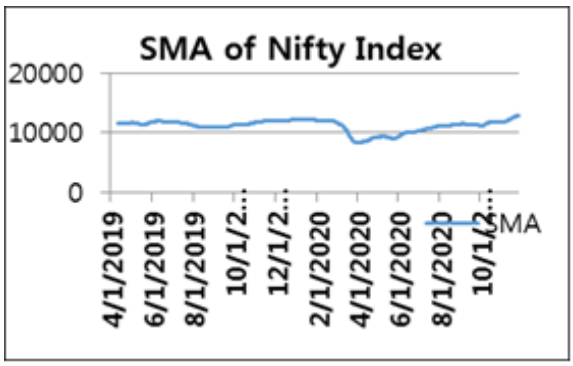
In this section, we will be considering the historical data of the Automobile sector for the current financial years. In particular, we are analyzing the movement of the stocks of the Maruti Suzuki Private Limited. The Nifty Index will hold the base and helps in better understanding the unwavering movement of the stocks, resulted by the Covid-19 pandemic. Frequency analysis, Simple Moving Average (SMA), Exponential Moving Average (EMA) are used to interpret the data and conclude.

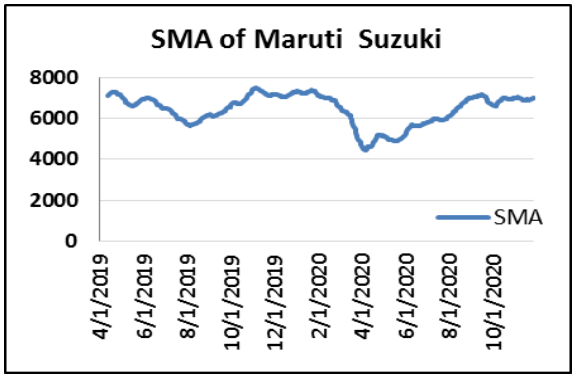
### 3.1. GRAPHICAL REPRESENTATION

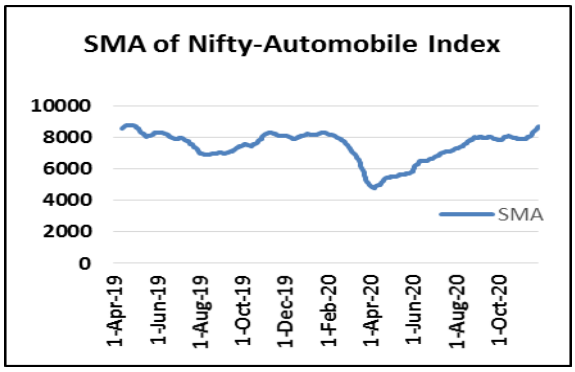
The data between 01.04.2019 and 30.12.2020, of the Nifty Index, NIFTY -Automobile Index and the Maruti Suzuki Limited in particular have been collected, and the SMA, EMA and MACD, is interpreted using these technical indicators.

*3.1.1. 10D – SMA*

The Simple Moving Average of the NIFTY-Automobile Index, Nifty index and the Maruti Suzuki Limited are depicted below.



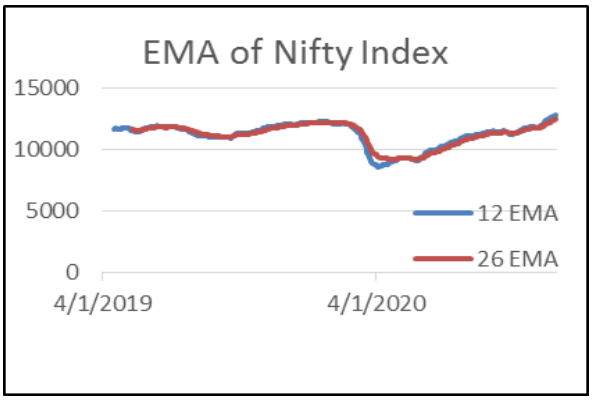



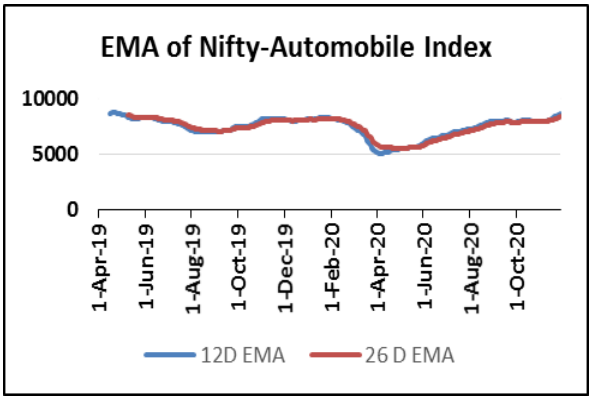



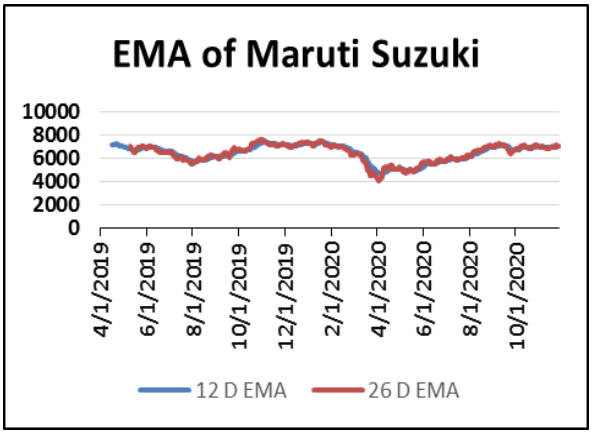

*3.1.2. 12D EMA & 26D EMA*

The Exponential Moving Average of the NIFTY-AUTO, NSE index and the Maruti Suzuki Industries Limited are depicted below.

EMA = (C – YEMA) * W.M. + YEMA



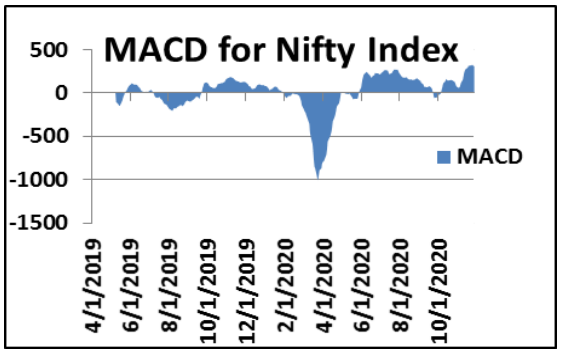



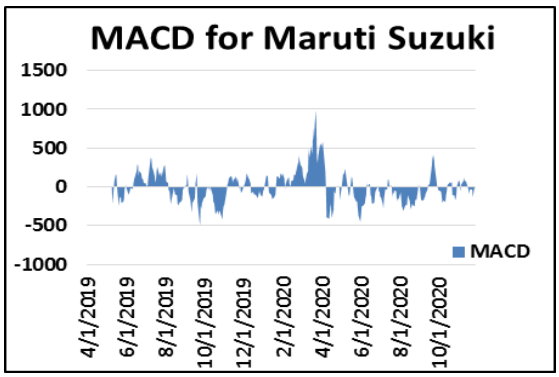



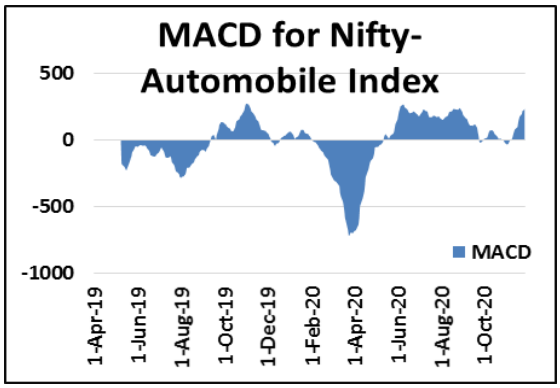

*3.1.3. MACD*

The Moving Average Convergence Divergence of the NIFTY-Automobile Index, Nifty index and the Maruti Suzuki Private Limited are

MACD = 12D EMA – 26D EMA



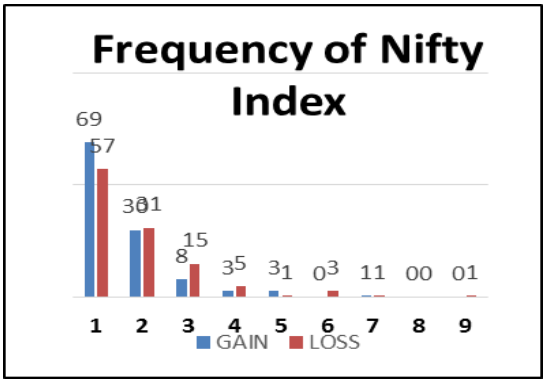

The graphs of MACD for all the three data has been interpreted and hence the result of the behaviour of the stocks is in a more bearish trend particularly in the month of April 2020 for the NIFTY-Automobile Index and Nifty Index, whereas Maruti Suzuki has experienced a bullish trend set during the same time. The pattern holds similar for both NIFTY-Automobile Index and Nifty Index, but it has upward trend set during January 2020- April 2020 for Maruti Suzuki. The reason behind due to the downtrend in Nifty Index and NIFTY-Automobile Index majority is contributed as the immediate impact of the imposition of lockdown and thus the market underwent a downward movement.

We also find that the stocks of both the indices of Nifty Index and NIFTY-Automobile Index show a gradual bullish trend gradually after June 2020, which implicates the cause due to the unavail of the public transport and the Maruti Suzuki were being traded in a bearish trend towards December 2020.

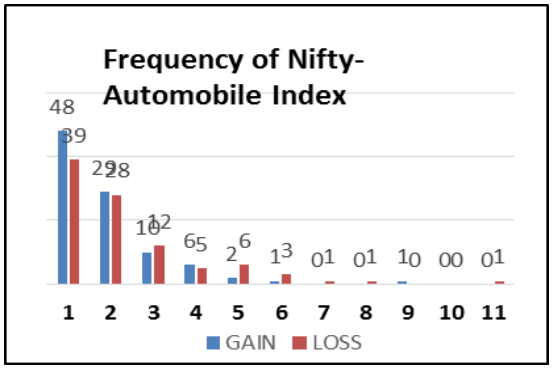
*3.1.4. Frequency Table*

The Frequency Table of the NIFTY-Automobile Index, Nifty index and the Maruti Suzuki Limited are depicted below. The frequency graph for the consecutive Gain and Loss are drawn for the period of lockdown between 01.04.2019 and 30.12.2020.

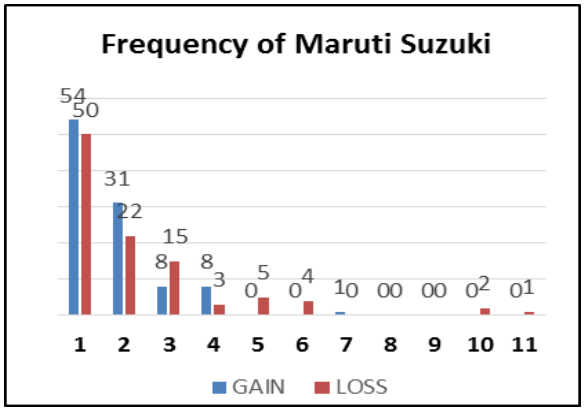
| Frequency Table of Nifty Index | | |
|---|---|---|
| **No. of Days** | **Gain** | **Loss** |
| 1 | 69 | 57 |
| 2 | 30 | 31 |
| 3 | 8 | 15 |
| 4 | 3 | 5 |
| 5 | 3 | 1 |
| 6 | 0 | 3 |
| 7 | 1 | 1 |
| 8 | 0 | 0 |
| 9 | 0 | 1 |
| | 187 | 223 |
| | Total | 410 |




| Frequency table of Nifty Automobile Index | | |
|---|---|---|
| **No. of Days** | **Gain** | **Loss** |
| 1 | 48 | 39 |
| 2 | 29 | 28 |
| 3 | 10 | 12 |
| 4 | 6 | 5 |
| 5 | 2 | 6 |
| 6 | 1 | 3 |
| 7 | 0 | 1 |
| 8 | 0 | 1 |
| 9 | 1 | 0 |
| 10 | 0 | 0 |
| 11 | 0 | 1 |
| | 185 | 225 |
| | Total | 410 |




| Frequency Table of Maruti Suzuki | | |
|---|---|---|
| **No. of Days** | **Gain** | **Loss** |
| 1 | 54 | 50 |
| 2 | 31 | 22 |
| 3 | 8 | 15 |
| 4 | 8 | 3 |
| 5 | 0 | 5 |
| 6 | 0 | 4 |
| 7 | 1 | 0 |
| 8 | 0 | 0 |
| 9 | 0 | 0 |
| 10 | 0 | 2 |
| 11 | 0 | 1 |
| | 179 | 231 |
| | Total | 410 |



The frequency tables and the graph discussed above provide us with a clear understanding of the fluctuating nature of the stock markets. We can see that nearly 50 % of it has been as a to – fro movement between Gain and Loss. The wavering sign is the nature of stocks is usual and holds similar trend set.

The highest gain frequency was found at the rate of 7 consecutive days performed by the Maruti Suzuki during the month of January 2020 and the Nifty Index highest gain frequency of 7 consecutive days in the month of November 2020. The first frequency of the Gain could be due to the serious impact of lockdown regulations imposed and the second frequency of the Loss could be due to the unstandardized economy in the people's livelihood as a deteriorating part of COVID crisis. The frequency of NIFTY-Automobile Index in these months were seen fluctuating at the rate of 1 day and 2 days.

The highest loss frequency can be found at the rate of 11 consecutive days which has also been performed by the NIFTY-Automobile Index and the Maruti Suzuki Private Limited. These frequencies were marked at the months of June and November 2019. Eventually, the wavering of frequency supposed post COVID times, are interpreted as the consequences of lockdown regulations aiming the safety and the reach to private vehicles and the unstable economic regression. The frequency of Nifty Index in these months were seen fluctuating at the rate of 1 day and 2 days.

## 4. INTERPRETATION

The Covid-19 pandemic is believed to be one of major consequences experienced in the history of mankind, enforcing a step down in the economy across the globe.

The Gross Domestic Product (GDP) growth rate was at a downline edge which further took a steep down as a cause of Covid-19. This pandemic has terminated the growth of various arena due to the restrictions. In context of those suppressed by this outbreak, the Automobile Index rose out to stand with a more stable performance with a positive sign of improvements. This lockdown has thus impacted the Automobile Index in optimistic line of backup, as a result of the increased demand for the private vehicles to hold safety during the pandemic times and also due to the unavail of public transport. The analysis reflects that the sector has been in an oscillatory movement at the initial stages of lockdown, during the month of April and May. While the Nifty Index has been performing in a current ground progress, the Automobile Index and in particular, the Maruti Suzuki Limited has performed really in an appreciative manner. This merely indicates that there is a relatively high demand for the automobiles during the lockdown.

According to our analysis, we have been at an interpretation that the sector has seen emergence of hike not only in the initial pandemic period, but in the subsequent continuation of days. In fact, we can clearly conclude that the Automobile Index experienced a highly significant volume around the month of April-June and the influential factor is highly due to the impact of regulations of COVID-19 pandemic.

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**Publisher**: Kongunadu Arts and Science College, Coimbatore.

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**REVIEW ARTICLE**

# A PHYTO PHARMACOLOGICAL REVIEW OF MEDICINALY IMPORTANT PLANT *SOLENA AMPLEXCAULIS* (CUCURBITACEAE)

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## ABSTRACT

*Solena amplexicaulis*, (Cucurbitaceae) commonly known as the creeping cucumber, native to tropical southern Asia. It is generally prescribed for wound healing by the local healers in western districts of Tamil Nadu. The fruits, leaves, roots and shoots have used as food and it is traditionally used as astringent, appetizer, carminative, cardiotonic, digestive, diuretic, expectorant, invigorating, purgative and stimulant. It have lot of medicinal uses such as antioxidant antidiabetic, antibacterial etc. The available reports on physicochemical, anti-microbial activity, anti-oxidant activity and pharmacological value of *Solena amplexicaulis* are discussed in this review.

**Keywords:** *Solena amplexicaulis,* pharmacological studies, phytochemical screening.

## 1. INTRODUCTION

Herbal plants are integral parts of the traditional medicine worldwide and most of the rural and urban population used these plants in many of their regular needs even today. The current researchers are more focused on natural chemicals than the synthetic chemicals due to their environmental, economical and health benefits. Plants produce many chemical compounds for its biological activities against microbes, insects and herbivors and these chemicals are called as phytochemicals. Herbal plants are a natural source of many important phytochemicals and widely used in pharmaceutical, food and cosmetic industries. A wide variety of herbal plants are available in the Indian subcontinent and they are the backbone of Indian traditional medicines such as Ayurveda Siddha and Unani.

*Solena amplexicaulis* is a perennial dioecious climber with tuberous root found throughout Asia mainly growing in hilly dry deciduous forests, scrub jungles. The tubers, leaves and seeds are extensively used in traditional system for various ailments like hepatosplenomegaly, spermatorrhoea, appetizer, cardiotonic, diuretic and thermogenic, haemorrhoids and invigorating [1]. The leaves have good anti-inflammatory activity and also prescribed for skin lesions and other skin diseases [2].

Due to the importance of *Solena amplexicalis* in modern medicine as a potential candidate for curing many diseases. In this regard, the phytochemical study, antimicrobial property, antioxidant property and the pharmacological value of this plant is explained in this review.

## 2. PLANT DESCRIPTION

*Solena amplexicaulis* is found in Sri Lanka, Pakistan, India, Nepal, Bangladesh, Myanmar, Indonesia, Vietnam and China at altitudes of up to 2,600 m (8,500 ft). It grows in a range of habitats including tropical mixed forests, thickets, hilly areas, semi-cultivated areas and roadside verges [3]. It also found in Chittagong, Chittagong Hill Tracts [2]. In India as the foothills of both Western and Eastern Ghats around 600m above in certain localities where dry deciduous forest/scrub jungles are available, it is present with less population size.



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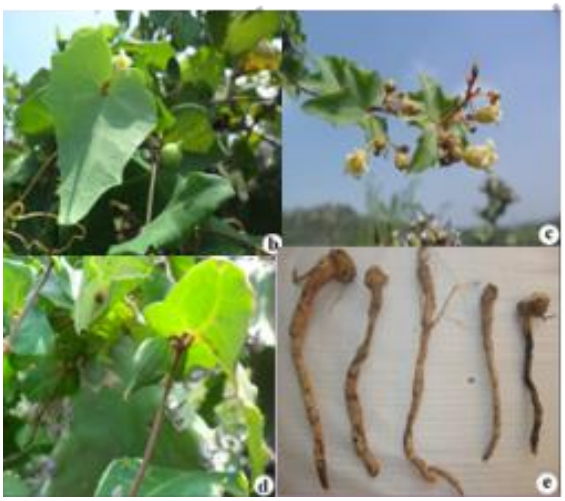


**Figure 1.** a) Habit b) Female flower, c) Male flower, d) Fruit, e) Tuber

*2.1. Distribution*

The systematic position of this species according to Bentham and Hooker (1862-1883) system of classification is given below:

| | | |
|---|---|---|
| Division | : | Phanerogams |
| Class | : | Dicotyledons |
| Subclass | : | Polypetalae |
| Series | : | Calyciflorae |
| Order | : | Passiflorales |
| Family | : | Cucurbitaceae |
| Genus | : | *Solena* |
| Species | : | *Amplexicaulis* |

*2.2. Synonyms*

The vernacular names are given in the Table 1

| S.No | Language | Vernacular Name |
|---|---|---|
| 1 | Bengali | Kudri |
| 2 | Hindi | Van kakdi, Amantamul, Ban kakra, Tarali |
| 3 | Kannada | Bimpuli |
| 4 | Malayalam | Nerinnampuli |
| 5 | Marathi | Gometi |
| 6 | Nepali | Ban kankro |
| 7 | Oriya | Kamaraja |
| 8 | Sanskrit | Amlavetasah |
| 9 | Tamil | Pulivanchi |
| 10 | Telugu | Adavi donda, Tigadonda |
| 11 | Urdu | Bankakra |

*2.3. Botanical description*

*Solena amplexicaulis* is a dioecious perennial climber with many spindle-shaped tuberous roots which are 1.5-2 cm in diameter and with slender branched furrowed, stems bearing simple tendrils; Leaf-stalk slender, 4-10 mm, finely velvet-hairy at first, becoming hairless after some time. Leaf blades are very variable may be polymorphous, ovate, suborbicular, oblong or narrowly lanceolate in shape, 3-5 angled or lobed, lobes are lathery, usually cordate at base, reticulately veined beneath, margins remotety denticulate, oblong-lance shaped, lance shaped, or triangular, 8-12 × 1-5 cm, below densely bristly or almost hairless, above densely bristly or scabrous, base heart-shaped, margin entire or toothed, tip blunt or tapering. Flowering and fruiting: May to January [3].

*2.4. Flowers*

Flowers are yellow or yellow-white in colour, petals are triangular in shape, 1-1.5 mm in size, tip of petals are blunt or pointed, filaments are threadlike, about 3 mm in length. Male flowers are umbellate or subumbellate, flower-cluster-stalk is very short, apically with 10-20-flowered. Flower-stalks are 2-8 mm in length, calyx tube about 3-5 mm in length and about 3 mm in diameter. Female flowers are usually solitary, flower-stalk is about 2-10 mm in length, finely velvet-hairy, calyx and flower of female flower is same as male flowers [3].

*2.5. Seeds*

Seeds are subcircular in outline, pale creamy brown with conspicuosly, nearly round or ovate in shape, 5-7 × 5-6.5 mm in size and smooth or slightly tuberculate [3].

## 3. TRADITIONAL USES

The unripe fruits of *Solena amplexicaulis* are used for making salads and in curries, and leaves, stem and tubers are used for edible purpose [4]. In Chhattisgarh, the fruits and roots are consumed to assist in the digestion of bushmeat. Creeping cucumber can be gathered from the wild or can be cultivated as a field crop and given suitable supports over which to climb [5].

In traditional medicine, the tuberous roots of *Solena amplexicaulis* are used to treat anorexia, digestive problems, flatulence, asthma, gonorrhoea and spermatorrhoea, and extracts of the leaves are widely used to treat inflammation. Whole plant used to cure jaundice [6]. The tubers, leaves and seeds of the plant are extensively used in traditional system for various ailments like gonorrhea, dyspepsia, asthma, appetizer, spermatorrhoea, thermogenic, hepatosplenomegaly, cardiotonic, diuretics, haemorrhoids and invigorating.

The whole plant is a potential source of natural antioxidant activity [7,8]. Plant pacifies vitiated kapha, vata, anorexia, dyspepsia, colic, asthma, cough, renal calculi, urinary retention and constipation. It is useful in paralytic disorder also [9].

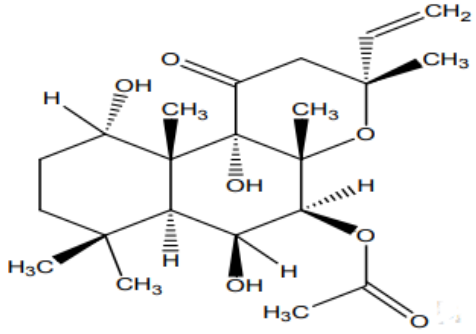
**Table 2. Ethnopharmacological uses of *Solena amplexicaulis***

| Part | **Value** | ***Reference*** |
|---|---|---|
| Whole plant | Jaundice, Swelling | [6] |
| Leave | Anti-inflammation | [1] |
| Root | Dyemenorrhea, Leucorrhea, Infertility of women. | [10] |
| The young unripe fruit | Used as vegetable(edible) | [4] |
| Whole plant | Cough, constipation | [9] |

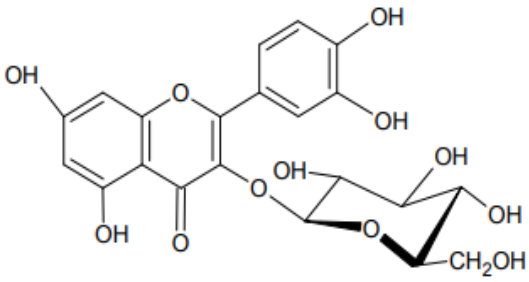
This plant is widely used in the tropical Asia and other localities in the management of various ailments, as summarized in Table 2. Its ethnomedicinal popularity has warranted the various activities of the plant to be documented in several research publications. In West Bengal Lodha people use the root medicinally for leucorrhea and infertility of women [4].

## 4. PHYTOCHEMICAL STUDY

Phytochemical screening of various extracts such as chloroform, ethanol, water of *Solena amplexicaulis* root, stem and leaf revealed the presence of secondary metabolites such as Steroids, triterpenoids, sugars [11] (Agarwal & Jain 2018), Reducing sugars, phenolic compounds, tannins, anthroquinone, amino acids, saponins [8]. Phytochemical analysis of dried powder of *Solena amplexicaulis* leaves showed the presence of carbohydrates, saponins, phytosterols and tannins, whereas the stem portion possess carbohydrates, saponins, phytosterols, tannins, flavonoids and cardiac glycosides [2]. Active chemical compounds such as (Figure 2) forskolin and isoquercetin were isolated [1,8] from the methanolic extract of leaf and tuber.




(a)




(b)

Figure 2: Structure of the isolated compounds a) Forskolin b) Isoquercetin from the methanolic extract of leaf and tuber part of *Solena amplexicaulis* Isoquercetin [1,8].

## 5. PHARMACOLOGICAL STUDY

*5.1. Antimicrobial study*

Karthika and Paulsamy [12] evaluated the aqueous and organic solvent extracts (hexane, benzene, chloroform and methanol) of aerial parts of S. amplexicaulis on 15 human pathogenic bacteria. The result showed that the chloroform and benzene extract of stem exhibited significant antibacterial activity compared to the leaves part. Ethanolic root-extract of the plant showed the

most promising result again both gram positive and gram negative bacteria [2].

*5.2. Antioxidant activity*

The investigation of ascorbic acid and ethanolic extract of *S. amplexicaulis* root was performed for its antioxidant activity. The findings of this study showed that the highest scavenging activity for ascorbic acid and ethanol was 98.66% and 93.97% at concentration 1000 µg/ml [2].

In vitro antioxidant activity of *S. amplexicalis* root was studied by using of various alcoholic and aqueous extracts of tuber the result showed good antioxidant activity and it were compared with synthetic (BHA and BHT) as well as natural antioxidants (rutin and quercetin) [8].

*5.3. Anti-inflammatory activity*

*S. amplexicaulis* produced a potent anti-inflammatory activity against the paw edema in Swiss albino rats [2]. The aqueous extracts of leaf was examined for anti-inflammatory activity by administration to Swiss albino rats against Diclofenac sodium drug (20mg/kg) as standard reference and normal saline as control by Randall and Baroth method. And its derivative exhibited anti-inflammatory activity [13]. The oral administration of forskolin (10 mg/kg) was studied for anti-inflammatory activity. The findings of this study show that potent anti-inflammatory activity by reducing paw edema (87.79%) than the crude extract (150 and 300 mg/kg) and it was comparable with the standard drug, indomethacin (10 mg/kg, 93.89%) [8].

*5.4. Antidiabetic activity*

According to Kabir *et al.* [2] the ethanolic extract of *S. amplexicaulis* showed higher percentage of α-amylase inhibitory The result shows that leaf and stem extracts have to possess anti-diabetic activity in terms of their α -amylase inhibition activity.

Venkatachalapathi *et al*. [14] evaluated the antidiabetic activity of crude methanol leaf extract of *S. amplexicaulis* (MeOHSa) and its isolated compound Forskolin in Wistar albino rats. The effect of MeOHSa and Forskolin on oral glucose tolerance in the normoglycemic rats were studied. The administration of the MeOHSa and Forskolin significantly ($P < 0.05$) improved the activities of enzymatic and non-enzymatic antioxidants in the diabetic induced rat.

*5.5. Hepatoprotective studies*

The investigation of methanolic extract of *S. amplexicalis* was carried out by administration albino rat and analyzed parameters include ALT, AST, ALP, TPL & ALB activity and histopathology of liver damage. This study creates the social awareness among the liver disorders patient who were infected by excess consumption of alcohol [15].

*5.6. Acute toxicity*

Kabir *et al.* [2] evaluated the ethanolic extract of the root of *S. amplexicaulis* on albino mice for acute toxicity study. The finding of the study showed the acute toxicity of the extract on mice administered orally in the range from 50 to 150 mg/kg bodyweight of mice. While it administered orally.

*5.7. Analgesic activity*

Analgesic activity of the ethanolic extract of *S. amplexicaulis* root was investigated on mice. Acetic acid induced writhing response model used for the assay of analgesic activity. The ethanolic extract of this plants exhibited the higher considerable degree of inhibition (26.22%, 14.63%, and 3.05%) which was found less than that of standard diclofenac sodium (51.83%) [2].

*5.8. Urinary stone prophylaxis activity*

Various parts of *S. amplexicaulis* such as stem, leaves and seed were investigated for the inhibition of the mineralization of urinary stone to reduce the deposition of Calcium phosphate and Calcium carbonate crystals. This study suggested that the fruit and seed extract exhibit the good urinary stone prophylaxis activity.

## 6. CONCLUSION

The extensive survey of literature revealed that *S.amplexicaulis* is an important source of many pharmacologically and medicinally important chemicals, especially forskolin and isoquercitin and various useful alkaloids. This plant is extensively studied for the various pharmacological activities like hepatoprotective, anti-inflammatory, anti-microbial and antibacterial activities. Although the results from this review are quite promising for the use of *S. amplexicaulis* as a multi-purpose medicinal agent, while this plant has been used successfully by tribes as a medicine for centuries, more clinical trials should be conducted to support its therapeutic use. The research on the

pharmacological value of this plant proves that it has valuable compounds for curing many diseases and thus it is a promising plant for future advanced medicine. The traditional uses, toxicity pharmacology and phytochemistry of *S. amplexicaulis* presented in this review could be helpful for future studies and research and new molecules could be discovered from this plant against the life threatening diseases like cancer. The plant has good future prospective for discovery of new molecules and pharmacological activities.

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DOI:10.26524/krj.2021.19

*Kong. Res. J.* 8 (2): 49-60, 2021
**Publisher**: Kongunadu Arts and Science College, Coimbatore.

https://www.krjournal.com

**RESEARCH ARTICLE**

# STUDIES ON THE ARBUSCULAR MYCORRHIZAL FUNGAL ASSOCIATION IN THE PLANT SPECIES OF THEERTHAMALAI HILLS, WESTERN GHATS OF DHARMAPURI DISTRICT TAMILNADU, INDIA

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## ABSTRACT

The present study to investigate the arbuscular mycorrhizal fungal root colonization and spore population of some medicinal plants species at Theerthamalai hills Western Ghats of Dharmapuri district, Tamil Nadu. Root and rhizosphere soil samples were collected during the month of August, 2010-March, 2011. From the surface to 20 cm depth as well as pH were also measured. Totally 42 plant species belonging to 24 families recovered Arbuscular mycorrhizal fungal spore and root colonization. The results of the present study arbuscular mycorrhizal fungal spore population in the rhizosphere soil and root colonization of all the plant species. The maximum spore population was found in the rhizosphere soil samples of the plant species *Leucas aspera* (386/100g of soil) which belongs to the family Lamiaceae and lowest spore population was observed in the *Wrightia tinctoria* (117/100g of soil) belongs to Apocyanaceae. The maximum AM fungal infection was found in roots of *Cassia auriculata* (63%) belongs to the family Fabaceae, while the lowest AM fungal association was found in the root of *Achyranthes aspera* (17%) belongs to the family Amaranthaceae. A total of 24 AM fungal species belonging to 4 genera were recorded from the rhizosphere soil samples of this study region. Among these genus *Glomus* was dominant had seen in rhizosphere soil samples in all the medicinal plant species.

**Keywords:** *Glomus* sp, Arbuscular mycorrhizal fungi**,** Medicinal plants, Theerthamalai hills.

## 1. INTRODUCTION

More than a century, observation has revealed that the roots of majority of land plants are associated with fungi. However, it is only in recent years that the significance of this association has emerged. To a great extent, most of the land plants roots associate with soil fungi. Soil is the habitat for plant roots, micro flora (bacteria, actinomycetes, fungi, and algae) microfauna and macrofauna. The zone of soil under the influence of root is called the "rhizosphere". This area of activated microbial populations can extend more than 5 mm from the root surface. However, the abundance and activity of soil microorganisms in general diminish with increasing distance from the root (Pankow *et al.,* 1991). It is now recognized that the "rhizosphere effect" is mainly due to the exudates from the roots, which attract soil microorganisms. These microorganisms play vital roles in physiological processes in the ecosystem of plants growing in soil.

Association of plant roots with fungi is termed as mycorrhizae. It is a marriage between two highly dissimilar organisms based on mutual exchange of nutrients. The plant root system is a major biotic component of soil providing energy for the majority of soil fauna and microflora (Freckman and Caswell, 1985). Soil microorganisms play important roles in plant-soil interactions. Microbes alter nutrient availability, immobilize heavy metals in soils, and bind soil particles in to stable aggregates (Shetty *et al*., 1994).

Arbuscular mycorrhizal fungi are soil microorganisms that establish mutual symbiosis with the majority of higher plants, providing a direct physical link between soil and plant roots. AM fungi geographically ubiquitous occur over a broad ecological range including associated agriculture, horticulture, pasture grasses, tropical plants and cereals (Qadri, 2004). Arbuscular Mycorrhizas improve the growth and nutrient uptake of plants and are formed in 80% of all land plants. Mycorrhizal associations appear to be the result of relatively diffuse co-evolutionary processes. While early events in the evolution of mycorrhizal symbiosis may have involved reciprocal to genetic changes in ancestral plants



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and free living fungi available evidence points largely to ongoing parallel evolution of the partners in response to environmental changes and non-mycotrophy evolved more recently (Cairney, 2000).

AM fungi play a vital role in primary and secondary succession of plant species, especially in low nutrient ecosystems (eg. coastal and sand dunes). The below-ground diversity of AM fungi is one of the major factors contributing to the maintenance of plant biodiversity and to the ecosystem functioning (Van der Heijden *et al*., 1998). Arbuscular mycorrhizal associations are beneficial for plants growing in various Indian semi-arid landscapes (Kaushick *et al*., 1992).

Majority of plants used for medicinal purposes grow in forests and very few of them are cultivated. The forest wealth with regard to plants of medicinal importance has not yet fully been tapped. There is an increasing world-wide interest in AM fungi (AM), which are universally present in all soils and in association with great variety of plants of different taxonomic groups (Sambandan *et al*., 1994). AM fungi are very common in tropical forests while ectomycorrhizae dominates in temperate and coniferous forests. AM fungi infect fine feeder roots and colonize the cortical region from where they extend their mycelia in rhizosphere soil. Besides, these fungi also protect the host plants from root pathogens and enhance drought resistance (Michelsen and Rosendahal, 1990; Verma and Jamaluddin, 1994).

Mycorrhiza allows the fungal symbionts to extract a greater amount of nutrients from the soil such as phosphorus, nitrogen, zinc, boron and colonized by AM fungi benefits a plant in a number of ways: increased nutrient uptake, increased disease resistance, enhanced water relations and increased soil aggregation (Newsham *et al.* 1994). The aim is to use mycorrhiza technology in improving phosphate availability, soil fertility and produce staple food crops in small farming. Maintenance of sustainable soil fertility depends greatly on the ability to harness the benefits of soil AMF, due to depleting phosphate mineral resources arising from the low availability of AMF (Irene and Thomas, 2006).

The great interest in AM in recent years has prompted numerous survey aimed at enumerating and assessing AM fungi in a particular region or in a natural environment. Hence in this present study was isolate and identification AM fungal spores from rhizosphere soils samples in the medicinal plant species in addition with root colonization in the study region.

## 2. MATERIALS AND METHODS

*2.1. Study area -Description*

Theerthamalai Hills, a range of mountains in the Eastern Ghats in Tamil Nadu South India. The name "Theerthamalai" derives from Tamil word 'Therrtham' meaning "Neer Aruvi" and 'Malai' meaning Hill, thus Theerthamalai Hill. They really consist of a forest-clad and grassy table land, with summits rising about 8000 ft. The highest peak of the Theerthamalai Hills 2,527 meters (8,842 ft), located in the Dharmapuri district (Fig. 1). Harur is located at 12°04′N 78°30′E12.07°N 78.5°E. It has an average elevation of 350 meters (1148 feet). Harur is situated along the Salem and (via Harur) Chennai State Highway 18.

The climate is generally warm. The hottest period of the year is between the months of March to May, reaching a maximum temperature of up to 32°C in April. The temperatures drop in December and the low temperatures continue up to February, touching a minimum of 14°C in January. The district has an average annual rainfall of 895.56 mm. The tropical forests here generally have short shrubs and thorned-plants. Temperature being increasing after March. May is the hottest maximum with a mean daily maximum temperature of 36.5ºC and minimum temperature of 28ºC. The maximum temperature may go up to 36.5ºC on same days. The maximum and minimum temperatures are 36.5ºC and 17.7ºC recorded during the month of May 2010 and January 2011 respectively (Table 1), (Fig 2).

*2.2. Sample collection*

The present study root and rhizosphere soils samples were collected from 25 plant species during the year August, 2010 to March, 2011. All the samples were placed in the polyethylene bags, labeled and then transported to the laboratory. The root samples were freshly processed, whereas rhizosphere soil samples were analyzed for mycorrhizal spore population and AM fungal root colonization.

*2.3. Estimation of AM fungal root colonization*

The fresh root samples were cleared and stained in tryphan blue following method of (Philips and Hayman's (1970). Root samples of each plant species were washed gently under tap water and cleared in 2.5% KOH, acidified in 5 N

HCL and stained in lacto glycerol with 0.05% Trypan blue. The stained roots were examined under a compound microscope (40x– 100x). The percentage of AM fungal infect ion was calculated using the formula:

$$\text{Percentage of infection (\%)} = \frac{\text{No. of root segments infected}}{\text{Total no of root segments observed}} \times 100$$

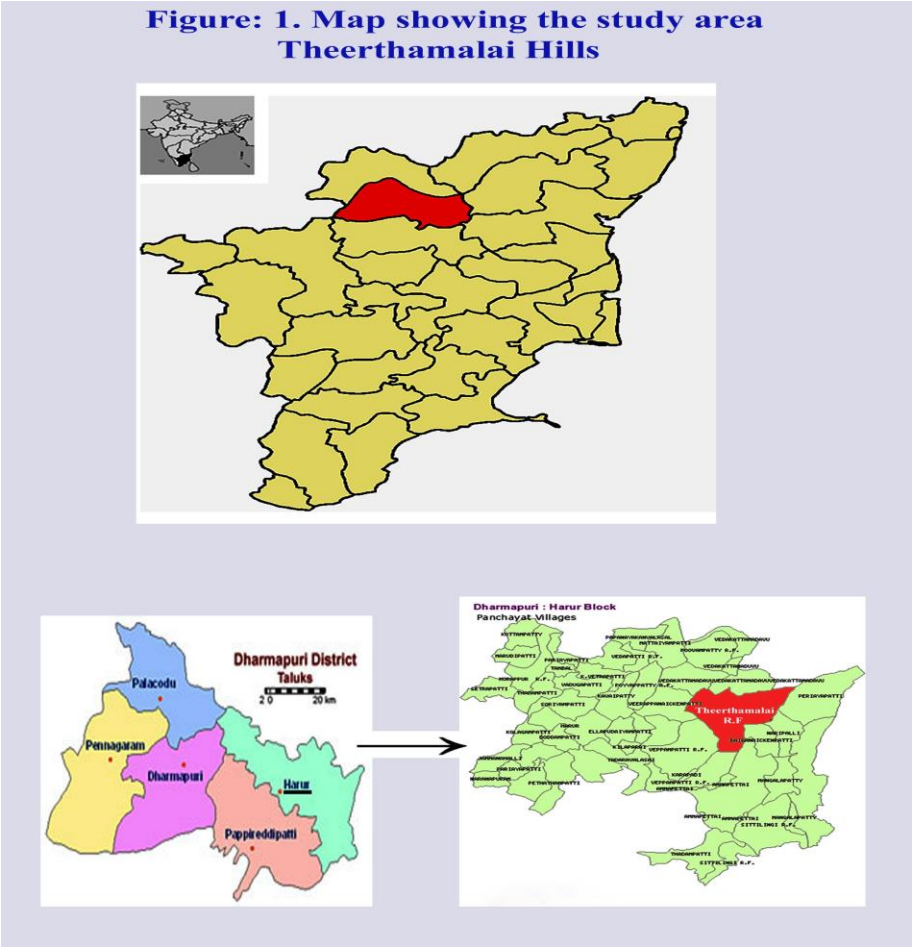


**Fig. 1. Map indicating the study area Theerthamalai hills, Dharmapuri district**

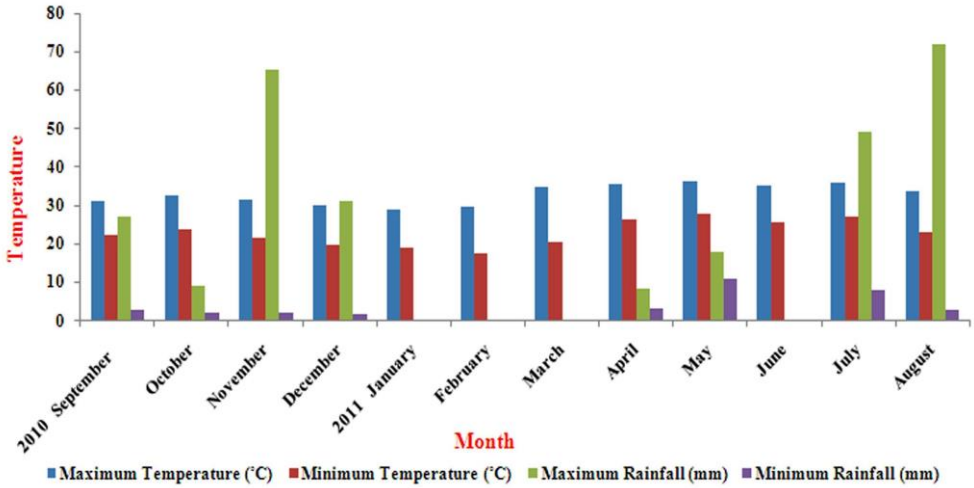


**Fig. 2. Meteorological factors of Theerthamalai hills during 2010-2011.**

*2.4. AMF spore identification*

AM fungal spores were extracted from 100 g rhizosphere soil by wet-sieving and decanting method (Gerdemann and Nicolson, (1963) through a series of 710 to 37µm size sieve filter. For the identification and nomenclature of these AM fungal spore synoptic keys developed by (Schenck, and Perez (1990; Raman, N. and V. Mohan Kumar, 1988; Schüßler Walker, 2010.) were used. The classification was based upon the color, shape, hyphae, structure, size, and cell wall thickness and spore diameter.

*2.5. Soil pH*

The pH of the rhizosphere soil samples was determined (soil-water suspensions 1:5) with the help of pH meter (Elico) and values were recorded.

*2.6. Available nitrogen (N)*

Available nitrogen in the soil was estimated following the method of Sankaram (1996). 20 g soil sample was taken in distillation flask and 200 ml of water, 100 ml of 0.32% Potassium permanganate solution and 100 ml of 2.5% NaOH were added. The sample was digested for 1 hour and distilled to 30 ml. The distillate was collected in 20 ml of 2% Boric acid and titrated against N/50 sulphuric acid using bromo-cresol green indicator (0.099 g bromo-cresol green and 0.066 g methyl red dissolved in 100 ml of 95% ethyl alcohol). From the titre value, the available nitrogen in the soil was calculated.

*2.7. Available Phosphorous (P)*

Available phosphorous in the soil was determined following the method of Olsen *et al*. (1954). Phosphorous from the soil was extracted by adding 50 ml of extracting solution (15 ml of ammonium fluoride solution, and 25 ml of 2 N HCl was added to distilled water to make up to 500 ml), to 1 g of the soil. The suspension was shaken for 1 min and the content was filtered using Whatmann No.1 filter paper. To 5 ml of the filtrate, 4 ml of ascorbic acid reagent (ammonium molybdate 12 g dissolved in 250 ml of water was added to 291 mg potassium antimony tartarate in 100 ml distilled water and 140 ml concentrated sulphuric acid and the volume was made up to 10 ml and left for 10 minutes. The colour intensity of the reaction mixture was read at 640 nm in a Beckman Du-40 spectrophotometer. The values were calculated from a standard graph, which was plotted with $KH_2PO_4$. The available phosphorous in the soil was calculated by multiplying the factor derived from the standard curve and dilution factor.

*2.8. Available potassium (K)*

The method of Sankaram (1996) was followed for the determination of available potassium in the soil. Soil sample, 5 g was taken, 250 ml neutral ammonium were added and stirred for 5 min. The suspension was filtered through Whatmann No.1 filter paper. The filtrate was collected and readings were taken in a flame photometer (Evans Electro Selenium Ltd). The instrument was checked with distilled water and KCl was used as standard.

*2.9. Available microelements*

Lindsay and Norvell's (1978) method was followed to estimate available microelements such as Zn, Mn, Cu and Fe. From the soil sample, 10 g soil was taken, 20 ml of DTPA (diethylene triamine penta-acetic acid) were added and stirred for 2 hour. The suspension was filtered through Whatmann No.1 filter paper. The filtrate was collected and readings were taken in atomic absorption spectrophotometer (AA 1475 Varian, USA). The following wavelengths were used: 213.86 nm for Mn, 324.75 nm for Cu and 248. 33nm for Fe.

## 3. RESULTS

In the present study results revealed that arbuscular mycorrhizal fungal (AMF) infections and spore population of totally 42 plant species belongs to 24 families in the plant from Theerthamalai hills during the year 2010 – 2011, (Table: 2). The pH, macro and micronutrient contents of rhizosphere soil samples were presented in (Table: 3). The pH of the soil samples varied from 4.7 to 7.9. The nitrogen content of soil samples ranged from 56 to 77 kg/ha. The phosphorus level of soil samples was 7.0 to 13.0 kg/ha. The potassium content in the soil samples ranged from110 to 135kg/ha.

*3.1. AM fungal spore population and root colonization*

In the present study totally 42 medicinal plant species belonging to 24 families were examined for AM fungal association. Of these the maximum spore population was displayed in the plant species of *Leucus aspara* (386/100g of soil) belongs to the family Lamiaceae and minimum spore was observed in *Wrightia tinctoria* (117/100g of soil) belongs to the family Apocyanaceae.

The highest AM fungal infection found in the roots of *Cassia auriculata* (63%) belongs to the family Fabaceae and lowest AM fungal infection was recorded in *Achyranthes aspera* (17%) belongs to the family Amaranthaceae.

**Table 3. pH, macro and micro elements contents of Rhizosphere soil samples of medicinal plant species at Theerthamalai hills.**

| S. No | Name of the plant | Soil Texture | pH | EC | Macronutrients Kg/Ha | | | Micronutrients (ppm) | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | N | P | K | Iron | Zinc | Mn | Cu |
| 1. | *Annona squamosa* L. | Sandy loam | 6.4 | 0.4 | 77 | 8.0 | 105 | 4.90 | 0.92 | - | 0.80 |
| 2. | *Capparis sepiaria* L. | Clay loam | 6.5 | 0.5 | 70 | 10.0 | 125 | 4.96 | 0.90 | - | 0.90 |
| 3. | *Sida acuta* Burm.f. | Sandy loam | 6.8 | 0.6 | 70 | 11.0 | 125 | 4.98 | 0.94 | - | 0.96 |
| 4. | *Chloroxylon swietenia* DC. | Sandy loam | 6.7 | 0.5 | 70 | 13.0 | 120 | 4.90 | 0.86 | - | 0.94 |
| 5. | *Murraya paniculata* (L.) Jack. | Sandy loam | 6.7 | 0.5 | 72 | 9.0 | 118 | 4.76 | 0.80 | - | 0.92 |
| 6. | *Cissus quadrangularis* L. | Clay loam | 6.7 | 0.5 | 63 | 8.0 | 110 | 4.74 | 0.86 | - | 0.90 |
| 7. | *Cardiospermum halicacabum* L. | Clay loam | 6.8 | 0.5 | 64 | 7.0 | 120 | 4.72 | 0.84 | - | 0.90 |
| 8. | *Dodonaea viscosa* Jack. | Sandy loam | 7.3 | 0.5 | 66 | 11.0 | 125 | 4.64 | 0.80 | - | 0.96 |
| 9. | *Abrus precators* L. | Sandy loam | 7.3 | 0.6 | 70 | 10.0 | 130 | 4.68 | 0.86 | - | 0.94 |
| 10. | *Cassia auriculata* L. | Sandy loam | 7.2 | 0.6 | 72 | 13.0 | 105 | 4.80 | 0.84 | - | 0.92 |
| 11. | *Cassia occidentalis* L. | Sandy loam | 6.8 | 0.7 | 64 | 11.0 | 110 | 4.90 | 0.80 | - | 0.90 |
| 12. | *Pongamia pinnata* (L.) Pierre | Clay loam | 7.0 | 0.5 | 63 | 15.0 | 125 | 4.92 | 0.90 | - | 0.94 |
| 13. | *Pterolobium hexapetalum* (Roth) Sant. & Wagh | Clay loam | 6.9 | 0.5 | 64 | 10.0 | 125 | 4.70 | 0.96 | - | 0.70 |
| 14. | *Tephrosia purpurea* (L.) Pers. | Clay loam | 6.5 | 0.7 | 67 | 9.0 | 120 | 4.64 | 0.90 | - | 0.64 |
| 15. | *Tamarindus indica* L. | Clay loam | 7.2 | 0.6 | 70 | 10.0 | 123 | 4.60 | 0.94 | - | 0.64 |
| 16. | *Acacia nilotica* (L.) Willd.ex Delile. | Clay loam | 7.8 | 0.7 | 70 | 11.0 | 125 | 4.70 | 0.94 | - | 0.60 |
| 17. | *Passiflora foetida* L. | Sandy loam | 6.7 | 0.5 | 72 | 9.0 | 118 | 4.76 | 0.80 | - | 0.92 |
| 18. | *Tecoma stans* (L.) Juss.ex Kunth. | Sandy loam | 6.7 | 0.5 | 63 | 8.0 | 114 | 4.74 | 0.83 | - | 0.91 |
| 19. | *Alangium salvifolium* (L.f.) Wangerin Lamarck. | Sandy loam | 6.8 | 0.6 | 65 | 7.0 | 120 | 4.72 | 0.85 | - | 0.93 |
| 20. | *Canthium parviflorum* Lam. | Sandy loam | 7.0 | 0.5 | 66 | 10.0 | 121 | 4.64 | 0.80 | - | 0.96 |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 21. | *Chomelia asiatica* Schumann ex Engl. | Clay loam | 7.1 | 0.7 | 70 | 10.0 | 130 | 4.68 | 0.86 | - | 0.94 |
| 22. | *Plumbago zeylanica* L. | Clay loam | 6.1 | 0.8 | 70 | 11.0 | 110 | 4.62 | 0.86 | - | 0.74 |
| 23. | *Carissa carandas* L. | Sandy loam | 5.3 | 0.7 | 66 | 10.0 | 117 | 4.66 | 0.84 | - | 0.60 |
| 24. | *Wrightia tinctoria* (Roxb) R. Br. | Clay loam | 7.4 | 0.6 | 65 | 11.0 | 132 | 4.62 | 0.86 | - | 0.78 |
| 25. | *Caralluma adscendens* Wall. | Clay loam | 7.9 | 0.8 | 59 | 9.0 | 129 | 4.79 | 0.86 | - | 0.68 |
| 26. | *Gymnema sylvestre* R.Br. | Clay loam | 5.7 | 0.7 | 68 | 8.0 | 111 | 4.75 | 0.92 | - | 0.60 |
| 27. | *Pergularia daemia* (Forssk.) Chiov. | Clay loam | 6.4 | 0.9 | 56 | 9.0 | 130 | 4.64 | 0.82 | - | 0.77 |
| 28. | *Evolvulus alsinoides* L. | Sandy loam | 4.8 | 0.5 | 69 | 11.0 | 140 | 4.71 | 0.81 | - | 0.76 |
| 29. | *Datura stramonium* L. | Sandy loam | 7.2 | 0.5 | 67 | 10.0 | 126 | 4.92 | 0.86 | - | 0.87 |
| 30. | *Solanum surattense* Burm.f. | Sandy loam | 6.3 | 0.6 | 72 | 9.0 | 110 | 4.75 | 0.89 | - | 0.92 |
| 31. | *Justicia tranquebariensis* L. | Sandy loam | 7.3 | 0.9 | 71 | 7.0 | 109 | 4.93 | 0.92 | - | 0.69 |
| 32. | *Leucas aspera* (Willd) Link | Sandy loam | 6.9 | 0.7 | 69 | 7.0 | 134 | 4.83 | 0.88 | - | 0.79 |
| 33. | *Ocimum sanctum* L. | Clay loam | 7.4 | 0.8 | 63 | 11.0 | 135 | 4.70 | 0.91 | - | 0.67 |
| 34. | *Lantana camara* L. | Sandy loam | 4.7 | 0.5 | 73 | 8.0 | 115 | 4.79 | 0.89 | - | 0.84 |
| 35. | *Achyranthes aspera* L. | Sandy loam | 5.5 | 0.6 | 65 | 10.0 | 121 | 4.84 | 0.81 | - | 0.92 |
| 36 | *Aerva lanata* (L.) Juss.ex Schult. | Clay loam | 4.8 | 0.7 | 72 | 11.0 | 121 | 4.62 | 0.83 | - | 0.91 |
| 37 | *Acalypa fruticosa* Forssk. | Clay loam | 7.2 | 0.6 | 63 | 9.0 | 118 | 4.71 | 0.82 | - | 0.86 |
| 38 | *Acalypha indica* L. | Clay loam | 6.3 | 0.8 | 65 | 8.0 | 120 | 4.75 | 0.93 | - | 0.94 |
| 39 | *Jatropha glandulifera* Roxb. | Clay loam | 7.2 | 0.7 | 72 | 9.0 | 132 | 4.70 | 0.91 | - | 0.71 |
| 40 | *Euphorbia hirta* L. | Sandy loam | 6.8 | 0.5 | 70 | 11.0 | 130 | 4.76 | 0.80 | - | 0.87 |
| 41 | *Aloe vera* (L.) Burm.f. | Clay loam | 7.0 | 0.5 | 66 | 10.0 | 128 | 4.98 | 0.84 | - | 0.89 |
| 42 | *Gloriosa superba* L. | Clay loam | 7.2 | 0.6 | 65 | 9.0 | 122 | 4.84 | 0.90 | - | 0.91 |

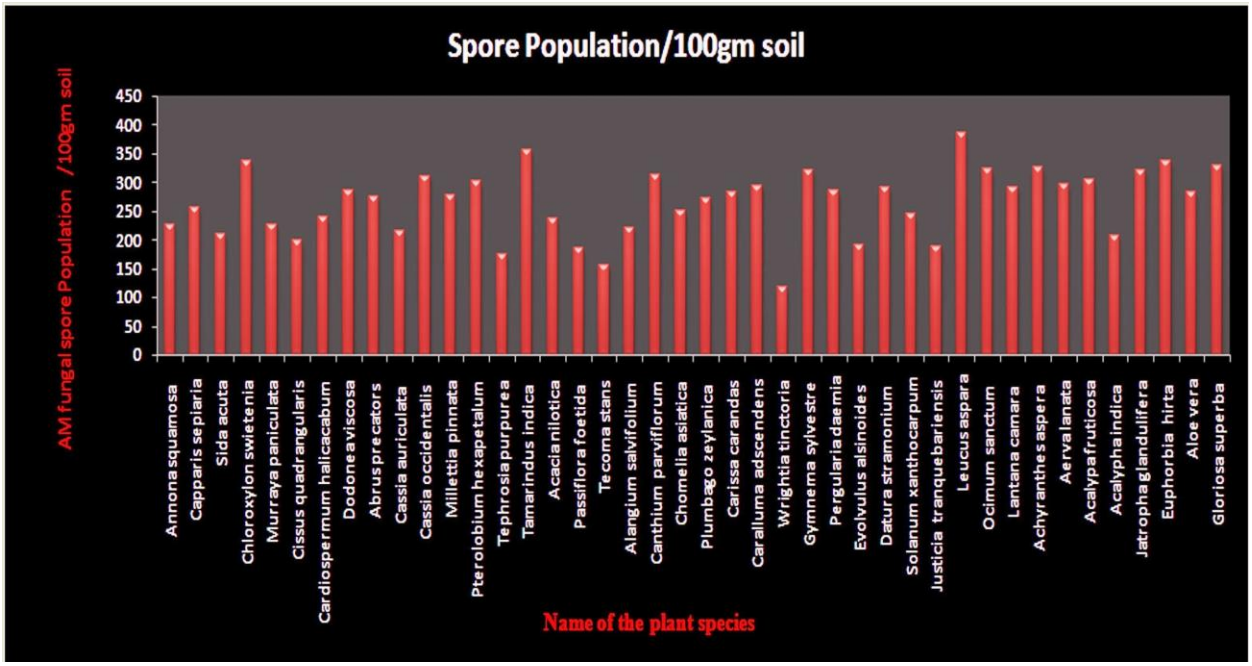


**Fig. 3. AM fungal spore population in the rhizosphere soil samples of Theerthamalai hills.**

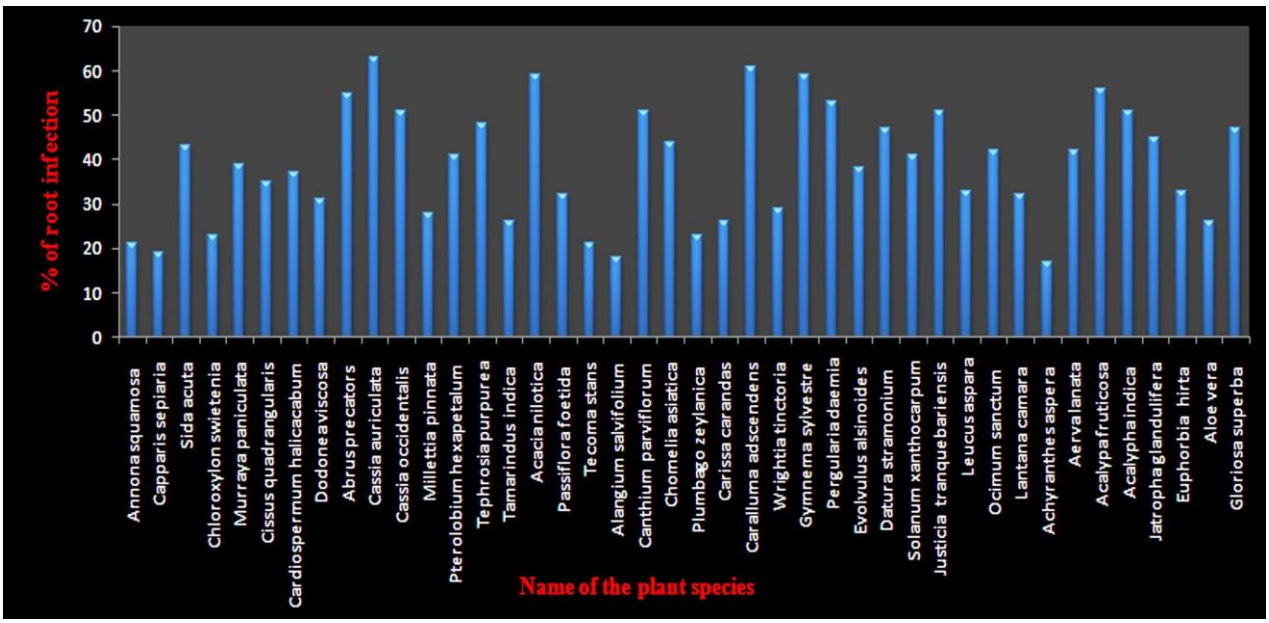


**Fig. 4. AM fungal infection in the root samples of Theerthamalai hills.**

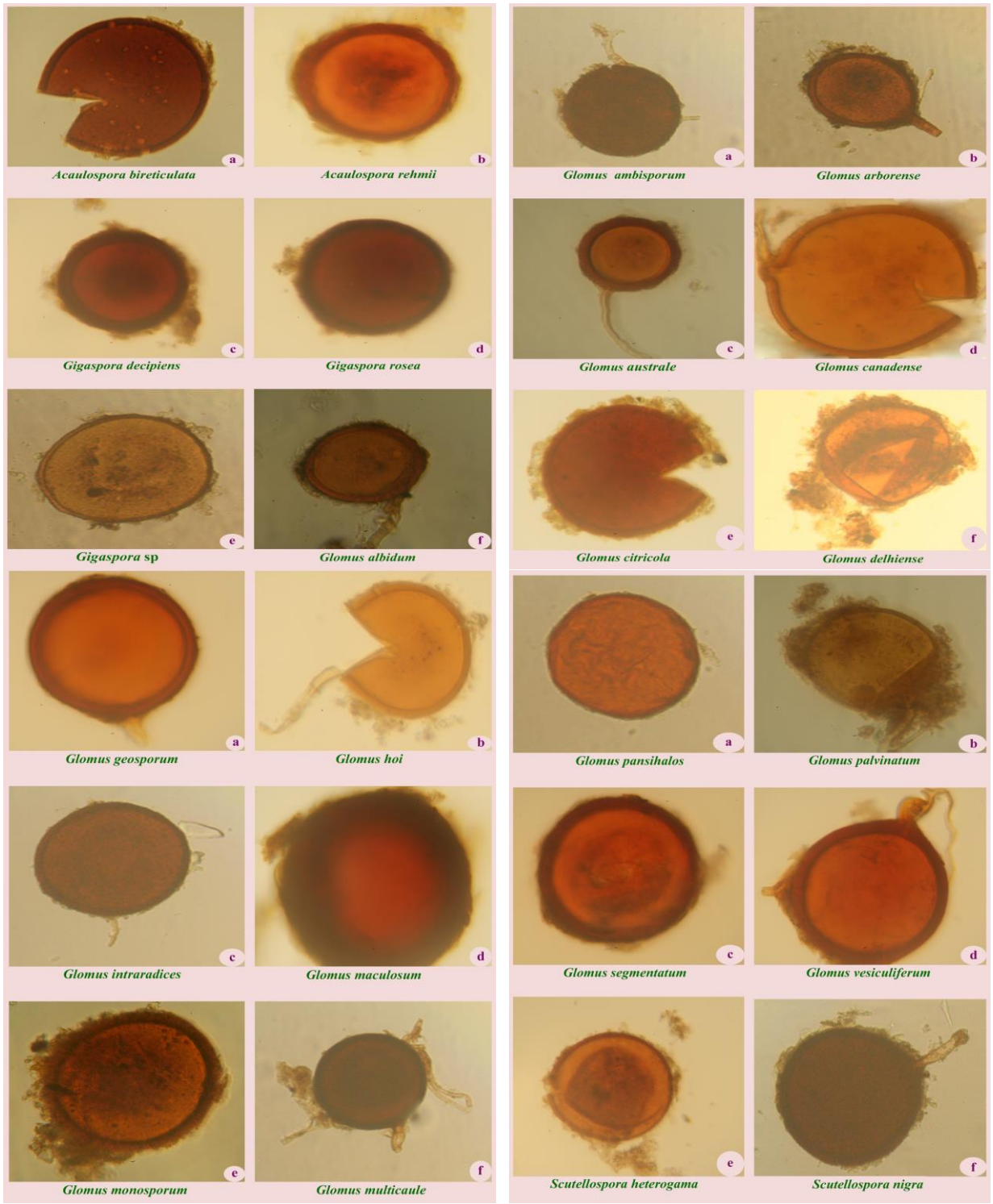


**Fig. 5. Various AM fungal spores identified by rhizosphere soil samples from this study region.**

All the 42 plant species colonized by AM fungal infection. Of these the following species such as *Annona squamosa* (21 %), *Capparis sepiaria* (29%), *Chloroxylon swietenia* (23%), *Pongamia pinnata* (28%), *Tamarindus indica* (26%), *Tecoma stans* (21%), *Alangium salvifolium* (18%), *Plumbago zeylanica* (23%), *Carissa carandas* (26%), *Wrightia tinctoria* (29%), *Achyranthes aspera* (17%), and *Aloe vera* (26%), were infected only less than 30% The other species like *Murraya paniculata* (39%), *Cissus quadrangularis* (35%), *Cardiospermum halicacabum* (37%), *Dodonaea viscosa* (31%), *Passiflora foetida* (32%), *Evolvulus alsinoides* (38%), *Leucas aspera* (33%), *Lantana camara* (32%), *Euphorbia hirta* (33%), infected less than 40% and above 30%.

The species such as *Pterolobium hexapetalum* (41%), *Tephrosia purpurea* (48%), *Chomelia asiatica* (44%), *Datura stramonium* (47%), *Solanum surattense* (44%), *Ocimum sanctum and Aerva lanata* (42%), *Jatropha glandulifera* (45%), *Gloriosa superba* (47%), showed less than 50%. The species like *Abrus precators* (55%), *Cassia occidentalis* (51%), *Acacia nilotica* (59%), *Canthium parviflorum* (51%), *Gymnema sylvestre* (59%), *Pergularia daemia* (53%), *Justicia tranqubariensis* (51%), *Acalypha indica* (51%), showed above 50% and less than 60%. The species like *Cassia auriculata* (63%), *Caralluma adscendens* (61%), recorded above 60% and less than 70% respectively.

*3.2. AM fungal spores recovered from rhizosphere soils samples in plant species*

In the present study first time the AM spore diversity isolated from medicinal plants species ttotally 3- AM fungal spore species was identified six genera from 40 plant species belongs to 7 families, Of these AM fungal spores of the genus *Glomus* was recorded as the most population, followed by *Aculospora*, *Gigaspora*, *Scutellispora*, *Sclerocystis* and *Entrophospora* are recorded in (Fig 6).

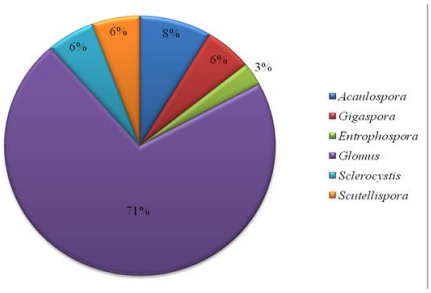


**Fig. 6.** Dominant AM fungal species identified from rhizosphere soils samples in the study region.

**4. DISCUSSION**

Totally 42 plant species belongs to 24 family were analyzed to determine for mycorrhizal infection and spore population in Theerthamalai hills for a period of one year (2010-2011). The factors like climatic and physico-chemical character of the soil were also studied. Generally in Theerthamalai Hills all the plant species have mycorrhizal association. Totally 42 plant species belongs to 24 families were surveyed for AM fungal infection. The range of spore population and rate of AM fungal infection was occurred variously. The root colonization ranged from 17 to 63%. The spore density in the present study from (117 to 386/100gm of soil) moderate to high lands.

Arbuscular mycorrhizal are ubiquitous and have a broad ecological range and also AM fungi usually associated with most plants and are important in Agriculture, Horticulture and Forestry. Nishi Mathur and Anil Iyar (1994) reported the *Simmondsia chinensis* in desert associated with AM fungi and noted that the percentage of root colonization could not related to spore population.

In general, mycorrhizal inoculation increased the percentage of mycorrhizal root colonization and spore numbers in soil. Chiramel *et al*., (2006) observed the highest percentage of root colonization in *Glomus intraradices* treated plants followed by those treated with *Gl. monosporum* and *G. leptotichum* wet land woody species. The same results were obtained in the present investigation. Mohan Kumar and Mahadevan (1986) analysis the AM fungal infection in *Acanthus ilicifolius* belongs to the family Acanthaceae from Mangrove forest, Pithchavaram, Tamilnadu. Surprisingly there is no mycorrhizal association was recorded in the roots. It may be due to the soil from mangrove forest was dry. Furthermore, soil moisture substantially reduced the mycorrhizal association due to insufficient availability of oxygen. The same findings no colonization was observed in the plant species of *Adathoda vasica* (Acanthaceae) by Radhika and Rodrigues (2010) from Western Ghats, Goa region. But in the present study reveals that the Acanthaceae members of *Justicia tranquebariensis* (51%), *Leucas aspara* (33%), *Ocimum sanctum* (42%). Colonization was observed and roots showed hyphae and intra cellular arbuscular and vesicles in the soil samples collected from Thirumoorthy hill areas. The similar finding was obtained in the Acanthaceae members of *Astracantha longifolia* (34%), marshy plants (Dharmarajan *et al.,* 1993) from in and around Poondi, Thanjavur District.

Mycorrhizal inoculation resulted in a significant increase in height, biomass and nutrient content of *W. chinensis* seedlings (Nisha and Rajeshkumar, 2010). This report supports earlier investigations in medicinal plants (Earanna *et al.,* 2002; Rajan *et al.,* 2004). *W. chinensis* plants inoculated with AM fungi showed a general increase in growth parameters such as plant height, total dry weight than those of the uninoculated plants. Beena *et al.,* (2001) observed that the AM fungal infection (47%) in Euphorbiaceae members *E. articulate* and the spore population (6/100 gm of soil) form Coastal sand dunes of West Coast of India. In the present study the same results was obtained that the AM fungal infection (33%) and spore population (336/100 g of soil) in other species of *Euphorbia hirta* belongs to Euphorbiaceae member.

The Fabaceae member *Cyperus arenarius* (60%), *Fimbristylis argenta* (22%), observed the AM fungal infection in Cyperaceae member by Beena *et al.,* 2001. But they did not observed any AM fungal infection in plant species of *Cyperus pedunculatus* belongs Cyperaceae member. In the present investigation, all the Fabaceae members, *Abrus precatorius* (51%), *Cassia auriculata* (63%), *C. occidentalis* (51%), *Pongamia pinnata* (28%) *and Pterolobium hexapetalum* (41%), showed fungal infection and spore population. The Solanaceae members *Datura stramonium* (47%) and *Solanum surattense* (41%) infected b AM fungi in the present investigation. Sadiq Gorsi (2002) also obtained the same results and his study revealed that the Solanaceae members of *Solanum nigrum* (30%) infected by Arbuscular mycorrhizae. The inoculation of AM and other beneficial soil microorganisms significantly increased the biomass of different medicinal plants (Sena and Das 1998, Kothari *et al.,* 1999). The same result was present in the present study. Akond and Khan (2001) reported 5% – 73% root colonization by AM fungi in timber yielding plants of Bangladesh which is it consistence with this present study.

Arbuscular mycorrhizal associations are beneficial for plant growing in various Indian semi-arid landscapes (Kaushick *et al*., 1992). Muthukumar *et al*., 1994 reported that the AM fungal infection in the plant species *Acacia eburnean* (70%) and *Mimosa pudica* (70%) belongs to the family Mimosaceae. The same results were obtained in *Acacia nilotica* (59%) the other species of Mimosaceae member. In the present investigation the Verbenaceae member *Lantana camara* (32%) infected by AM fungal infection and also the spore population (289/100gm of soil). But in contrast, Beena *et al*., (2001) reported there is no mycorrhizal infection and spore population present in the different plant species of *Phyla nodiflora* belongs to Verbenaceae member.

Santhoshkumar and Nagarajan (2017) reported that arbucular mycorrhizal fungal association in the rhizosphere soils and root colonization of some medicinal plant Species in Sirumalai hills Eastern Ghats of Dindugul District, and they were identified totally 39 AM fungal species belonging to six genera were recovered the rhizosphere soil samples from the study region. The genus *Glomus* was dominate as followed by *Acaulospora*, *Sclerocystis*, *Entrophospora* and *Gigaspora* was recorded. In the present study observed that, arbuscular mycorrhizal fungi colonized all the medicinal plant species and the three stages of root colonization viz., hyphal, arbuscular and vesicular colonization were recorded. AM spore populations also showed variation in the rhizozphere soil of the shrubs and tree species.

## 5. CONCLUSION

The present study revealed that the all plant species had AM fugal spore density and root colonization. In this symbiotic association of AM fungi is absorb the soil nutrients, zinc, copper especially phosphorous and also increased plant resistance to various stresses like drought, salt and heavy metal. Hence in this present study in baseline data, further more research is needed to AMF inoculum in green house condition increased in biomass and plant growth productivity.

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DOI:10.26524/krj.2021.20

*Kong. Res. J.* 8 (2): 61-68, 2021
**Publisher**: Kongunadu Arts and Science College, Coimbatore.


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**RESEARCH ARTICLE**

# TRIANGULAR INTUITIONISTIC FUZZY SEQUENCING PROBLEM

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**ABSTRACT**

In this paper, we discuss different types of fuzzy sequencing problem with Triangular Intuitionistic Fuzzy Number. Algorithm is given for different types of fuzzy sequencing problem to obtain an optimal sequence, minimum total elapsed time and idle time for machines. To illustrate this, numerical examples are provided.

**Keywords:** Triangular Intuitionistic Fuzzy Number, Optimal sequence, Total elapsed time, Idle time, Score function and Ranking.

## 1. INTRODUCTION

Sequencing Problem is the problem of determining an appropriate order for a series of tasks to be performed on a finite number of service facilities so as to minimise the total time taken for finishing all the jobs. Johnson [1] has given a method of scheduling jobs in two machines. Its primary objective is to determine an optimal sequence of jobs and to reduce the total amount of time it takes to complete all the jobs. It also reduces the amount of idle time between the two machines. Furthermore, Johnson's method has been extended to 'm' machines problem with an objective to complete all the jobs in a minimum duration. But it is difficult to apply those ordinary approaches to real life situations. In reality, it is observed that the information available is of imprecise nature and there is an uncertainty in the problem. In order to handle this uncertainties, we use fuzzy sets which was introduced by Zadeh [2]. Here, we represent these uncertainties in terms of triangular intuitionistic fuzzy number. Atanassov [3] introduced the concept of Intuitionistic Fuzzy Sets, which is a generalization of the concept of fuzzy sets. Nagoorgani and Ponnalagu [4] have proposed a new algorithm to solve an Intuitionistic Fuzzy Linear Programming Problem. Nagoorgani and Ponnalagu [5] defined a new operation on triangular fuzzy number for solving fuzzy linear programming problem. Radhakrishnan and Saikeerthana [6] have solved problems on Game Theory using interval parameters. Radhakrishnan and Saikeerthana [7] have discussed and solved problems related to Critical Path Method and Programme Evaluation Review Technique with intervals and also with Conversion of fuzzy parameters(triangular and trapezoidal) into intervals using $\alpha - \text{cut}$ s. Radhakrishnan and Saikeerthana [8] solved fuzzy sequencing problem with triangular fuzzy numbers. Radhakrishnan and Saikeerthana [9] have discussed single machine sequencing problem using fuzzy parameters.

The rest of this paper is framed as follows:

In section 2, basic definitions, arithmetic operations of triangular intuitionistic fuzzy numbers, ranking and score functions are given as preliminaries. In section 3, algorithm for solving different types of fuzzy sequencing problem is provided. In section 4, numerical examples illustrating the algorithm are given. Finally, the conclusion.

## 2. PRELIMINARIES

### *2.1. Fuzzy set*

A fuzzy set $\tilde{A}$ in X is a set of ordered pair defined by $\tilde{A} = \{(x, \mu_{\tilde{A}}(x)); x \in X, \mu_{\tilde{A}}(x) \in [0,1]\}$ where $\mu_{\tilde{A}}(x)$ is a membership function.

### *2.2. Fuzzy Number*

A fuzzy set Ã defined on a set of real number R is said to be a fuzzy number, if its membership function $\mu_{\tilde{A}}(x)$: R→ [0, 1] that satisfies the following properties.

a. $\tilde{A}$ is convex.



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i.e.,
$\mu_{\tilde{A}}\{\lambda x_1 + (1-\lambda)x_2\} \geq \min\{\mu_{\tilde{A}}(x_1), \mu_{\tilde{A}}(x_2)\} \forall\, x_1, x_2 \in R$ and $\lambda \in [0,1]$.

b. $\tilde{A}$ is normal i.e., there exists an element $x_0 \in \tilde{A}$ such that $\mu_{\tilde{A}}(x_0) = 1$.

c. $\mu_{\tilde{A}}(x)$ is piecewise continuous.

*2.3. Intuitionistic Fuzzy Set*

Let X is a non-empty set. An Intuitionistic Fuzzy Set is defined as $\tilde{A} = \{(x, \mu_{\tilde{A}}(x), \vartheta_{\tilde{A}}(x)); x \in X\}$ which assigns to each element x, a membership degree $\mu_{\tilde{A}}(x)$ and a non- membership degree $\vartheta_{\tilde{A}}(x)$ under the condition $0 \leq \mu_{\tilde{A}}(x) + \vartheta_{\tilde{A}}(x) \leq 1$, for all $x \in X$.

*2.4. Intuitionistic Fuzzy Number*

An Intuitionistic Fuzzy Number $\tilde{A}^I$ is defined as follows:

i. an intuitionistic fuzzy subset of the real line,

ii. normal, that is, there is some $x_0 \in R$ such that $\mu_{\tilde{A}^I}(x_0) = 1$, $\vartheta_{\tilde{A}^I}(x_0) = 0$,

iii. A convex set for the membership function $\mu_{\tilde{A}^I}(x)$,
i.e.,
$\mu_{\tilde{A}^I}\{\lambda x_1 + (1-\lambda)x_2\} \geq \min\{\mu_{\tilde{A}^I}(x_1), \mu_{\tilde{A}^I}(x_2)\} \forall\, x_1, x_2 \in R$ and $\lambda \in [0,1]$.

iv. A concave set for the non-membership function $\vartheta_{\tilde{A}^I}(x)$,
i.e.,
$\vartheta_{\tilde{A}^I}\{\lambda x_1 + (1-\lambda)x_2\} \leq \max\{\vartheta_{\tilde{A}^I}(x_1), \vartheta_{\tilde{A}^I}(x_2)\} \forall\, x_1, x_2 \in R$ and $\lambda \in [0,1]$.

*2.5. Triangular Intuitionistic Fuzzy Number*

A Triangular Intuitionistic Fuzzy Number $\tilde{A}^I$ is an intuitionistic fuzzy set in R with the following membership function $\mu_{\tilde{A}^I}(x)$ and non-membership function $\vartheta_{\tilde{A}^I}(x)$,

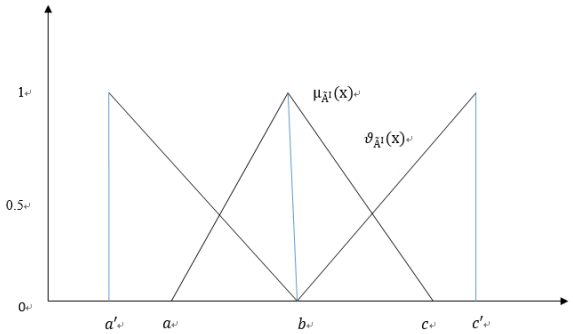
$$\mu_{\tilde{A}^I}(x) = \begin{cases} \frac{x-a}{b-a}, & a \leq x \leq b \\ \frac{x-c}{b-c}, & b \leq x \leq c \\ 0, & otherwise \end{cases}$$

and

$$\vartheta_{\tilde{A}^I}(x) = \begin{cases} \frac{b-x}{b-a'}, & a' \leq x \leq b \\ \frac{x-b}{c'-b}, & b \leq x \leq c' \\ 1, & otherwise \end{cases}$$

Where $a' \leq a \leq b \leq c \leq c'$ and $\mu_{\tilde{A}^I}(x) + \vartheta_{\tilde{A}^I}(x) \leq 1$ or $\mu_{\tilde{A}^I}(x) = \vartheta_{\tilde{A}^I}(x)\ \forall\, x \in R$

This Triangular Intuitionistic Fuzzy Number is denoted by
$\tilde{A}^I = (a, b, c; a', b, c') = \{(a, b, c); (a', b, c')\}$




Membership and Non-membership functions of Triangular Intuitionistic Fuzzy number

*2.6. Arithmetic Operations of Triangular Intuitionistic Fuzzy Number*

Let $\tilde{A}^I = \{(a, b, c); (a', b, c')\}$ and $\tilde{B}^I = \{(d, e, f); (d', e, f')\}$. Then

i. Addition:
$\tilde{A}^I + \tilde{B}^I = \{(a+d, b+e, c+f); (a'+d', b+e, c'+f')\}$

**ii.** Subtraction:
$\tilde{A}^I - \tilde{B}^I = \{(a-f, b-e, c-d); (a'-f', b-e, c'-d')\}$

*2.7. Score function*

Let $\tilde{A}^I = \{(a, b, c); (a', b, c')\}$ be a Triangular Intuitionistic Fuzzy Number, then we define a Score function for membership and non-

membership values respectively as $S(\tilde{A}^{I\alpha}) = \frac{a+2b+c}{4}$ and $S(\tilde{A}^{I\beta}) = \frac{a'+2b+c'}{4}$

*2.8. Ranking using Score function*

Let $\tilde{A}^I = \{(a,b,c);(a',b,c')\}$ and $\tilde{B}^I = \{(d,e,f);(d',e,f')\}$ be two Triangular Intuitionistic Fuzzy Numbers and $S(\tilde{A}^{I\alpha})$, $S(\tilde{A}^{I\beta})$ *and* $S(\tilde{B}^{I\alpha})$, $S(\tilde{B}^{I\beta})$ be the scores of $\tilde{A}^I$ and $\tilde{B}^I$ respectively

i. If $S(\tilde{A}^{I\alpha}) \leq S(\tilde{B}^{I\alpha})$ and $S(\tilde{A}^{I\beta}) \leq S(\tilde{B}^{I\beta})$ then $\tilde{A}^I < \tilde{B}^I$.
ii. If $S(\tilde{A}^{I\alpha}) \geq S(\tilde{B}^{I\alpha})$ and $S(\tilde{A}^{I\beta}) \geq S(\tilde{B}^{I\beta})$ then $\tilde{A}^I > \tilde{B}^I$.
iii. If $S(\tilde{A}^{I\alpha}) = S(\tilde{B}^{I\alpha})$ and $S(\tilde{A}^{I\beta}) = S(\tilde{B}^{I\beta})$ then $\tilde{A}^I = \tilde{B}^I$.

## 3. FUZZY SEQUENCING PROBLEM

The Sequencing Problem with uncertain processing time is termed as fuzzy sequencing problem. Algorithms for different types of fuzzy sequencing problems are proposed to sequence the jobs to be processed in various machines, with minimum total processing time. The assumptions for the classical problem are also applicable for fuzzy sequencing problem.

*3.1. Algorithm for solving different types of fuzzy sequencing problem*

*3.1.1. Processing 'n' jobs on two machines*

Let $A_1'$, $A_2'$... $A_n'$ be the processing times of 'n' jobs on Machine 1 and $B_1'$, $B_2'$...$B_n'$ be the processing times of 'n' jobs on Machine 2. The problem is to find the order in which the 'n' jobs are to be processed through two machines with the minimum total elapsed time.

**Procedure:**

**Step 1**: Use the ranking function to identify the minimum processing time from the given list of processing times $A_1'$, $A_2'$... $A_n'$ and $B_1'$, $B_2'$... $B_n'$.

**Step 2:** If the minimum processing time is $A_s'$ (i.e., job number 's' on machine 1) then do the $s^{th}$ job first in the sequence. If the minimum processing time is $B_t'$ (i.e., job number 't' on machine 2) then do the $t^{th}$ job last in the sequence.

**Step 3:**

a) If there is a tie in minimum processing of both machines (i.e., $A_s' = B_t'$), process the $s^{th}$ job first and $t^{th}$ job last in the sequence.

b) If the tie for the minimum occurs among the processing time on Machine 1, choose the job corresponding to the minimum of processing time on Machine 2 and process it first.

c) If the tie for the minimum occurs among the processing time on Machine 2, choose the job corresponding to the minimum of processing time on Machine 1 and process it last.

**Step 4:** Cancel the jobs already assigned and repeat steps 2 to 4 until all the jobs have been assigned.

The resulting order will minimise the total elapsed time and it is known as optimal sequence.

**Step 6:** After obtaining an optimal sequence as stated above, the minimum total elapsed time and also the idle time on machines 1 and 2 are calculated as follows:

**Minimum Total elapsed time** = Time out of the last job on machine 2.

**Idle time for machine 1** = Total elapsed time - time when the last job is out of machine 1

**Idle time for machine 2** = Time at which the first job on machine 1 finishes in a sequence

$$+\sum_{i=2}^{n}\left\{\begin{matrix}(\text{time when the } i^{th} \text{ job starts on machine 2})\\ -(\text{time when the } (i-1)^{th} \text{ job finishes on machine 2})\end{matrix}\right\},$$

*3.1.2. Processing 'n' jobs on three machines*

Let $A_1'$, $A_2'$... $A_n'$ be the processing times of 'n' jobs on Machine 1, $B_1'$, $B_2'$... $B_n'$ be the processing times of 'n' jobs on Machine 2 and $C_1'$, $C_2'$... $C_n'$ be the processing times of 'n' jobs on Machine 3. There is no standard procedure to obtain an optimal sequence for processing 'n' jobs on 3 Machines. So, we have to convert this type of problems into a two machine problem by satisfying any one or both of the following conditions.

1. Min $(A_i') \geq$ Max $(B_i')$, for i= 1, 2 .... n
2. Min $(C_i') \geq$ Max $(B_i')$, for i= 1, 2 .... n

We use ranking function to determine the minimum or maximum processing time. If one of the above conditions is satisfied, we introduce two new machines G and H such that the processing times on G and H are given by

$G = A_i' + B_i'$, for i= 1, 2... n

$H = B_i' + C_i'$, for i= 1, 2… n
Now we can proceed to determine the optimal sequence using 3.1.1.
After obtaining an optimal sequence, the minimum total elapsed time and also the idle time on machines 1, 2 and 3 are calculated as follows:

**Minimum Total elapsed time** = Time out of the last job on machine 3.
**Idle time for machine 1** = Total elapsed time - time when the last job is out of machine 1
**Idle time for machine 2** = (Total elapsed time - time when the last job is out of machine 2)
+ Time at which the first job in a sequence finishes on machine 1

$$+\sum_{i=2}^{n}\left\{\begin{array}{l}\left(\text{time when the } i^{th} \text{ job starts on machine 2}\right)\\ -\left(\text{time when the } (i-1)^{th} \text{ job finishes on machine 2}\right)\end{array}\right\}$$

**Idle time for machine 3** = Time at which the first job in a sequence finishes on machine 2

$$+\sum_{i=2}^{n}\left\{\begin{array}{l}\left(\text{time when the } i^{th} \text{ job starts on machine 3}\right)\\ -\left(\text{time when the } (i-1)^{th} \text{ job finishes on machine 3}\right)\end{array}\right\}$$

*3.1.3. Processing 'n' jobs on 'm' machines*
Let there be 'n' jobs which are to be processed through 'm' machines $M_1$, $M_2$ …$M_m$ in the order $M_1, M_2 \ldots M_m$ and $T_{ik}$ be the time taken by the $i^{th}$ job on $k^{th}$ machine.

**Procedure**
**Step 1:** Use ranking function to identify Min $T_{i1}$ (Minimum time for the first machine), Min $T_{im}$(Minimum time on the last machine) and Max ($T_{ik}$) for k=2, 3… m-1 and i=1, 2 …n (Maximum time on intermediate machines).

**Step 2:** Check the following conditions:
(i) $\text{Min}(T_{i1}) \geq \text{Max}(T_{ik})$
(ii) $\text{Min}(T_{im}) \geq \text{Max}(T_{ik})$

**Step 3:** If the conditions in step 2 are not satisfied, the problem cannot be solved by this method, hence go to next step.

**Step 5:** Convert the 'n' job 'm' machine problem into 'n' job two machine problem by considering two machines P and Q such that
$P_{ij} = T_{i1} + T_{i2} + \ldots + T_{i(m-1)}$
$Q_{ij} = T_{i2} + T_{i3} + \ldots + T_{im}$

**Step 6:** Now we can proceed to determine the optimal sequence using 3.1.1. After obtaining an optimal sequence, the minimum total elapsed time and also the idle time on machines can be determined.

## 4. NUMERICAL EXAMPLES

*4.1. There are five jobs, each of which is to be processed through two machines $M_1$, $M_2$ in the order $M_1M_2$. Processing time (in hours) is given below.*

| Jobs | Machine ($M_1$) | Machine ($M_2$) |
|---|---|---|
| **A** | **(2, 3, 4 ; 1,3,5)** | **(2, 4, 6 ; 1, 4, 7)** |
| **B** | **(6, 8, 10 ; 4, 8, 12)** | **(5, 10, 15 ; 3, 10, 17)** |
| **C** | **(3, 5, 7 ; 1, 5, 9)** | **(3, 6, 9 ; 2, 6, 10)** |
| **D** | **(4, 7, 10 ; 3, 7, 11)** | **(3, 5, 7 ; 1 ,5, 9)** |
| **E** | **(2, 4, 6 ; 1, 4, 7)** | **(6, 8, 10 ; 4, 8, 12)** |

**Obtain the optimal sequence and also determine the minimum total elapsed time and idle time for each of the machine.**

Solution:

| Jobs | A | B | C | D | E |
|---|---|---|---|---|---|
| Order of Cancellation | (1) | (5) | (3) | (4) | (2) |

Optimal Sequence: A-E-C-B-D

To find the total elapsed time:

| Jobs | Machine($M_1$) | |
|---|---|---|
| | **Time in** | **Time out** |
| A | (0, 0, 0; 0, 0, 0) | (2, 3, 4; 1, 3, 5) |
| E | (2, 3, 4; 1, 3, 5) | (4, 7, 10; 2, 7, 12) |
| C | (4, 7, 10; 2, 7, 12) | (7, 12, 17; 3, 12, 21) |
| B | (7, 12, 17; 3,12, 21) | (13, 20, 27; 7, 20, 33) |
| D | (13, 20, 27; 7, 20, 33) | (17, 27, 37; 10, 27, 44) |

| Jobs | Machine($M_2$) | |
|---|---|---|
| | Time in | Time in |
| A | (2, 3, 4; 1, 3, 5) | (4, 7, 10; 2, 7, 12) |
| E | (4, 7, 10; 2, 7, 12) | (10, 15, 20; 6, 15, 24) |
| C | (10, 15, 20; 6, 15, 24) | (13, 21, 29; 8, 21, 34) |
| B | (13, 21, 29; 8, 21, 34) | (18, 31, 44; 11, 31, 51) |
| D | (18, 31, 44; 11, 31, 51) | (21, 36, 51; 12, 36, 60) |

| Jobs | Idle Time ($M_1$) | Idle Time ($M_2$) |
|---|---|---|
| A | - | (2, 3, 4; 1, 3, 5) |
| E | - | (-6, 0, 6; -10, 0, 10) |
| C | - | (-10, 0, 10; -18, 0, 18) |
| B | -- | (-16, 0, 16; -26, 0, 26) |
| D | | (-26, 0, 26; -40, 0, 40) |
| | (-16, 9, 34; -32, 9, 50) | - |
| Total | (-16, 9, 34; -32, 9, 50) | (-56, 3, 62; -93, 3, 99) |

Minimum total elapsed time = (21, 36, 51; 12, 36, 60) hours
Idle time for Machine $M_1$ = (-16, 9, 34; -32, 9, 50) hours
Idle time for Machine $M_2$ = (-56, 3, 62; -93, 3, 99) hours

*4.2. There are seven jobs, each of which is to be processed through three machines $M_1$, $M_2$ and $M_3$ in the order $M_1M_2M_3$. Processing time (in hours) is given below.*

| Jobs | Machine ($M_1$) | Machine ($M_2$) | Machine ($M_3$) |
|---|---|---|---|
| **A** | **(2, 3, 4; 1, 3, 5)** | **(2, 4, 6; 1, 4, 7)** | **(3, 6, 9; 1, 6, 11)** |
| **B** | **(7, 8, 9; 6, 8, 10)** | **(2, 3, 4; 1, 3, 5)** | **(4, 7, 10; 2, 7, 12)** |
| **C** | **(4, 7, 10; 2, 7, 12)** | **(1, 2, 3; -1, 2, 5)** | **(3, 5, 7; 1, 5, 9)** |
| **D** | **(2, 4, 6; 1, 4, 7)** | **(3, 5, 7; 1, 5, 9)** | **(7, 11, 15; 5, 11, 17)** |
| **E** | **(8, 9, 10; 7, 9, 11)** | **(-1, 1, 3; -2, 1, 4)** | **(3, 5, 7; 1, 5, 9)** |
| **F** | **(7,8,9;6,8,10)** | **(2,4,6;1,4,7)** | **(3,6,9;1,6,11)** |
| **G** | **(4,7,10;2,7,12)** | **(2,3,4;1,3,5)** | **(10,12,14;9,12,15)** |

**Obtain the optimal sequence and also determine the minimum total elapsed time and idle time for each of the machine.**

Solution:

Since the problem is a three machine problem, we convert this into a two machine problem.
For that, it has to satisfy any one or both of the following conditions

i. $\text{Min}(M_1) \geq \text{Max}(M_2)$

ii. $\text{Min}(M_3) \geq \text{Max}(M_2)$

Here Min ($M_1$) = (2, 3, 4; 1, 3, 5) and Max ($M_2$) = (3, 5, 7; 1, 5, 9) = Min ($M_3$).

(i.e.,) i. Min ($M_1$) $\geq$ Max ($M_2$)

ii. Min ($M_3$) = Max ($M_2$)

Therefore, the second condition is satisfied. We convert the problem into a two machine problem as H and K. The processing time of the twomachines H and K for 7 jobs are as follows:

H= $M_1$+ $M_2$ and K= $M_2$+ $M_3$

| Job | H | K | Order of Cancellation |
|---|---|---|---|
| A | (4, 7, 10; 2, 7, 12) | (5, 10, 15; 2, 10, 18) | (2) |
| B | (9, 11, 13; 7, 11, 15) | (6, 10, 14; 3, 10, 17) | (6) |
| C | (5, 9, 13; 1, 9, 17) | (4, 7, 10; 0, 7, 14) | (3) |
| D | (5, 9, 13; 2, 9, 16) | (10, 16 ,22; 6, 16, 26) | (4) |
| E | (7, 10, 13; 5, 10, 15) | (2, 6, 10; 1, 6, 13) | (1) |
| F | (9, 12, 15; 7, 12, 17) | (5, 10, 15; 2, 10, 18) | (7) |
| G | (6, 10, 14; 3, 10, 17) | (12, 15, 18; 10, 15, 20) | (5) |

| | |
|---|---|
| | 260, 7, 274) |

Optimal sequence: A-D-G-F-B-C-E

| Job | Machine ($M_1$) | | |
|---|---|---|---|
| | Time in | Time out | Idle time |
| A | (0, 0, 0; 0, 0, 0) | (2, 3, 4; 1, 3, 5) | - |
| D | (2, 3, 4; 1, 3, 5) | (4, 7, 10; 2, 7, 12) | - |
| G | (4, 7, 10; 2, 7, 12) | (8, 14, 20; 4, 14, 24) | - |
| F | (8, 14, 20; 4, 14, 24) | (15, 22, 29; 10, 22, 34) | - |
| B | (15, 22, 29; 10, 22, 34) | (22, 30, 38; 16, 30, 44) | - |
| C | (22, 30, 38; 16, 30, 44) | (26, 37, 48; 18, 37, 56) | - |
| E | (26, 37, 48; 18, 37, 56) | (34, 46, 58; 25, 46, 67) | - |
| | | | (-21, 13, 47; -45, 13, 71) |
| Total | | | (-21, 13, 47; -45, 13, 71) |

| Job | Machine ($M_2$) | | |
|---|---|---|---|
| | Time in | Time out | Idle Time |
| A | (2, 3, 4; 1, 3, 5) | (4, 7, 10; 2, 7, 12) | (2, 3, 4; 1, 3, 5) |
| D | (4, 7, 10; 2, 7, 12) | (7, 12, 17 ; 3, 12, 21) | (-6, 0, 6; -10, 0, 10) |
| G | (8, 14, 20; 4, 14, 24) | (10, 17, 24; 5, 17, 29) | (-9, 2, 13; -17, 2, 21) |
| F | (15, 22, 29; 10, 22, 34) | (17, 26, 35; 11, 26, 41) | (-9, 5, 19; -19, 5, 29) |
| B | (22, 30, 38; 16, 30, 44) | (24, 33, 42; 17, 33, 49) | (-13, 4, 21; -25, 4, 33) |
| C | (26, 37, 48; 18, 37, 56) | (27, 39, 51; 17, 39, 61) | (-16, 4, 24;- 31, 4, 39) |
| E | (34, 46, 58; 25, 46, 67) | (33, 47, 61; 23, 47, 71) | (-17, 7, 31;- 36, 7, 50) |
| | | | (-24, 12, 48; -49, 12, 73) |
| Total | | | (-92, 37, 166; -186, 37, 260) |

| Job | Machine ($M_3$) | | |
|---|---|---|---|
| | Time in | Time out | Idle Time |
| A | (4, 7, 10; 2, 7, 12) | (7, 13, 19; 3, 13, 23) | (4, 7, 10; 2, 7, 12) |
| D | (7, 13, 19; 3, 13, 23) | (14, 24, 34; 8, 24, 40) | (-12, 0, 12; -20, 0, 20) |
| G | (14, 24, 34; 8, 24, 40) | (24, 36, 48; 17, 36, 55) | (-20, 0, 20; -32, 0, 32) |
| F | (24, 36, 48; 17, 36, 55) | (27, 42, 57; 18, 42, 66) | (-24, 0, 24; -38, 0, 38) |
| B | (27, 42, 57; 18, 42, 66) | (31, 49, 67; 20, 49, 78) | (-30, 0, 30; -48, 0, 48) |
| C | (31, 49, 67; 20, 49, 78) | (34, 54, 74; 21, 54, 87) | (-36, 0, 36; -58, 0, 58) |
| E | (34, 54, 74; 21, 54, 87) | (37, 59, 81; 22, 59, 96) | (-40, 0, 40; -66, 0, 66) |
| Total | | | (-158, 7, 172; - |

Minimum total elapsed time = (37, 59, 81; 22, 59, 96) hours
Idle time for Machine $M_1$ = (-21, 13, 47; -45, 13, 71) hours
Idle time for Machine $M_2$ = (-92, 37, 166; -186, 37, 260) hours
Idle time for Machine $M_3$ = (-158, 7, 172; -260, 7, 274) hours

*4.3. Obtain an optimal sequence for the following sequencing problem of four jobs and four machines when passing is not allowed, of which processing time (in hours) are given below. Also calculate the minimum total elapsed time and idle time for each of the machines.*

| Job | Machine ($M_1$) | Machine ($M_2$) | Machine ($M_3$) | Machine ($M_4$) |
|---|---|---|---|---|
| A | (10,13,16;9,13,17) | (3,8,13;2,8,14) | (6,7,8;5,7,9) | (12,14,16;10,14,18) |
| B | (11,12,13;10,12,14) | (3,6,9;1,6,11) | (3,8,13;2,8,14) | (17,19,21;15,19,23) |
| C | (5,9,13;4,9,14) | (6,7,8;5,7,9) | (3,8,13;2,8,14) | (14,15,16;12,15,18) |
| D | (3,8,13;2,8,14) | (4,5,6;3,5,7) | (3,6,9;1,6,11) | (14,15,16;12,15,18) |

Solution:
To find an Optimal Sequence, we convert the 4 Machine problem into a 2 Machine problem. For that, it has to satisfy any one of the following conditions.

i) Min ($M_1$) ≥ Max ($M_2$ , $M_3$)
ii) Min ($M_4$) ≥ Max ($M_2$ , $M_3$)

Here Min ($M_1$) =(3, 8, 13; 2, 8, 14) and Min ($M_4$) =(12, 14, 16; 10, 14, 18)
Max ($M_2$) = (3, 8, 13; 2, 8, 14) = Max ($M_3$)
Max ($M_2$, $M_3$) = (3, 8, 13; 2, 8, 14)
i.e.,

i) Min ($M_1$) = Max ($M_2$, $M_3$)
ii) Min ($M_4$) ≥ Max ($M_2$, $M_3$)

Here both the conditions are satisfied. Now, we convert this problem into a 2 machine problem as G and H. The processing time of machines G and H are obtained as follows:
G = $M_1$+ $M_2$+ $M_3$
H= $M_2$+ $M_3$+ $M_4$

| Jobs | G | H | Order of cancellation |
|---|---|---|---|
| A | (19, 28, 37; 16, 28, 40) | (21, 29, 37; 17, 29, 41) | (4) |
| B | (17, 26, 35; 13, 26, 39) | (23, 33, 43; 18, 33, 48) | (3) |
| C | (14, 24, 34; 11, 24, 37) | (23, 30, 37; 19, 30, 41) | (2) |

| | | | |
|---|---|---|---|
| D | (10, 19, 28; 6, 19, 32) | (21, 26, 31; 16, 26, 36) | (1) |

Optimal Sequence: D-C-B-A

To find total elapsed time:

| Job | Machine ($M_1$) | | |
|---|---|---|---|
| | Time in | Time out | Idle time |
| D | (0, 0, 0; 0, 0, 0) | (3, 8, 13; 2, 8, 14) | - |
| C | (3, 8, 13; 2, 8, 14) | (8, 17, 26; 6, 17, 28) | - |
| B | (8, 17, 26; 6, 17, 28) | (19, 29, 39; 16, 29, 42) | - |
| A | (19, 29, 39; 16, 29, 42) | (29, 42, 55; 25, 42, 59) | - |
| | | | - |
| Total | | | (12, 40, 68; -4, 40, 84) |

| Job | Machine ($M_2$) | | |
|---|---|---|---|
| | Time in | Time out | Idle time |
| D | (3, 8, 13; 2, 8, 14) | (7, 13, 19; 5, 13, 21) | (3, 8, 13; 2, 8, 14) |
| C | (8, 17, 26; 6, 17, 28) | (14, 24, 34; 11, 24, 37) | (-11, 4, 19; -15, 4, 23) |
| B | (19, 29, 39; 16, 29, 42) | (22, 35, 48; 17, 35, 53) | (-15, 5, 25; -21, 5, 31) |
| A | (29, 42, 55; 25, 42, 59) | (32, 50, 68; 27, 50, 73) | (-19, 7, 33; -28, 7, 42) |
| | | | (-1, 32, 65; -18, 32, 82) |
| Total | | | (-43, 56, 155; -80, 56, 192) |

| Job | Machine ($M_3$) | | |
|---|---|---|---|
| | Time in | Time out | Idle time |
| D | (7, 13, 19; 5, 13, 21) | (10, 19, 28; 6, 19, 32) | (7, 13, 19; 5, 13, 21) |
| C | (14, 24, 34; 11, 24, 37) | (17, 32, 47; 13, 32, 51) | (-14, 5, 24; -21, 5, 31) |
| B | (22, 35, 48; 17, 35, 53) | (25, 43, 61; 19, 43, 67) | (-25, 3, 31; -34, 3, 40) |
| A | (32, 50, 68; 27, 50, 73) | (38, 57, 76; 32, 57, 82) | (-29, 7, 43; -40, 7, 54) |
| | | | (-9, 25, 59; -27, 25, 77) |
| Total | | | (-70, 53, 176; -117, 53, 223) |

| Job | Machine ($M_4$) | | |
|---|---|---|---|
| | Time in | Time out | Idle time |
| D | (10, 19, 28; 6, 19, 32) | (24, 34, 44; 18, 34, 50) | (10, 19, 28; 6, 19, 32) |
| C | (24, 34, 44; 18, 34, 50) | (38, 49, 60; 30, 49, 68) | (-20, 0, 20; -32, 0, 32) |
| B | (38, 49, 60; 30, 49, 68) | (55, 68, 81; 45, 68, 91) | (-22, 0, 22; -38 ,0, 38) |
| A | (55, 68, 81; 45, 68, 91) | (67, 82, 97; 55, 82, 109) | (-26 ,0, 26; -46, 0, 46) |
| Total | | | (-58, 19, 96; -110, 19, 148) |

Minimum Total elapsed time = (67, 82, 97; 55, 82, 109) hours

Idle time for machine $M_1$ = (12, 40, 68; -4, 40, 84) hours

Idle time for machine $M_2$ = (-43, 56, 155; -80, 56, 192) hours

Idle time for machine $M_3$ = (-70, 53, 176; -117, 53, 223) hours

Idle time for machine $M_4$ = (-58, 19, 96; -110, 19, 148) hours

## 5. CONCLUSION

In this paper, we have solved different types of fuzzy sequencing problem using triangular intuitionistic fuzzy numbers. With the help of the proposed algorithm we obtained an optimal sequence and total elapsed time to process all jobs through machines, without converting the problem into a classical sequencing problem. The concept of fuzzy sequencing problem

provides an efficient framework in solving the real-life problems.

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DOI:10.26524/krj.2021.21

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**Publisher**: Kongunadu Arts and Science College, Coimbatore. https://www.krjournal.com

**RESEARCH ARTICLE**

# A STUDY ON IN VITRO ANTIOXIDANT ACTIVITY OF AQUEOUS SEED EXTRACT OF *SESBANIA SESBAN* (L) MERR.

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## ABSTRACT

The present study was done to investigate the *in vitro* antioxidant activity of aqueous extract of *Sesbania sesban* seeds. The assays such as DPPH, Chelation, ferrous ion, ABTS, Superoxide radical, hydroxyl radical assay, FRAP assay and total antioxidant activity were done to assess the antioxidant potential of the seed extract. The extract was tested at a concentration range of 100 – 500 µg/ml for all the assays and the values were compared with a standard. The results obtained showed that the radical scavenging activity was in a dose dependent manner and found to increase with increase in concentration of the extract. The IC50 value was calculated for the assays and tabulated for inference. Different assays revealed different levels of radical scavenging potential of the extract and exhibited as a better antioxidant source for therapeutic applications.

**Keywords:** *Sesbania sesban*, antioxidant, aqueous extract, radical scavenging, dose dependent.

## 1. INTRODUCTION

The traditional medicine all over the world is nowaday revealed by an extensive activity of researches on different plant species and their therapeutic principles. Plants contain phytochemicals with various bioactivities including antioxidant, anti-inflammatory and anticancer activities. Currently, about 25% of the active component was identified from plants that are used as prescribed medicines [1].

Free radicals and reactive oxygen species (ROS) such as superoxide, hydroxyl and peroxyl radicals are normal by-products of aerobic metabolism produced *in vivo* during oxidation. These ROS are generated in the mitochondria and microsome organelles under normal physiological conditions. They can also be produced externally by exposure to radiation, toxic chemicals, cigarette smoking and alcohol consumption, and by eating oxidized polyunsaturated fats. Overproduction of ROS can result in oxidative damage to various biomolecules including lipids, proteins, DNA and cell membranes. They also lead to the development of a variety of diseases such as coronary heart diseases, cancer, diabetes, hypertension and neurodegeneration. While compounds capable of scavenging free radicals possess great potential in ameliorating these diseases, most of the ROS are scavenged by endogenous defense enzymes such as catalase, superoxide dismutase and peroxidase-glutathione system. However, the activities of these endogenous defense systems may not be sufficient to mop up the free radicals [2].

Antioxidants can protect the human body from free radicals and reactive oxygen species (ROS) effects. Antioxidant agents are well known to retard the progress of many chronic diseases as well as lipid peroxidation [3].

Currently, there is a great interest in the study of antioxidant substances mainly due to the findings concerning the effects of free radicals' in the organism. Phenolic plant compounds have attracted considerable attention for being the main sources of antioxidant activity, in spite of not being the only ones. The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. In addition, they have a metal chelation potential. The antioxidant activities of phenolics play an important role in the adsorption or neutralization



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of free radicals. Several synthetic antioxidants are commercially accessible but have been reported to be toxic. Plants have been reported to exhibit antioxidant activity due to the presence of antioxidant compounds such as phenolics, proanthocyanidins and flavonoids [4].

Commonly used synthetic antioxidants include butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propylgallate (PG) and tertbutylhydroxyltoluene (TBHQ). Though important, they are known to constitute potential health risks and toxic effects. Their applications are, therefore, strongly restricted. Hence, the need to search, develop and utilize more effective antioxidant from natural origin. Inspite of our dependence on modern medicine and the tremendous advances in synthetic drugs, a large number of the world populations (80% of people) cannot afford the products of the western pharmaceutical industry and have to rely upon the use of traditional medicines, which are mainly derived from plant material [5].

Fabaceae, which is the third largest family among the angiosperms after Orchidaceae (orchid family) and Asteraceae (aster family), consists of more than 700 genera and about 20,000 species of trees, shrubs, vines, and herbs and is worldwide in distribution. *Sesbania sesban* (L) Merr belongs to fabaceae family has a long history of use in India, grows in a wide range of soils from loose sands to heavy clays. Root and bark used as bitter tonic used in debility nervous disorders and act as a CNS stimulant. Root of plant used as dysuria, retention of urine, hepatoprotective activity. Leaves are used as anthelmintic activity [6].

Therefore, this study has been designed to evaluate the *invitro* antioxidant activity of the aqueous seed extract of *Sesbania sesban.*

## 2. MATERIALS AND METHODS

### *2.1. Plant material and Preparation of extract*

The plant was authenticated with Botanical Survey of India, Southern Regional Centre, Coimbatore. Seeds of *Sesbania sesban* were shade dried and healthy seeds were selected and grinded well to a coarse powder. Aqueous extract was prepared with 10g of the powdered sample in 300 ml of water. The extract that was obtained was condensed in an oven and was preserved in an air tight container and stored at $4^0$C for further use.

### *2.2. In vitro* antioxidant activity of aqueous extract of seeds of *Sesbania sesban*

#### *2.2.1. Superoxide Radical Scavenging Assay [7]*

Superoxide radical $O_2^-$ scavenging capacity of aqueous extract was examined by a pyrogallol autooxidation system. The reaction mixture contained 70µl 10mM pyrogallol, 4.5ml 50mM Tris Hcl (pH 8.2) and 0.5ml various concentrations of samples. The absorbance at 325nm was recorded immediately at 30 seconds and then recorded once every minute. The scavenging rate was obtained according to the formula: $O_2^-$, scavenging rate (%) = $[1-(A_1-A_2)/A_0]$ X 100, where $A_0$ was the absorbance of the control (without extract), $A_1$ was the absorbance in the presence of the extract; $A_2$ was the absorbance of without pyrogallol.

#### *2.2.2. Hydroxyl Radical Scavenging Assay [8]*

Two sets were prepared for the hydroxyl radical scavenging effect, one with extract and sodium salicylate and the other with extract but without sodium salicylate. One set of the tubes containing the mixture 0.5ml of $FeSO_4$, 0.35 ml of $H_2O_2$, 0.5 ml of the extract and 0.15 ml of sodium salicylate and the other set of tubes containing all the components except sodium salicylate were incubated at $37^0$C for 1 hr. The absorbance was read at 562nm.

#### *2.2.3. DPPH Radical Scavenging Activity [9]*

Various concentrations of the extract (1.0 ml) were mixed with 1.0ml of methanolic solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.135 mM. The mixture were shaken vigorously and left to stand for 30 min in dark, and the absorbance was measured at 517 nm. BHA was used as control. The percentage of DPPH decolorization of the sample was calculated according to the equation:

% decolorization = $[1-(ABS_{sample}/ABS_{control}]$ x 100

#### *2.2.4. Reducing Power Assay [10]*

The reaction mixture contained 2.5 ml of various concentrations of methanol extract of the sample, 2.5 ml of 1% potassium ferricyanide and 2.5 ml of 0.2 M sodium phosphate buffer. The control contained all the reagents except the sample. The mixture was incubated at 50°C for 20 min and were terminated by the addition of 2.5 ml of 10% (w/v) of trichloroacetic acid, followed by

centrifugation at 3000 rpm for 10 min. 5.0 ml of the supernatant upper layer was mixed with 5.0 ml of deionized water and 1.0 ml of 0.1% ferric chloride. The absorbance was measured at 700 nm against blanks that contained distilled water and phosphate buffer. Increased absorbance indicates increased reducing power of the sample.

*2.2.5. Ferrous Ion Chelating Assay [11]*

The reaction mixture contained 1.0 ml of various concentrations of the extract, 0.1 ml of 2 mM $FeCl_2$ and 3.7 ml methanol. The control contained all the reaction reagents except sample. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine. After 10 min at room temperature, the absorbance of the mixture was determined at 562 nm against a blank. A lower absorbance of the reaction mixture indicated a higher $Fe^{2+}$ chelating ability. The capacity to chelate the ferrous ion was calculated by

% chelation = [1-(ABS $_{sample}$/ABS $_{control}$] x 100.

*2.2.6. ABTS Radical Scavenging Activity [12]*

Samples were diluted to produce 0.2 to 1.0 mg/ml. The reaction was initiated by the addition of 1.0 ml of diluted ABTS to10 µl of different concentration of the sample or 10 µl of methanol as control. The absorbance was read at 734 nm and the percentage inhibition was calculated. The inhibition was calculated according to the equation

$I = A_o - A_1/A_o \times 100$, where $A_o$ is the absorbance of control reaction, $A_1$ is the absorbance of test compound.

*2.2.7. Ferric reducing antioxidant power (FRAP) assay [13]*

A solution of 20 mM $FeCl_3 \cdot 6H_2O$, 300 mM acetate buffer (3.1 g $C_2H_3NaO_2 \cdot 3H_2O$ in 16 ml $C_2H_4O_2$, pH 3.6) and 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl) was prepared. At the time of establishing the assay, 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$ was mixed to prepare the FRAP solution. Plant extract (150 µl) was mixed with 2850 µl of FRAP solution and incubated at room temperature in the dark for 30 min. Absorbance of the intense blue-coloured product (ferrous tripyridyltriazine complex) was measured at 593 nm. The observed absorbance of the sample was calculated by putting the values on a linear standard curve plotted between 200 µM to 1000 µM $FeSO_4$.

*2.2.8. Total Antioxidant Capacity Assay [14]*

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample analyte and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH. The reagent solution consists of 0.6 M $H_2SO_4$, 28.0 mM sodium phosphate and 4.0 mM ammonium molybdate. An aliquot of 0.1 ml of sample was combined with 1 ml of reagent solution. The tubes were capped and incubated in a thermal block at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank. The blank solution contained 1 ml of reagent solution and the solvent used for the sample, and it was incubated under the same conditions as the rest of the samples.

## 3. RESULTS AND DISCUSSION

The *in vitro* antioxidant activity of aqueous seed extract of *Sesbania sesban* was done using different assays and the results are tabulated and discussed as follows.

**Table 1. Percentage inhibition activity of aqueous extract of seeds of *Sesbania sesban***

| Sample concentration (µg/ml) | DPPH | Chelation | ABTS | Superoxide radical | Hydroxyl radical |
|---|---|---|---|---|---|
| **100** | 21.70 ± 0.92 | 13.27 ± 0.85 | 12.39 ± 1.02 | 28.16 ± 0.69 | 16.60 ± 0.52 |
| **200** | 39.06 ± 1.25 | 22.24 ± 1.43 | 35.68 ± 1.50 | 39.43± 1.72 | 29.31± 1.37 |
| **300** | 56.43 ± 1.54 | 58.44± 2.70 | 55.66 ± 2.84 | 54.92± 1.50 | 43.19± 1.94 |
| **400** | 70.09 ± 1.86 | 66.67 ± 3.52 | 71.18 ± 3.98 | 67.60± 2.01 | 76.32 ± 2.25 |
| **500** | 79.09 ± 2.48 | 81.72 ± 4.87 | 81.71± 4.37 | 78.87± 0.74 | 83.88± 4.68 |

Value expressed as mean ± SD (n = 3)

In the DPPH test, the stable, nitrogen centered, coloured, DPPH free radical is reduced either by hydrogen donor or antioxidant to a non-radical DPPH-H and the decrease in colour of DPPH radical is monitored over a time period. The seed extract exhibited stronger radical scavenging ability and its 50% inhibition reached at 265 ± 12.5 µg/ml which indicates its good antioxidant potential. The percentage inhibition ranged from 21.70 ± 0.92 to 79.09 ± 2.48 % corresponding to 100-500 µg/ml of the extract.

Metal chelating activity is significant since it reduces the concentration of the catalyzing transition metal in lipid peroxidation [15]. It has been reported that the chelating agents, which forms σ bonds with the metal are effective as secondary antioxidants, because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion [16]. The $EC_{50}$ value was found to be 275 ± 12.75 µg/ml.

ABTS is a compound frequently used in phytomedicine research to measure the antioxidant properties of plants for better elucidation of their biological properties. The radical is generated through the oxidation of ABTS to an intensely coloured nitrogen centered cation by reacting with potassium persulfate for 12-14 h. The $IC_{50}$ values of the plant extracts were also determined for $ABTS^{+}$ which ranges at 275 ± 20.5 µg/ml.

Superoxide anion radical is one of the strongest reactive oxygen species among the free radicals that are generated. The scavenging activity of this radical by the plant extract compared favourably with the standard reagents such as gallic acid suggesting that the plant is also a potent scavenger of superoxide radical [4]. The extract was found to have the property to scavenge the superoxide radical at various concentrations ranging from 100-500 µg/ml. The activity increased with increase in concentration of the extract and reached its $EC_{50}$ value of 275 ± 17.06 µg/ml.

The hydroxyl radical (OH) is said to be detrimental and initiates auto-oxidation, polymerization and fragmentation of biological molecules. The identification of compounds that have excellent hydroxyl scavenging activity would be significant for some diseases caused by oxidative stress. It has been demonstrated that plants contain many natural antioxidants compounds which have been identified as hydroxyl radical scavengers. Therefore, OH scavenging effects of *S. sesban* aqueous extract were assessed in the present study. The result shows that the scavenging activity of seed extract are significant. The percentage inhibition was at rise with increase in concentration of the extract. The 50% percentage inhibition was at 340 ± 15.2 µg/ml.

**Table 2. $EC_{50}$ (µg/ml) values of aqueous extracts of *Sesbania sesban***

| Antioxidant assay | $EC_{50}$ (µg/ml) |
|---|---|
| DPPH | 265 ± 12.50 |
| Chelation | 275 ± 12.75 |
| ABTS | 275 ± 20.50 |
| Superoxide radical | 276 ± 17.06 |
| Hydroxyl radical | 340 ± 15.20 |
| Reducing Power | 300 ± 15.15 |
| FRAP | 250 ± 14.01 |

$EC_{50}$ value represents scavenging efficiency at 50% concentration of the extract on DPPH, ABTS radical, OH radical, chelation of ferrous ions, superoxide radical, hydroxy radical, and by reducing power and FRAP assay
Value expressed as mean ± SD (n = 3).

**Table 3. Total antioxidant activity of the aqueous extracts of *Sesbania sesban* by phosphomolybdenum assay**

| | mg/g (GAE) | mg/g (AAE) |
|---|---|---|
| Total antioxidant activity | 5.84 ± 0.05 | 16.90 ± 0.13 |

Total Antioxidant Capacity (TAC) assay by phosphomolybdenum method that based on the reduction of Mo (VI) to Mo (V) by the sample analyte and subsequent formation of a green phosphate/Mo (V) complex at acidic pH, usually detects antioxidants such as some phenolics, ascorbic acid, α-tocopherol and carotenoids [14]. The total antioxidant activity of the aqueous seed extract of *Sesbania sesban* was determined and was found to be 5.84 ± 0.05 mg/g of gallic acid equivalent (GAE) and 16.90 ± 0.13 mg/g of ascorbic acid equivalent (AAE). The values

represent a significant antioxidant potential of the extract.

**Table 4. Scavenging capacity of the aqueous extracts of *Sesbania sesban***

| Sample concentration (µg/ml) | Reducing Power($A_{700nm}$) | FRAP($A_{540nm}$) |
|---|---|---|
| **100** | 0.153± 0.008 | 0.282± 0.01 |
| **200** | 0.241± 0.01 | 0.415± 0.02 |
| **300** | 0.512± 0.03 | 0.627± 0.02 |
| **400** | 0.685± 0.03 | 0.795± 0.04 |
| **500** | 0.889± 0.04 | 0.971± 0.05 |

Value expressed as mean ± SD (n = 3).

The table.4 shows the scavenging capacity of the aqueous extracts of *Sesbania sesban* assessed by Reducing Power and FRAP assay. The reducing capacity of the extract, another significant indicator of antioxidant activity was also found to be appreciable. In the reducing power assay, the presence of antioxidants in the sample would result in the reduction of $Fe^{3+}$ to $Fe^{2+}$ by donating an electron. Increasing absorbance indicates an increase in reductive ability. The 50% inhibition was found at 300 ± 15.15 µg/ml. The extract was found to be effective at all concentrations. The results show that there was increase in reducing power of the plant extract as the extract concentration increases [4].

In FRAP assay, reduction of the ferric-tripyridyltriazine to the ferrous complex forms an intense blue colour which can be measured at a wavelength of 593 nm. The intensity of the colour is related to the amount of antioxidant reductants in the samples [17]. Since FRAP assay is easily reproducible and linearly related to molar concentration of the antioxidant present, thus it can be reported that extract of *S. sesban* may act as a free radical scavenger, capable of transforming reactive free radical species into stable non radical products. The antioxidant potentials of the extract of seeds of *S. sesban* were estimated from their ability to reduce TPRZ-Fe (III) complex to TPTZ-Fe (II) at 593 nm and its antioxidant activity increased proportionally with the polyphenol content [18]. The 50% inhibition was found at 250 ± 14.01µg/ml and the scavenging activity was a dose dependent manner.

## 4. CONCLUSION

The present study demonstrates the *invitro* antioxidant activity of aqueous seed extract of *Sesbania sesban.* Various assays supported the free radical scavenging potential and antioxidant potential of the extract at various concentrations. As per the literature available and the results of the present study, the *Sesbania sesban* seed extract can serve as a potent antioxidant source. A validation of the purified compounds from the extract need to be done for making it to be used for pharmaceutical and therapeutic applications.


## ACKNOWLEDGEMENT

The authors are thankful to Management of Kongunadu Arts and Science College (Autonomous), Coimbatore, Tamil Nadu, India for the facilities offered to carry out the research work.


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