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RESEARCH ARTICLE

DIVERSITY AND DISTRIBUTION OF ANTS (HYMENOPTERA: FORMICIDAE) IN KATANCHIMALAI REGION OF COIMBATORE DISTRICT, TAMIL NADU

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ABSTRACT

The study examined the diversity of ants in the Kattanchimalai region, Coimbatore District, Tamil Nadu, as there is no adequate information pertaining on ant diversity of this region. The present study was carried out during November 2020 to February 2021. We have sampled ants by employing intensive all out search method. The sampled specimens representing 35 species belonged to 12 genera and five subfamilies. The most diverse subfamily was Formicinae (4 genera with 16 species), followed by Myrmicinae (5 genera with 12 species), Pseudomyrmicinae (1 genera with 4 species) followed by Dolichoderinae (2 genera with 2 species). The smallest number of species belonged to the Ponerinae (1 genus with 1 species). Among the sampled genera, the highest number of species representation was *Camponotus* with 4 species. Few ant genera as *Crematogaster* and *Pheidole* of Myrmicinae, *Camponotus* of Formicinae and *Leptogenys* of Ponerinae were mostly found everywhere. Some genera viz*; Oecophylla*, *Anoplopsis, Paratrechina* of Formicinae subfamily and *Tetraponera* of Pseudomyrmicinae are represented by one species each.

Keywords: Ants, Formicinae, Myrmicinae, Camponotus, Periyanaickenpalayam

1. INTRODUCTION

Ants are found everywhere, except in Iceland, green-land and Antarctica [1], In India, Himalaya and the Western Ghats harbor a large number of ant species, 656 species from 88 genera were recorded from Himalaya, and 455 species from 75 genera were recorded from the Western Ghats, especially in Tamil Nadu, 184 species from 51 genera were recorded . But the number of species declines with increasing latitude, altitude and aridity [2]. Currently, they are 15,983 extant and species are subspecies as per the recent classification [3]. They are grouped in to 20 subfamilies, with 464 genera. Ants are conspicuous and important parts of virtually all terrestrial ecosystems [1,4,5]. Toward understanding the function of ant communities, ecologists have often used single linear measures of size [6]. India, a few reports on ant ecology Ants diversity exit [7]. The Kattanchimalai region is semi forest area and part of the Western Ghats in Coimbatore district, it is near to the Karamadai region, and the study area is full of fertile area.

The ants are everywhere except polar region and these are sub-terrestrial or ground insects mainly. The literature on ant ecology suggests that there are 11000 plants on the earth that depends on ants for pollination, seed dispersal and soil recycling to increase the soil fertility.

A significant focus for present-day myrmecologists is the assessment of biodiversity, community composition, biogeography, and other basic investigations of the ecology of a regional ant biota. The Myrmicinae is the largest subfamily of the Formicinae, With 138 genera followed by Formicinae that have 39 genera and Ponerinae which have 25 genera [8]. Indian ants fauna, represent diversity, includes 12 known subfamilies like; Aenictinae, Amblyoponinae,

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Cerapachyinae, Dolichoderinae, Dorylinae, Ectatomminae, Formicinae, Leptanillinae, Myrmicinae, Poneriae, Porceratiina and Pseudomyrmicinae. Rothney [9] worked on Indian ants and later on Forel [10] contributed comprehensive work on Formicidae of India and Ceylon.

Bingham [11] published his valuable work in Fauna of British India, Hymenoptera, and VO'I. 2, including Burma and Ceylon and gave details about distribution of species included. Successive workers like Ali [12], Brown Jr [13], Bolton [14], Baroni Urbani [15], Chapman and Capco [16], Chhotani and Maity [17] , Collingwood [18], Dutta and Raychaudhuri [19], Devi and Singh [20], Donisthorpe [21, 22], Ghosh [23], Imai et al. [24], JerdO'n [25], Kugler [26], Kurian [27], Karavaiev [28] , Mathew and Tiwari [29] , Reddy et al. [30] , Roonwal [31] , Ramdas et al. [32], Saunders [33], Smith, F. [34], Smith, M. R. [35] , Sykes [36], Sheela and Narendran [37], Shivashankar [38] , Taylor [39], Tiwari [40], Verghese et al. [41] , Veeresh et al. [42] recorded 12 species under 10 genera from Orissa. No comprehensive work on Ants fauna of Coimbatore has been done since then, except a few scattered works. Recently, these subfamilies Martialinae has been added to the family Formicidae. All the ant species fall into the single family Formicidae. This family included in the super family vesipedae of the order hymenoptera, which is placed in the class insect.

Ants can build their nests in leaf litter, rotting logs, underneath the soil, within woody stems or under the rocks and they can also establish fungal gardens in the soils. During activities associated with gallery building of nests by ants favor the mixing of organic matter in the soil, as well as increase the aeration properties of soils. The aim of the study is biodiversity richness of the ants in the biogeographically and size of the ants were observed.

2. MATERIALS AND METHODS

2.1. Study area

The field work was conducted in the Kattanchimalai region, Coimbatore district, Tamil Nadu. Coimbatore lies at 11°1′6″N, 76°58′21″E, in south India at 411 meters (1349 ft) above sea level on the banks of the Noyil River, in south western Tamil Nadu. The average annual rainfall is around 700 mm (27.6 in) with the northeast and the southwest monsoons contributing to 47% and 28% respectively to the total rainfall. Periyanaickenpalayam is a neighborhood in Coimbatore in the Indian state of Tamil Nadu. It is located along National Highway NH 67, Mettupalayam road, an arterial road in Coimbatore.

Fig 1 shows the study area map of Kattanchimalai region.

2.2. Collection method

We employed all out search method for the collection of ants in November 2020 to February 2021. Ants were collected using a brush and forceps during day time in between 11am to 4 pm twice in every month. 2.3 Preservation method Ant's species were preserved in 70% ethanol in

plastic vials at the Department of Zoology, Kongunadu College of arts and science. The stored ant specimens were then counted and identified up to genus level (some to species level) using microscope. Species identification was carried out under the help of the keys of "Ants identification guide" [54], collected ants were identified up to the genus level by using based on literature (29, 14, 11, 1]. Identified specimens will be kept in the air tight insect wooden box. Ant species were listed and each species was counted to calculate and compared composition, richness, species diversity, trees association, habitat type and identification of ants.

2.3. Statistical analysis

Relative density of the species was calculated by the formula,

Relative Density (%) = (Number of individuals of one species / Number of individuals of all species) X=100.

(SDI), and Shannon-Wiener index. SDI is a measure of diversity which takes into account the number of species present, as well as the relative abundance of each species.

SDI is calculated using the formula,

Where, $D = \Sigma n (n-1)/ N (N-1)$

n=total number of organisms of a particular species and N=total number of organisms of all species. Subtracting the value of Simpson's index from 1, gives Simpson's Index of Diversity (SID). Shannon-Wiener index (H') is another diversity index and is given as follows $H' = - \Sigma$ Pi ln (Pi), Where, Pi=S/N; S=number of individuals of one species, N=total number of all individuals in the sample, ln=logarithm to base e. Dominance index is a measure of how dominants (or similar), (D) follows the formula D=n (100/N), where n=individual number, N=total number of species.

3. RESULTS

Ant diversity in the Kattanchimalai region, Coimbatore district, Tamil Nadu has been analyzed in this study. During this study a total of 35 ant species are belonging to 12 genera and five subfamilies. Subfamily Formicinae were represented by 16 species and 4 genera followed by Myrmicinae were 12 species and 5 genera, Subfamily Pseudomyrmicinae consists of 4 species and 1 genera and Doilchoderinae represented by 2 species and 1 genus. The most number of genus was *Camponotus* with 13 species were observed.

Among these species *Camponotus compressus* was high compare to other species and noticeably found in everywhere in study site. The species of *Oecophylla* and *Crematogaster* were dominant on tree trunk which nested on trees. Few ant genera as *Crematogaster* and *Pheidole* of *Myrmicinae*, *Camponotus* and *Polyrhachis* of Formicinae and *Leptogenys* of Ponerinae are mostly found everywhere. The Table 1 (Figure 1) shows detailed distribution of diversity of ants. A number of factors seem to be involved in the increased diversity. It includes food resources, nesting habit etc. The environs of the study area are rich in ant species deserve. To date, no research has been conducted on the diversity of ants.

The above information will be useful for the preparation of a management plan for the myrmecologists. Total 35 ant species were recorded in the study area during this study. Among them *Polyrhachis spp, Crematogaster spp,* Myrmicinae*, Pheidole spp*., (Forel 1902), *Leptogenys* sp. 3 and *Tetraponera sp. 2* are rarely found the study area are represented in the list.

4. DISCUSSION

In the present study, 35 species of ants in 12 genera representing five subfamilies namely Formicinae, Myrmicinae, Ponerinae, Dolichoderinae and Pseudomyrmicinae were recorded. Out of five subfamily, Formicinae is the most abundant having 16 species in 3 genera. This subfamily is widely distributed in all geographic regions. This correlated with the present study, because, we similarly collected the utmost number of ant species from Formicinae subfamily in Kattanchimalai region. The Formicinae and Myrmicinae are the largest ant subfamilies in the world and the dominant groups in most terrestrial

habitats. The prevalence of these subfamilies has been reported to increase with increasing aridity [44, 45].

The Formicinae were the most abundant in the study area. The extreme dominance exhibited by Formicinae sub family with seven species in this study. Formicinae show a significant difference between the seasons. Humidity may influence the nest building. The genus *Camponotus* were record of four species. *Camponotus* was a frequently occurring species in everywhere. The *Camponotus* had the greatest individual numbers. These ants are called as carpenter ants because of their "Nesting behaviours" [46].

Table 1. Showing the list of identified ant species and their distribution in Kattanchimalai region, Coimbatore district

Family	Genus	Species	Common Name	Size
Formicinae	*Componotus*	*radiates*	Carpenter ant	1 cm and 0.5 cm
	Camponotus	*compressus*	Common godzilla ant	1.2 cm and 0.4 cm
	Camponotus	*irritans*	Giant honey ant	1 cm and 1.5 cm
	Camponotus	sp.	Carpenter ant	1 cm and 1.2 cm
	Camponotus	sp.	Carpenter ant	1.2 cm to 1 cm
	Camponotus	*parius*	Common black ant	1.2 cm
	Camponotus	*sericeus*	Ant	1.0 cm
	Camponotus	*maculatus*	Corpenterant	1.2 cm to 1 cm
	Camponotus	*sp.(flying)*	Corpenterant	1.2 cm to 1.0 cm
	Camponotus	*fabricius*	Ant	1.2 cm to 1.5 cm
	Camponotus	sp.	Corpenterant	1.2 cm to 1.0 cm
	Camponotus	sp.	Corpenterant	1.1 cm
	Camponotus	sp.	Corpenterant	1.2 cm
	Oecophylla	*smargdina*	Weaver ant,	1.2 cm to 1.0 cm
	Anoplolepsis	*gracillipes*	Yellow crazy ant	1.2 cm 1.0 cm
	Paratrechina	*logicornis*	Longhorn crazy ant	1.2 cm, 0.2 cm, 1.2 cm and 1.0 cm
Myrmicinae	*Monomorium*	*minimum*	Little black ant	1.2 cm,1 cm,0.5 cm
	Monomorium	*pharaonis*	Pharaoh ant	1.2 cm, 1.0 cm, and 1.3 cm
	Monomorium	*destructor*	Distructive trilling ant	1.2 cm and 1.3 cm
	Tetramorium	sp.	Pavement ant	1.3 cm, 1.2 cm, 1.0 cm
	Crematogaster	*subnuda*	Crematogaterini	1.3 cm,1.2 cm,1.0 cm
	Crematogaster	sp.	Crematogaterini	1.2 cm,1.0 cm,1.3 cm
	Crematogaster	sp.	Crematogaterini	1.2 cm, 1.0 cm, 1.2 cm
	Solenopsis	*invicta*	Red imported fire Ant	1.2 cm,1.0 cm, 0.5 cm, and 1 cm
	Solenopsis	*germinate*	Tropical fire ant	1.2 cm, 1.0 cm, 1.2 cm
	Solenopsis	*diplorhoptom*	Thief ant	1.3 cm, 1.0 cm, 1.2 cm
	Phediole	*magacephala*	African Big Headed ant	1.2 cm, 0.5 cm, 0.3 cm
	Phediole	sp.	Big Headed ant	1.5 cm
Dolichoderinae	*Tapinoma*	*indicum*	Odour ant	1.2 cm, 0.5 cm
	Tapinoma	*sessile*	Ant	1.3 cm
Ponerinae	*Lepitogenys*	*processionalis*	Processionant	1.3 cm, 1.0 cm
Pseudomyrmicinae	*Tetraponera*	*nigra*	Ant	1.3 cm
	Tetraponera	*nigra (male)*	Ant	1.2 cm; 1.0cm
	Tetraponera	*rufonigra*	Bicoloured arboreal ant	1.2 cm, 0.5 cm
	Tetraponera	*allaborans*	Ant	1.2 cm ,0.5 cm

The subfamily Myrmicinae, having 12 species in five genera, subfamily Dolichoderinae and Pseudomyrmicinae were recorded only the one genera for each with two and four species respectively, while the subfamily Ponerinae subfamily were one genera and one species reported in Kattanchimalai region. Overall abundance pattern in different sites varied considerably due to their habitat - heterogeneity and species composition. This was evident in certain sampling sites 1, 11 and 14 were common species viz., Dolichoderinae, *Camponotus* variegates, Myrmicaria *brunnea, Pheidole spp* dominated. As observed by many workers [47] species abundance pattern indicated a relatively small proportion of abundant species against large number of rare species. Secondly, the subfamilies such as Myrmicinae, Ponerinae, Formicinae were dominant. As observed by many workers [48] species abundance pattern indicated a relatively small proportion of abundant species against large number of rare species. Species richness is typically recorded to change across tropical forest disturbance gradients [49-51]. In Kattanchimalai region, four types of habitats were survey to find out the suitable area for ant species. Few ant genera as Crematogaster with most abundant record of seven species and genera Aphaenogaster, Myrmicaria and Monomorium of Myrmicinae, *Camponotus* and *Polyrchis* of Formicinae and *Leptogenys* of Ponerinae are mostly found everywhere, commonly found in all the habitats and most localities.

The workers of L. umbratus live entirely subterranean in symbiosis with root aphids [52] and S. debile forages mostly underground or in the litter layer with a small home range [53]. Subfamily Formicinae under genera *Camponotus, spp* which contains 37.14%, *Oecophylla, spp* which contains 2.85%, *Paratrechina spp* among with 2.85% and *Anoplolepis spp* contains 2.85%. In Formicinae subfamily, genera Camponotus was maximum in Kattanchimalai region followed by Myrmicinae subfamily into five genera including *Monomorium* spp with 8.57%, *Tetramorium* spp with 2.85%, *crematogaste*r spp consists of 8.57%, *Solenopsis spp* with 8.57% and *phediole spp* with consists of 5.27%. In Dolichoderinae and Pseudomyrmicinae subfamily, genera were tapinoma spp with 5.71% and tetraponera constitute 11.42%. *Tetramorium spp, Lepidogenys spp* also noted in minimum level. During comparison of tapinoma and tetraponera species tetraponera species were rich in Kattanchimalai region. Ponerinae subfamily, genera lepidogenys spp which contains 2.85% was observed during the present study. Sornapriya J et al., 2018, in Periyanaickenpalayam we observed thirty five species observed [55]. In 2019 we revealed the higher abundance of butterflies and ants among the insects were noted and the total 28 number of different types of insects were recorded in KonguNadu Arts and Science college campus [56]. Individual ant species was noted in Periyanaickenplayam area during the year of 2019 [57]. Twenty three species were identified among the 4 subfamilies reported were subfamily Formicinae was dominated with 10 species followed by Myrmicinae with 9 species, Dolichoderinae and Pseudomyrmicinae with 2 species each was noted in Karamadai region [58].

5. CONCLUSION

The present investigation on diversity of ants in the Kattanchimalai region, Coimbatore district clearly indicated that the richness of ants fauna in the city. The present study showed that the 35 species of ants belonging under the 5 subfamilies and 12 genera of Ants species and also large number of *Camponotus* and *Monomorium* genera were observed in Kattanchimalai region, Coimbatore district, Tamil Nadu. The Kattanchimalai, region mostly affected by anthropogenic pressure like deforestation, human population and vehicles pollution causing the diversity of ants in our area.

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RESEARCH ARTICLE

ETHNOMEDICINAL PLANTS USED BY MALAYALI TRIBALS IN YERCAUD HILLS, SALEM DISTRICT, TAMIL NADU, INDIA

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ABSTRACT

The present study highlights the importance of some medicinal plants in the health care system of Malayali tribal community of Yercaud Hills, Salem district, Tamil Nadu, India. Ethnomedicinal information was collected from malayali tribes through personal interviews and group discussions with 8 randomly selected informants. Use value (UV), fidelity level (FL) and Informant Consensus factor (ICF) were determined. During the data collection 20 species distributed in 16 families for treating 44 different ailments. Ethnomedicinal plants like *Abrus pulchellus*, Wall, *Andrographis paniculata*, Wall.ex.Nees, *Asclepias curassavica*, L, *Asparagus racemosus*, Wild, *Azadirachta indica*, A. Juss, *Cassia fistula*, Linn, *Centella asiatica*, Urb, *Corallocarpus epigaeus*, Hook.f, *Curculigo orchioides*, Gaertn, *Emblica officinalis*, Gaerth,*Enicostemma littirale*, Blume, *Hemidesmus indicus*, R. Br, *Holarrhena pubescens* (Buch.Ham.) Wall.ex.G.Don, *Leucas aspera*, Spreng, *Mimosa pudica*, Linn, *Myrica esculenta*, Buch. Ham, *Pergularia daemia* (Forsk.) chiov, *Terminalia bellerica*, Roxb, *Terminalia chebula*, Retz and *Toddalia asiatica*, Lamk were documented during the study. UV of the encountered plant species ranged from 0.38 to 1.13. The uppermost FCI value is reported for Ejaculation of semen and Bone fracture. In the present investigation, the FL varied from 50 to 100%.

Keywords: Ethnomedicinal plants, Malayali tribals, Yercaud Hills Use value (UV), Fidelity level (FL) and Informant Consensus factor (ICF).

1. INTRODUCTION

India is one of the 17th mega biodiversity countries of the world which resides a gargantuan diversity of plants, animals and microbes. When peoples were appearing on this earth has been crucial with the plant kingdom for their day today needs such as food, medicine, clothing, shelter and other requirements. Now days in many developing countries peoples are take modern medicine for their illnesses, but in many rural areas indigenous medicine based folk remedies has been played an important role in the health care system. In India the indigenous people are exercise a divergence of herbals for impressive curing of various diseases [1,-2] and it is known that India has the second largest tribal population in the world after Africa [3]. Today traditional medicine and ethnobotanical information play an important role in scientific research and conservation programs in different parts of the world [4].

Eastern Ghats ecosystem contains more than 2500 species of angiosperms which compose about 13 % of the flowering plants in India [5]. Yercaud hills is the major point in the Eastern Ghats, located in the forest types range from evergreen to moist deciduous with the altitude of 1515 meters (4970 Ft). The temperature ranges from 13^0 C to 29^0 C on the peaks and 25^0 C to 40^0 C at the foot hills. The average annual rainfall is

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around 1500 mm – 1750 mm [6]. The soil is deep and non-calcareoces [7]. Malayali tribals are typically hill tribals present in the foot hills of Yercaud hills. The main aim of the present study is highlights the importance of some medicinal plants in the health care system of Malayali tribals community of Yercaud Hills.

2. MATERIALS AND METHODS

Frequent field surveys were carried out in Yercaud hills in different seasons during 2014 - 2016. Interview and data gathering methods were followed by Schultes [8] and Jain [9]; Jain and Rao [10]. The voucher specimens were collected and identified by referring to standard floras [11,12].

2.1. Data analysis

2.1.1. Use Value (UV)

The relative importance of each species used in the study area was quantitatively evaluated following the method developed by Phillips and Gentry[13,14].

UV = ∑U/ N, Where, UV is the use value of species; U is the number of use reports cited for a particular species and n is the total number of informants interviewed. In general, UV is high, if there are more use report citations (When there are more uses and all the informants agree with it) for a given species and low when there are few reports.

2.1.2. Informant consensus factor (ICF)

Informant consensus factor (ICF) was calculated to evaluate if there was a consensus in the knowledge of plants used in the ailment group between healers in the study area. The ICF was calculated using the following formula [15].

ICF = Nur - Nt/Nur-1 where Nur refers to the number of use reports for a particular ailment category and Nt refers for a particular ailment category by all informants.

2.1.3. Fidelity level (FL)

The fidelity level (FL), the percentage of informants claiming the use of a certain plants for the same major purpose, was calculated according to the following formula [16].

FL (%) = Np / N × 100. Where Np is the number of informants that calming a use of a plant species to treat particular diseases and N is the number of informants that use the plants as a medicine to treat any given disease.

3. RESULTS AND OBSERVATIONS

3.1. Utilization of plant species as traditional medicine by Malayali tribes in Yercaud Hills

The investigation revealed that the traditional healers of Yercaud hills used 20 species of plants encompassing to 16 families to treat 44 different types of ailments. Most of the recorded medicinal herbs are harvested from natural environment in the different location of the yercaud hills by traditional healers. The day before they collect the plants, they pray to the plant and tie a thread that has been dipped in turmeric around the plant. The next day, they hymn a mantra before harvesting. The reported twenty important medicinal herbs in the present survey were arranged in alphabetical order according to their botanical name. The botanical name of each plant is followed by the local name, family and ethnomedicinal uses are listed in the Table 1.

During the survey we noted single plant may use for curing many ailments such as *Abrus pulchellus* is used to treat female infertility, easy delivery and rashes, *Andrographis paniculata* is used to treat centipede bite, scorpion sting, snake bite, diabetes, fever, small pox and cure blister. *Asclepias curassavia* used for curing migraine pain, cycosis, normal delivery, lumbago, dysentery and excessive bleeding after delivery. *Asparagus racemous* used for increase the sperms count, epididymitis and diabetes. *Azadirachta indica* recommended for female infertility, snake bite, mosaic and small pox, *Cassia fistula* used for curing snake bite, chest pain, diabetes and blister, *Centella asiatica* used for body pain, diabetes, menstrual disorder, increase sexual capacity and sperm count, female infertility, hemorrhoids, *Corallocarpus epigaeus* is used for treating antidote for beetle bite, centipede bite, scorpion sting and snake bite, *Curculigo orchioides* oral administration of rhizome powder can used for curing diabetes, neurotic problems, ejaculation of

semen, epididymitis, increase sperm count, erysipelas and kidney stone, *Emblica officinalis* used to cure dental ache, whoop cough, diabetes, liver problem and reduces the weight, *Enicostemma littorale* to treat the body pain, fever, beetle bite, centipede bite, snake bite, chest pain, dymenorrhoea and blister, *Hemidesmus indicus* is consumed for curing constipation, abdominal pain, kidney stone, chest pain and snake bite, bark of *Holarrhena pubescens* administer orally to cure hemorrhoids, trismus and bone fracture, *Leucas aspera* Oral administration to cure paralysis, migraine pain, rashes, chest pain ear ache. *Mimosa pudica* consumed to cure wound, beetle bite, female infertility, epididymitis and hemorrhoids. *Myrica esculenta* Oral administration of bark powder good for bone fracture, diabetes, female infertility and over bleeding during menstruation period. Consumption of leaves of *Pergularia daemia* to cure body pain, snake bite, irregular menstruation, normal delivery and lumbago. Fruit powder of *Terminalia bellerica* administers orally to cure diabetes, dysentery, colitis and reduce the Weight. Oral administration of fruit powder of *Terminalia chebula* used to treat diabetes, colitis, dysentery, hemorrhoids, dental ache, chest pain, whoop cough and reduce weight and *Toddalia asiatica* is used to cure snake bite, centipede bite, chest pain, cold, erysipelas leprosy.

3.2. Data analysis

The present work was the first ever study to record quantitative data of the medicinal flora of the region, including Use Value, Informant Consensus Factor and Fidelity Level.

3.2.1. Use Value (UV)

As indicated in table 2, UV of the encountered plant species ranged from 0.38 to 1.13. The highest UV was found for *Centella asiatica* (1.13) while lowest was for *Abrus pulchellus, Asparagas racemous* and *Holarrhena pubescens (0.38).* Other important plant species with high use value were *Curculigo orchioides (1), Enicostemma littorale (1), Terminalia chebula, Andrographis paniculata (0.88), Asclepias curassavia (0.75), Leucas aspera (0.75), Toddalia asiatica* (0.75), *Emblica officinalis* (0.63), *Hemidesmus indica* (0.63), *Terminalia bellerica* (0.5), *Myrica esculenta* (0.5), *Mimosa pudica* (0.5), *Corallocarpus epigaeus* (0.5) and *Cassia fistula* (0.5). It was also observed that the highest use values were due to the high number of use reports in the study area.

3.2.2. Informant Consensus Factor

The inhabitants used medicinal plants in the treatment of 44 different types of ailments. The important disorders were Ejaculation of semen, Bone fracture, snake bite, Chest pain, Female infertility, skin diseases, diabetes, Menstrual disorder, Hemorrhoids, Body pain and sperm count. To determine the informant consensus factor (FCI), all the reported ailments were first grouped into 11 different disease categories on the basis of their use reports (Table 3). The uppermost FCI value is reported for Ejaculation of semen and Bone fracture (1), followed by Snake bite (0.86), chest pain (0.8), female infertility and skin diseases (0.75), Diabetes (0.71), Menstrual disorder and Hemorrhoids (0.66), Body pain and sperm count (0.5). These results show that Ejaculation of semen and Bone fracture were especially common in the study area.

3.2.3. Fidelity level (FL)

Fidelity level highlights the medicinal flora, Medicinal plants with maximum curative properties have the highest fidelity level, i.e., 100%. In the present investigation, the FL varied from 50 to 100%. The plant species most commonly utilized in the research area with 100% fidelity levels were *Abrus pulchellus, Andrographis paniculata, Corallocarpus epigaeus, Asparagus racemous, , Curculigo orchioides*, which were used to treat Female infertility, snake bite, increase sperm count and ejaculation of semen respectively. The FL determined for *Enicostemma littorale* (Body pain), *Centella asiatica* (Menstrual disorder), *Emblica officinalis* (Whoop cough and diabetes), *Myrica esculenta* (Bone fracture and diabetes), *Toddalia asiatica* (Snake bite), *Holarrhena pubescens* (hemorrhoids), *Pergularia daemia* (Snake bite) and *Leucas aspera* (Rashes) were 93, 88, 75, 75, 71, 60, 60 and 50% respectively (Table 4).

Table 1. List of Ethnomedicinal plants used by Malayali tribes in Yercaud Hills

S. No	Botanical Name	Local Name	Family	Ethnomedicinal uses
1	*Abrus pulchellus* Wall.	Vellaikuntumani	Fabaceae	Oral administration of seed powder along with the honey to cure the female infertility, Seed paste with a glass of milk it causes easy delivery and Both seeds and leaves paste with hot water administered orally to cure rashes.
2	*Andrographis paniculata* Wall. Ex. Nees.	Siriyananagai	Acanthaceae	Oral administration of whole plant parts powder along with the hot water to cure centipede bite, whole plant parts powder administrated orally to cure scorpion sting, consumption of leaves powder to cure snake bite, Oral administration of leaves powder to reduce the diabetes, Oral administration of leaves decoction to cure fever, consumption of leaves infusion to cure small pox and whole plant parts powder mixed with castor oil applied externally to cure blister.
3	*Asclepias curassavia* L.	Mokkutipoodu	Asclepidaceae	Leaves juice extracts poured in the noise to relief the migraine pain, fresh leaves are added to the boiling water while taking bath to cure cycosis, oral administration of leaves juice to causes normal delivery, crushed leaves along with milk administered orally to cure lumbago, leaves paste administered orally to arrest dysentery and seed paste along with the hot water administered orally to cure excessive bleeding after delivery.
4	*Asparagus racemous* Willd.	Thanerivittankilangu	Liliaceae	Rhizome powder along with milk to increase the sperms count, oral administration of rhizome powder along with pepper and garlic to cure epididymitis and consumption of rhizome powder is reduced the diabetes.

5	*Azadirachta indica* A. Juss.	Vembu	Meliaceae	Oral administration of bark powder along with jiggery to cure female infertility, consumption of bark powder to cure snake bite, leaves paste mixed with common salt apply whole body while taking bath; this process continues for one month to cure mosaic and leaves paste administered externally to cure small pox.
6	*Cassia fistula* Linn.	Konnei	Caesalpiniaceae	Oral administration of bark powder along with pepper and garlic with hot water to cure snake bite, infusion of bark is consumed orally to cure chest pain, oral administration of bark powder to reduced diabetes and root powder mixed with castor oil administered externally to cure blister.
7	*Centella asiatica* Urb.	Vallarai	Apiaceae	Leaves are added to the boiling water and the vapor is inhaled to relief the body pain, oral administration of leaves powder can reduce the diabetes, oral administration of leaves powder is used to cure menstrual disorder, oral administration of leaves powder with milk used to cure the lacking in manly sexual capacity, oral administration of whole plant parts powder with cow milk to increase the sperm count, oral administration of whole plant parts powder is used to cure female infertility, consumption of whole plant parts powder with curd to cure burning sensation during urination, oral administration of whole plant parts powder is used to cure hemorrhoids and whole plant parts paste with milk administered orally to cure ejaculation of semen.

8	*Corallocarpus epigaeus* Hook.f.	Keradankilangu	Cucurbitaceae	Oral administration of rhizome powder with hot water to cure beetle bite, consumption of rhizome powder with hot water to cure centipede bite, rhizome powder administered orally to cure scorpion sting and rhizome powder with hot water administered orally to cure snake bite.
9	*Curculigo orchioides* Gaertn.	Nilapanaikilangu	Amaryllidaceae	Oral administration of rhizome powder can reduce diabetes, rhizome powder administered orally to cure neurotic problems, oral administration of rhizome powder with milk to cure ejaculation of semen, Oral administration of rhizome paste is used to cure epididymitis, rhizome powder with milk to administered orally to cure the lacking in manly sexual capacity, oral administration of rhizome powder with cow milk to increase the sperm count, rhizome paste administered externally to cure erysipelas and oral administration of rhizome powder with hot water to cure kidney stone.
10	*Emblica officinalis* Gaertn.	Nelli	Euphorbiaceae	Fruit paste keep inside the mouth to cure whoop cough, fresh fruit placed on the painful teeth to cure dental ache, oral administration of fruit powder can reduce diabetes, bark powder administered orally to cure liver problems and fruit powder with hot water administered orally; it reduces the weight.
11	*Enicostemma littorale* Blume.	Vellaragu	Gentianaceae	Leaves are added to the boiling water and the vapor is inhaled to relief the body pain, oral administration of leaves decoction to cure fever, whole plant parts powder with hot water to administer orally to cure beetle bite, oral

				administration of whole plant parts powder with hot water to cure centipede bite, whole plant parts powder administered orally to cure snake bite, oral administration of whole plant parts powder with hot water to cure chest pain, whole plant part powder along with pepper and nigella to administered orally to cure dymenorrhoea and oral administration of leaves powder is used to cure blister.
12	*Hemidesmus indicus* R. Br.	Nannari	Asclepidaceae	Oral administration of leaves powder used to cure constipation, entire aerial parts powder administered orally to cure abdominal pain, oral administration of whole plant parts powder with hot water to cure kidney stone, whole plant parts powder with hot water administered orally to cure chest pain and root powder administered orally to cure snake bite.
13	*Holarrhena pubescens* (Buch.Ham.) Wall.ex.G.Don.	Kudasapali	Apocynaceae	Bark powder along with the seeds of cumin and garlic with hot water to administer orally to cure hemorrhoids, consumption of bark paste with mother milk to cure trismus and oral administration of bark powder to cure bone fracture.
14	*Leucas aspera* Spreng	Thumbai	Mimosaceae	Oral administration of whole plant parts powder to cure paralysis, leaves juice poured into the noise to relief the migraine pain, leaves are added to the boiling water and the vapor is inhaled to relief the body pain, leaves paste administered externally to cure rashes, leaves powder with hot water administered orally to cure chest pain and leaves juice extract poured into the ear to cure ear ache.

15	*Mimosa pudica* Linn.	Thottalsurungi	Mimosaceae	Leaves paste is applied over wound, whole plant parts powder along with the turmeric mixed with coconut oil to applied beetle bitten area, oral administration of leaves paste is used to cure female infertility, whole plant parts consumed in the form of pill to cure epididymitis and leaves paste with milk administered orally to cure hemorrhoids.
16	*Myrica esculenta* Buch.Ham.	Kudumaruthamaram	Combretaceae	Oral administration of bark powder with cow milk is good for bone fracture, bark powder with hot water administered orally can reduce the diabetes, bark powder with hot water administered orally to cure female infertility and oral administration of bark powder with hot water is used to cure over bleeding during menstruation period.
17	*Pergularia daemia* (Forsk.) chiov.	Velliparuthi	Asclepidaceae	Leaves are added to the boiling water and the vapor is inhaled to relief the body pain, whole plant parts powder administered orally to cure snake bite, leaves powder administered orally to cure irregular menstruation, leaves juice mixed with castor oil administered orally; it causes normal delivery and consumption of leaves juice with milk to cure lumbago.
18	*Terminalia bellerica* Roxb.	Thanrikkai	Combretaceae	Oral administration of fruit powder can reduce diabetes, fruit powder with cow milk to administer orally to arrest dysentery, fruit powder with hot water to administer orally to cure colitis and oral administration of fruit powder is used to reduce the Weight.
19	*Terminalia chebula* Retz.	Kadukkai	Combertaceae	Oral administration of fruit powder can reduce diabetes, fruit powder with hot water to administer orally to cure colitis,

				fruit powder with cow milk taken orally; it arrest dysentery, fruit powder along with cumin seeds administered orally to cure hemorrhoids, fresh fruit placed on the painful teeth it reduced the dental ache, bark powder administered orally to cure chest pain, fruit paste keep inside the mouth cure whoop cough and fruit powder with hot water is administered orally to reduce weight.
20	*Toddalia asiatica* Lamk.	Mulaikaradanmullu/ Milagaranai	Rutaceae	Oral administration of root powder with hot water is used to cure snake bite, root powder with hot water to administer orally to cure centipede bite, infusion of bark is taken orally good for chest pain, roasted seed paste administered orally for cold, bark paste applied externally for erysipelas and root powder mixed with oil applied externally for leprosy.

Table 2. Use Value (UV) of ethnomendicinal plants used by Malayali Tribes in Yercaud Hills

S. No	Botanical Name	Vernacular Name	Family	Ailment category	UV value
1	*Abrus pulchellus* Wall.	Vellaikuntumani	Fabaceae	Female infertility, easy delivery and rashes	0.38
2	*Andrographis paniculata* Wall. Ex. Nees.	Siriyananagai	Acanthaceae	Centipede bite, scorpion sting, snake bite, diabetes, fever, small pox and blister	0.88
3	*Asparagus racemous* Willd.	Thanerivittankilangu	Liliaceae	Increase sperm count, epididymitis and diabetes	0.38
4	*Centella asiatica* Urb.	Vallarai	Apiaceae	Body pain, diabetes, menstrual disorder, sexual capacity, sperm count, female infertility, burning sensation during urination, hemorrhoids and ejaculation of semen	1.13

5	*Corallocarpus epigaeus* Hook.f.	Keradankilangu	Cucurbitaceae	Beetle bite, centipede bite, scorpion sting and snake bite	0.5
6	*Curculigo orchioides* Gaertn.	Nilapanaikilangu	Amaryllidaceae	Diabetes, neurotic problem, ejaculation of semen, epididymitis, sexual capacity, increase sperm count, erysipelas and kidney stone	1
7	*Emblica officinalis* Gaertn.	Nelli	Euphorbiaceae	Whoop cough, dental ache, diabetes, liver problem and weight loss	0.63
8	*Enicostemma littorale* Blume.	Vellaragu	Gentianaceae	Body pain, fever, beetle bite, centipede bite, snake bite, chest pain, dymenorrhoea and blister	1
9	*Holarrhena pubescens* (Buch.Ham.) Wall.ex.G.Don.	Kudasapali	Apocynaceae	Hemorrhoids, trismus and bone fracture	0.38
10	*Leucas aspera* Spreng	Thumbai	Mimosaceae	Paralysis, migraine pain, body pain, rashes, chest pain and ear ache.	0.75
11	*Myrica esculenta* Buch.Ham.	Kudumaruthamaram	Combretaceae	Bone fracture, diabetes, female infertility and over bleeding during menstruation	0.5
12	*Pergularia daemia* (Forsk.) chiov.	Velliparuthi	Asclepidaceae	Body pain, snake bite, irregular menstruation, normal delivery and lumbago	0.63
13	*Toddalia asiatica* Lamk.	Mulaikaradanmullu/ Milagaranai	Rutaceae	Snake bite, centipede bite, chest pain, cold, erysipelas and leprosy	0.75

Table 3. Informant Consensus Factor value of Major ailments

S. No	Major ailment	ICF
1.	Ejaculation of semen	1
2.	Bone fracture	1
3.	Snake bite	0.86
4.	Chest pain	0.8
5.	Female infertility	0.75
6.	Skin diseases (Rashes, blister, erysipelas and leprosy)	0.75
7.	Diabetes	0.71
8.	Menstrual disorder	0.66
9.	Hemorrhoids	0.66
10.	Body pain	0.5
11.	Sperm count	0.50

Table 4. Most commonly used medicinal plants and their major uses with their fidelity level

S. No	Botanical Name	Major ailment	Fidelity level (%)
1.	*Abrus pulchellus* Wall.	Female infertility	100
2.	*Andrographis paniculata* Wall. Ex. Nees.	Snake bite	100
3.	*Asparagus racemous* Willd.	Increase the sperm count	100
4.	*Centella asiatica* Urb.	Menstrual disorder	88
5.	*Corallocarpus epigaeus* Hook.f.	Snake bite	100
6.	*Curculigo orchioides* Gaertn.	Ejaculation of semen	100
7.	*Emblica officinalis* Gaertn.	Whoop cough and Diabetes	75
8.	*Enicostemma littorale* Blume.	Body pain	93
9.	*Holarrhena pubescens* (Buch.Ham.) Wall.ex.G.Don.	Hemorrhoids and blister	60
10.	*Leucas aspera* Spreng	Rashes	50
11.	*Myrica esculenta* Buch.Ham.	Bone fracture and diabetes	75
12.	*Pergularia daemia* (Forsk.) chiov.	Snake bite	60
13.	*Toddalia asiatica* Lamk.	Snake bite, erysipelas and leprosy	71

4. CONCLUSION

The study depicts Salem district of Tamil Nadu revealed in the field of folk medicine. The survey of the report includes both common and serious health issues such as Diabetes, Body pain, snake bite, Skin diseases and Mensural disorders Therefore, documentation of traditional knowledge is the only way out to preserve the knowledge base protect the medicinal plants resources endemic to this area. Clinical study to prove the validity of the recorded treatments could spread indigenous herbal knowledge worldwide; hence, action should be taken to conserve herbal knowledge, as well as the medicinal plants.

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RESEARCH ARTICLE

NORDHAUS-GADDUM INEQUALITIES FOR ANTI FUZZY GRAPH

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ABSTRACT

The objective of this paper is to finds the lower and upper bounds of Nordhaus-Gaddum inequalities of fuzzy chromatic number for anti-fuzzy graph. This paper analyzes the chromatic index of complementary anti fuzzy graphs in some cases. A theorem is proved for anti-fuzzy graph to be k-critical. Examples are provided to derive the vertex coloring of these graphs.

Keywords: Anti fuzzy graph (AFG), Complete Anti fuzzy graph, Self-Complementary Anti fuzzy graph, Fuzzy chromatic number, Bound of N-G inequality.

1. INTRODUCTION

A mathematical representation helps to make out the problem in a difficult situation. The best reachable solution is to convert the difficulties into graph. Fuzzy graph theory has used to representation of many decision making problems in vague environment which have several applications. The main part of the difficulty is considered as vertices and their connection between theses vertices are considered as edges. These are represented with fuzzy value [o to 1] to determine the vagueness. Some time, vagueness exists in a relation that attains maximum value. This kind of form is known as Anti Fuzzy Graph. Graph coloring is to assign colors to certain elements of a graph subject to constraints. Vertex coloring is the most common graph coloring technique which used in various research areas of computer science such as data mining, image segmentation, clustering, image capturing and networking etc., In 1956, [7] E. A. Nordhaus and J. W. Gaddum established the inequality for the bounds of chromatic numbers to the graph and its complement when $|v(G)| = n$. Based on these results, we find the upper and lower boundary of fuzzy chromatic number to the anti-fuzzy graph and its complement. These results mainly focused by computing vertex coloring in a strong, complete and self-complementary anti fuzzy graphs.

2. PRELIMINARIES

Some defined definitions are list out here to focus the needed result. Undirected simple and connected graphs are considered for this work. G(σ, μ) is a fuzzy graph $G_A(\sigma, \mu)$ is an anti-fuzzy graph with primary set S. S is a fuzzy subset of non-empty set is a mapping $\sigma: S \to [0,1]$, and a fuzzy relation μ on fuzzy subset σ, is a fuzzy subset of $S \times S$.

A Fuzzy Graph G(σ, μ) with $\sigma: S \to [0,1]$ and $\mu: S \times S \to [0,1]$ *such that* μ (x,y) ≤ min($\sigma(x), \sigma(y)$) $\forall$ x, y in S.

Anti-Fuzzy Graph G_A(σ, μ) consists of $\sigma: S \to [0,1]$ & $\mu: S \times S \to [0,1]$ such that μ(x,y) ≥ max($\sigma(x), \sigma(y)$) $\forall$ x, y in S. The degree of a vertex $\sigma(x)$ of an anti-fuzzy graph $G_A(\sigma, \mu)$ is defined by, $d_{G_A}(\sigma(x)) = \Sigma_{x \neq y}\, \mu(x,y)$. $G_A(\sigma, \mu)$ is called strong if μ(x,y) = max($\sigma(x), \sigma(y)$) for all

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(x,y) in μ. $G_A(\sigma,\mu)$ is called complete if μ(x,y) = max($\sigma(x),\sigma(y)$) for all x, y in σ.

The complement of Anti fuzzy graph $\bar{G}_A(\bar{\sigma},\bar{\mu})$ is derived from an fundamental Anti fuzzy graph $G_A(\sigma,\mu)$ where $\sigma = \bar{\sigma}$ and $\bar{\mu}$ (x,y) $= \mu$ (x,y) – $\max(\sigma(x),\sigma(y))$, if μ(x,y) > 0. If $G_A = \overline{G_A}$ then G_A is said to be a self-complementary anti fuzzy graph.

In a crisp graph, a graph is said to be k-critical if, $\chi(H) < \chi(G)$ for every proper sub graph H of G. A k-Chromatic graph that is critical is called k-critical. Every k – chromatic graph has a k-critical sub graph. Vertex coloring is mapping $\beta: V \to N$ such that $\beta(i) \neq \beta(j)$ where i and j are the adjacent vertices in G. A graph is k-colourable if it admits k-colorings. The chromatic number $\chi(G)$, of a graph G is the minimum k for which G is k-colorable.

The fuzzy vertex coloring of a fuzzy graph was defined by [10]Eslahchi and Onagh. If a fuzzy graph is k-fuzzy coloring then it satisfied the follows.

i). Let $\Delta = \{\gamma_1,\dots\gamma_k\}$ be the k- colouring such that $\cup\Delta = \sigma$

ii). $\gamma_i \wedge \gamma_j = 0$ and

iii). For every adjacent vertices u,v of G,
min $\{\gamma_i(u),\gamma_i(v)\}$=0 (1≤ i ≤ k).

[11] M.A. Rifayathali, A.Prasanna and S.Ismail Mohideen defined the coloring and chromatic number of anti-fuzzy graphs using β-cuts. A set of β-cut, generated by an anti-fuzzy set A, where β ∈ [0,1] is a fixed numbers, is defined as
$A_\beta = \{x \in X / \mu_A(x) \le \beta, \forall\beta \in [0,1]\}$.
The family of β-cut sets A_β is monotone, that is for $\beta, \delta \in [0,1]$ and $\beta \ge \delta$ and $A_\beta \supseteq A\delta$.

3. CHROMATIC INDEX OF ANTI FUZZY GRAPH

[11] Definition 3.1: Let $\{G_\beta = (V_\beta, E_\beta)/\beta \in [0,1]\}$ be the family of β-cuts of G, where the β-cut of an anti- fuzzy graph is the crisp graph $G_\beta = (V_\beta, E_\beta)$ with $V_\beta = \{v_i/v_i \in V, \sigma(v_i) \le \beta\}$, $E_\beta = \{(v_iv_j)/(v_iv_j) \in E, \mu(v_iv_j) \le \beta\}$.

To determine chromatic number of AFG, family of β- cuts of G is determined and then the chromatic number χ_β of each G_β is determined by using crisp k- vertex coloring C_β^k. The Chromatic number of G is defined through a monotone family of sets.

[11] Definition 3.2 : For an anti-fuzzy graph $G = (V, E)$, its chromatic number is the anti-fuzzy number $\chi(G) = \{(x, k(x))/x \in X\}$, where X = {1, … , $|V|$}, $k(x) = \inf\{\beta \in [0,1]/x \in A_\beta\}$, $x \in X$ and $A_\beta = \{\chi_0, \dots, \chi_\beta\}, \beta \in [0,1]$.

4. CHROMATIC NUMBER OF SELF COMPLEMENTARY ANTI FUZZY GRAPH

This section observes the features of self-complementary anti fuzzy graph and derives the fuzzy chromatic number for complement of anti-fuzzy graph.

Proposition 4.1

For a self-complement AFG, the underlying graph and its complement must be strong. But every strong AFG need not be self-complementary.

Proposition 4.2

If self-complementary exists in AFG then it must be a v-nodal anti fuzzy graph with effective edges.

Proof: Every n-vertex self-complementary graph has exactly n(n-1)/4 edges and it must have the diameter either 2 or 3. [5]In complementary AFG, the vertices are isomorphic to each other by the property of complement. That is, $\sigma = \bar{\sigma}$.

if μ(x,y) > 0,

$\bar{\mu}$(x,y) = μ(x,y) – $\max(\sigma(x),\sigma(y))$.

To claim the isomorphism, μ(x,y) must be equal to 0. From this,

$\bar{\mu}$(x,y) = $\max(\sigma(x),\sigma(y))$.

Every (3x3 rook's graph) Paley graph is the best example of self-complementary graphs.

Example 4.5 with $\sigma(G_A)$ = 0.8 for all σ provides the self-complement.

Proposition 4.3

A Self complementary graph doesn't exist in a complete anti fuzzy graph. Since the complement of complete anti fuzzy graph is a null graph.

There is no possible to finds an isomorphism between them.

Proposition 4.4

If AFG preserves the self-complement, then their chromatic number are same. Converse part of this statement is not necessarily true.

Proof:

If AFG preserves self-complement then, $G_A(\sigma, \mu) = \bar{G}_A(\bar{\sigma}, \bar{\mu})$. That is, there exists an isomorphism between σ and μ. obviously, $\chi(G_A) = \chi(\bar{G}_A)$.

Example-4.5 Computation of fuzzy chromatic index using the β-cuts

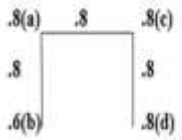


Fig 1: Anti Fuzzy Graph $G_A(\sigma,\mu)$

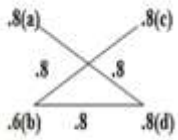
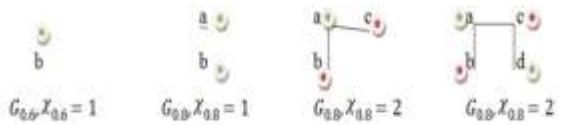


Fig 2: Complement of Anti Fuzzy Graph $\bar{G}_A(\bar{\sigma},\bar{\mu})$

In the above example, there are four crisp graphs in $G_\beta = (V_\beta, E_\beta)$.



$G_{0.6}, \chi_{0.6} = 1$ $G_{0.8}, \chi_{0.8} = 1$ $G_{0.8}, \chi_{0.8} = 2$ $G_{0.8}, \chi_{0.8} = 2$

The chromatic number of AFG = $\chi\beta = \{(1,0.6), (2,0.8)\}$

That is their chromatic index,

$\chi(G_A) = \chi(\bar{G}_A) = \{(1, 0.6), (2, 0.8)\}$.

5. CHROMATIC NUMBER OF COMPLETE ANTI FUZZY GRAPH AND ITS COMPLEMENT

This section observes the chromatic number of a complete AFG and compared this result with its complementary graph. From this observation, a circumstance is provided for the computation of chromatic number.

Proposition 5.1

In AFG, the following observation holds.

1. For every vertex graph, chromatic number by vertex coloring is 1.
2. For every edge graph, chromatic number (vertex coloring) is 2. Since the vertices are adjacent.
3. For any AFG, $1 \leq \chi(G_A) \leq n$.

Proposition 5.2

For $|\sigma(G_A)| = n$, anti-fuzzy graph, the following relation holds.

1. Fuzzy chromatic number

   $\chi(G_A) = n$, if AFG is complete.

   $\chi(G_A) < n$, otherwise

2. If G_A is complete then,

   $\chi(\bar{G}_A) = 1$ and $\chi(G_A) > \chi(\bar{G}_A)$.

3. G_A is k-critical graph when *it* is complete.

Proof:

1. For a complete AFG, all the edges are effective for all σ in G_A. Hence every vertex needs to assign distinct colors. That is, $\chi(G_A) = n$. If AFG is not complete then at least one of edge is not effective. ie., $\chi(G_A) < n$.
2. Complement of complete FG does not have edges. That is, all the vertices are distinct. Clearly, $\chi(\bar{G}_A) = 1$. From 1 &2, we concluded that, the chromatic number of complete AFG is finer than its complement.
3. In complete AFG, we cannot obtain the self-complementary. There is no possible to exist isomorphism between $\chi(G_A)$ and $\chi(\bar{G}_A)$. This implies that $\bar{G}_A$ is a subgraph of Anti Fuzzy Graph.

This says that, $\chi(\bar{G}_A) < \chi(G_A)$. Hence, G_A is k-critical graph.

Proposition 5.3

A complete AFG with 'n' vertices and its complement satisfies,

1. $\chi(G_A) = n+1 - \chi(\bar{G}_A)$

$$2.\ \chi(G_A) = \frac{n}{\chi(G_A)}$$

Proof:

By proposition 5.2, if AFG is complete then, $\chi(G_A)$ = n and $\chi(\bar{G}_A)$ = 1. This says that, $\chi(G_A) + \chi(\bar{G}_A)$ = n+1 and $\chi(G_A) \cdot \chi(\bar{G}_A)$ = n.

Proposition 5.4

NORDHAUS-GADDUM NEQUALITIES FOR ANTI FUZZY GRAPH

The chromatic number of any AFG G_A with 'n' vertices and its complement $\bar{G}_A$ satisfies,

1. $n+1 \le \chi(G_A) + \chi(\bar{G}_A) \le 2n$
2. $n \le \chi(G_A) \cdot \chi(\bar{G}_A) \le n^2$

Proof:

Consider any AFG need not be strong or complete. Take every vertices is adjacent to each other with anti-fuzzy values. Then $\chi(G_A)$ = n. For any edge membership value $\chi(\bar{G}_A)$ is not more than 'n'. That is, $\chi(\bar{G}_A) \le n$. This implies that,

$$\chi(G_A) + \chi(\bar{G}_A) = n + (\le n) \le 2n$$

$$\chi(G_A) \cdot \chi(\bar{G}_A) = n \cdot (\le n) \le n^2$$

Example: 5.5

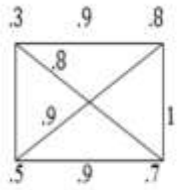


Fig 3: Anti Fuzzy Graph $G_A(\sigma, \mu)$

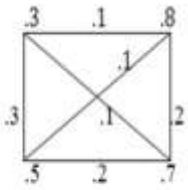


Fig 4 : Complement of Anti Fuzzy Graph $\bar{G}_A(\bar{\sigma}, \bar{\mu})$

From this example,

$$\chi(G_A) = \chi(\bar{G}_A) \rightarrow \chi(G_A) + \chi(\bar{G}_A) \le 2n$$

$$\rightarrow \chi(G_A) \cdot \chi(\bar{G}_A) \le n^2$$

5. CONCLUSION

The chromatic number of an anti-fuzzy graph is derived based on vertex coloring. This paper concludes that the chromatic number of self-complementary graph is same. The sum and product of chromatic number of complete anti-fuzzy graph is determined. For $|G_A(\sigma, \mu)| = n$, lower and upper bounds for the Nordhaus - Gaddum inequality is, $n+1 \le \chi(G_A) + \chi(\bar{G}_A) \le 2n$ and $n \le \chi(G_A) \cdot \chi(\bar{G}_A) \le n^2$.

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RESEARCH ARTICLE

DEPICTION OF SOCIETAL EVILS IN CHETAN BHGAT'S REVOLUTION 2020

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ABSTRACT

This paper focuses on Chetan's thoughts where he conveys in his writings that in a developing country youngsters are failing to get recognize Chetan bhagat the creative writer in the field of Indian English Literature has portrayed the mechanical life of youngsters in his novel Revolution 2020. The paper brings forth the empathetic life of youngsters and the political problems in a vivid manner. It shows the life of the protagonist and the burden faced by them by relatives and society.

Keywords: Societal, Contemporary, Getrecogorize, Developing and Burden

1. INTRODUCTION

Chetan Bhagat is usually considered to speak on behalf of the Indian entrepreneurial community. Every one of his novels has been bestsellers and has gained huge readerships worldwide. His novels have known to rock Indian sensibility, by providing thought provoking insights as a result of delving into the ever changing paradigms of human conditions that find it entangled in the maze of love, corruption and ambition in his novel, Revolution 2020. This research paper attempts in examining a detailed account of the happenings of Indian society, mainly in the fields of education and politics.

The various ingredients that make contemporary Indian society seem despicable were pointed out by Bhagat in his fifth novel, Revolution 2020. Chetan Bhagat delves deep into a very serious malaise of Indian society – corruption. Corruption has existed since time immemorial and has pervaded every facet of Indian society, from getting a certificate, to getting a government job or getting approvals to start a school or college. The bribes paid could run into many lakes of rupees. The despicable and uncouth reality of Indian society has been laid threadbare in this novel. The novel talks about how the basic inception of corruption in modern times can be traced to opportunistic leaders, who are consumed by ambition, and use corruption as a means to fuel their ambitions. The bleakness existing in Indian society, according to Bhagat, is due to political bankruptcy, social sins, malpractices and crimes of various hues. Revolution 2020 discusses in detail about how the Indian government aids in corruption. Bhagat's book talks about how government employees and political leaders actually operate, which lead to untold corruption everywhere. Corruption, the dark side of Indian society that Indians are generally accustomed to, manifests itself in the rotten educational system, coupled with the dishonesty and bribery that exist in public life forms the essence of this novel(2). The protagonist comes from a humble background that faces tremendous pressures in life. The novel describes him as an ordinary person with a slight paunch. It goes on to add that the "rest of him was whitish complexion, modest five feet seven-inch height, (and) his side-parted hair was reassuringly normal. His father didn't give him any pocket money and he didn't have much in his own pocket." (1) The protagonist lost his mother at the age of four, and his father owned only disputed agricultural land. Like Samir in Five Point Someone, the protagonist of Revolution 2020 is forced by his father to pursue

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engineering. Bhagat points out those students in India are not encouraged to opt for careers or studies based on their passion or choice. Rather, it is the will of the parent that is forced down upon their throats. The protagonist says, “I would be one of those unfortunate cases who had done well, but not well enough “(3). They become distraught when they are not able to fulfill their parents' desires that were imposed upon them. As a result, students have to lie to their parents when they don't do well, for they wouldn't want to see their parents getting hurt. Parents on the other hand are also insensitive, who tend to scold them rather that reassuring them when things don't go as planned. Bhagat infers that this could be one of the reasons why young people contemplate suicide. The plot revolves around the protagonist, his friends Raghav and the local beauty Aarti who is a District Magistrate's daughter. The three of them belong to middle class backgrounds. The protagonist's father is an ailing retired teacher, who is not inclined to spend all of his money on his medication, as the well-being of his son; the protagonist occupies a higher priority. The protagonist wants to enter one of the prestigious IITs. He has to do the JEE, the entrance test, in order to get into one of them. But he is not successful. His father sends him to Kota, Rajasthan, which is renowned for institutes that impart training for one to clear the JEE. Yet, the protagonist fails in his subsequent sitting for the JEE (4). His father passes away in the meanwhile. He starts to live by himself. His relatives do not seem very interested in taking him under their wings. Societal Evils in Contemporary India from Chetan Bhagat's Perspective in Revolution 2020 . They give him a little bit of money and gradually move out of his life. Bhagat highlights a major problem that exists among the young who are abandoned due the loss of their loved ones – no one in society cares too much for them! The young are running out of role models who can mould them to become leaders who not only follow their passion, but also make this world a better place. The protagonist, who has lost both of his parents, is now an orphan, who is despised by his relatives. It is at this juncture of his life, that through a friend of his, he is introduced to a political leader Shuklaji. Shuklaji has been portrayed as one who fits the stereotype that one usually finds in Indian movies – corrupt, loaded with unaccounted cash or black money and quite heartless. Shuklaji is corruption personified. Revolution 2020 rubs it in when it comes to corrupt politicians. According to Bhagat, politics is only for criminals and criminals are meant to be in politics. He goes on further to point out that elections have become associated with a host of criminal activities. Shuklaji finds a way to launder his black money – give some of it to the protagonist to start a college named Gunga Tec (5). Chetan Bhagat, in his novel Revolution 2020 talks about how meaningless education has become. But, it also highlights that everything in life is not that depressing. Revolution 2020 is a story of a young person's ambition and love in the background of corruption in Indian society and how youth react to these other very important facets – ambition and romance. Bhagat adroitly delineates the issues of contemporary Indian society which affect Indian youth through his characters in this novel: The way different characters and situations are developed by the novelist against complex sociocultural practices offers the readers an opportunity to see people and social institutions in an interactive mode. Whether it is the characters that are being in even a shade of change in the social practices or the social practices which facilitate or thwart the growth of human personality avenues for social criticism are opened. (6) In all of Chetan Bhagat's novels the stories revolve around the young person. His novels have attained popularity among this section of society as he is able to weave in a very seamless fashion everything that is happening in today's India from the perspective of the young person. At the same time, he is able to infuse interest in the reader by highlighting on the various challenges that a young person faces – anger, competition, bitterness, romance, sacrifice and rash decision making. His novels present a clear picture regarding his concerns in the current scenario prevailing in 21st century India. Most of what he wants to convey is through the eyes of the protagonist of his various novels and through leading newspapers. The youth of India form a significant proportion of Indian society and cannot be ignored. The aspirations of the youth need to be understood and Chetan Bhagat has

attempted to give voice to it through his writings. The dark and grim reality of Indian society is also being pointed out in his novels, which the young person can relate to. Revolution 2020 is another such book. The reason why his book click with his readers is because Bhagat does not seem to give answers to the various challenging situations that a young person faces. Rather, he faithfully tries to highlight it to the world. The young person feels that he or she has a voice in Chetan Bhagat's novels, as young people's views are not being seriously considered. It is the elder's voice that matters. Various issues are brought are brought to the limelight in this book. Revolution 2020 forces the reader to shine a light at the evil tentacles of corruption that is destroying the nation's conscience. When this is coupled with unbridled ambition, all boundaries are being crossed. Bhagat does not justify it, but raises this very pertinent question. The young person is forced to confront this ugly dual reality, and come up with ways to counter it. Does the title of the book offer some clues into the time frame by when it could be eradicated – 2020? Chetan Bhagat, in his novel Revolution 2020 very clearly elucidates the fact that the weak stem of 'love' ends up being the only consolation for human survival in the midst of the creepers of corruption that have been watered by unbridled ambition. Can the 'weak stem of love' strengthen to a point where in a potent, unadulterated, unselfish love could solve the problem? By highlighting the sickness, and stating that there is a glimmer of hope in love, Bhagat seems to remind his readers that true love will love their child unconditionally. He seems to go on further by adding that true love for friends and country will be willing to sacrifice. The 'weak stem of love' can be strengthened to uproot evils of unethical behavior, corruption and other criminal tendencies by the young person.

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RESEARCH ARTICLE

PHYTOCHEMICAL SCREENING AND FTIR ANALYSIS OF ETHANOLIC STEM EXTRACT OF VINCETOXICUM SUBRAMANII (A.N.HENRY) MAVE & LIEDE

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ABSTRACT

Medicinal plants have been used in the treatment of various diseases as they possess potential pharmacological activities including antineoplastic, antimicrobial, antioxidant, anti-inflammatory, analgesics, anti-diabetic, anti-hypertensive, antidiarrheal and other activities. There is continuous and urgent need to discover new active biological compounds with diverse chemical structures and novel mechanism of action because there has been an alarming increase in the incidence of new and re-emerging infectious diseases. An active compound of the medicinal plant has become a promising acquaintance in the development of phytomedicine to combat various diseases or disorder. The present investigation was carried out to assess the qualitative phytochemical analysis of stem of *Vincetoxicum subramanii* The phytochemical screening of stem extracts revealed the presence of steroids, saponins, alkaloids, flavonoids, glycosides, phenolic compounds, tannins and terpenoids in ethanolic extracts. The major functional group present in this plant was determined by FTIR analysis showed the existence of functional groups such as alkanes, aromatic compound, aromatics, carboxylic acid, phenol, aromatics ester and alkene compounds.

Keywords: *Vincetoxicum subramanii, Tylophora subramanii,* Functional group, FTIR, Stem extract.

1. INTRODUCTION

Phytomedicine, the art of using plants to treat diseases in known from time immemorial. Right from the first health problem encountered by early human and until the recent corona viral infections that occurred as a pandemic, plants are one of the important resource's humans seek for medicine and treatment, the plants have provided many renowned drugs that act as life saviour at various situations to encounter the health problems. Apparently, there is a growing concern in the field of phytomedicine to develop the insights of the therapeutic potential of traditionally used and economically underused plants. Herbal interventions are much demanded in the globalized world since the existing conventional therapies and their synthetic drugs are of high cost and provide only symptomatic reliefs associated with detrimental side effects (Srivastava *et al.,* 2019). Consequently, the need and utility of the plant-based drugs and phytotherapeutics have increased considerably.

The current study focuses on evaluating the phytochemical constituents and exploring the FTIR profile of *Vincetoxicum subramanii* stem extract. The plant *Vincetoxicum subramanii* belongs to the family apocynaceae. The genus *Vincetoxicum* N.M. Wolf. (Apocynaceae) comprises nearly 100 species which are distributed throughout Asia, Europe, Japan and North America. Leaves, rhizome and dry seeds of *Vincetoxicum* species have various usages in folk medicine due to medicinal purposes.

Tylophora subramanii is the synonym name for that plant. It is a native plant of southern India commonly found in evergreen forest areas of Theni, Tirunelveli and Kanyakumari districts of Tamil Nādu up to 1200 m elevation (Ravichandran *et al*.,

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2016). The plant have been used for treating various diseases like asthma, leukorrhea, dysentery, fever and headache (Vimalpriya *et al.,* 2022). The plant is used to cure nervous disorders among Kani tribe community of Agastiyamalai hills in Tamil Nadu. The plant is having watery latex in all over the body to have a number of secondary metabolites and high hydrocarbon content. Hence the present study was aimed to identify the bioactive phytocompounds present in the ethanol stem extract of such a medicinally important herb *Vincetoxicum subramanii*

1. PLANT DESCRIPTION

Habit: Climbing Undershrub

Leaves: Leaves simple, opposite-decussate; lamina ovate or ovate oblong (10-14 cm L and 8-10 cm b)

Flower: Monoecious, Flowers in axillary or lateral, umbellate cymes;

Fig. 1. Habit of *Vincetoxicum subramanii*
a) Flowering twig, b) Inflorescence,
c) Folicle fruit

Calyx : 5- lobed, lobes lanceolate, glandular at base.

Corolla: - reddish-brown, ovate-deltoid, rounded at base, shorter than the staminal column.

Anthers: erect, with small inflexed membranous appendages.

Ovary : bicarpellate, many-ovuled;

Style : apex pentagonal, flat.

Fruit: Follicles

Habitat: Rare; in edges and openings of evergreen forests.

Flowering: July - October.

Distribution: India (Kerala & Tamil Nadu)- Endemic.

Classification

Kingdom: Plantae

Class: Dicotyledonae

Subclass: Gamopetale

Order: Gentianales

Family: Apocynaceae

Genus: Vincetoxicum

Species: *Vincetoxicum subramanii*

Common name: Subramani's Ipecac

Synonym: Tylophora subramanii

3. MATERIALS AND METHODS

3.1. Collection of Plant Material

Vincetoxicum subramanii was collected from Megamalai Wildlife Sanctuary of Theni district, Tamil Nadu, India. Plant specimen was identified by Dr. Ravichandran. Senior Preservation Assistant, Botanical Survey of India, Southern Regional Centre, Coimbatore.

3.2. Preparation of Plant Extracts

The shaded dried leafs were powdered in the medical grinder. 50 grams of leaf powder was weighed, 500 ml of different solvents (hexane, chloroform, acetone, ethanol and distilled water) used for soxhlet and Maceration extraction. The solvents were then evaporated under reduced pressure and died using a rotary evaporator at 55℃. Dried extracts were stored in labelled sterile flasks at 5℃ in the refrigerator, until when required for use (Karthika *et al*., 2021).

3.3. Qualitative screening of Phytochemical Compounds

Plants are the resource of primary and secondary metabolites namely alkaloids, terpenoids, flavonoids, saponins, coumarins, glycosides, phenolics, carboxylic acids, amino acids, sugars, proteins etc. these phytochemicals have significant biological functions and also which contribute specific characteristic and property of the plant (Shyam Praveen., *et al.,* 2022). Here Preliminary qualitative phytochemical screening was carried out with the following methods.

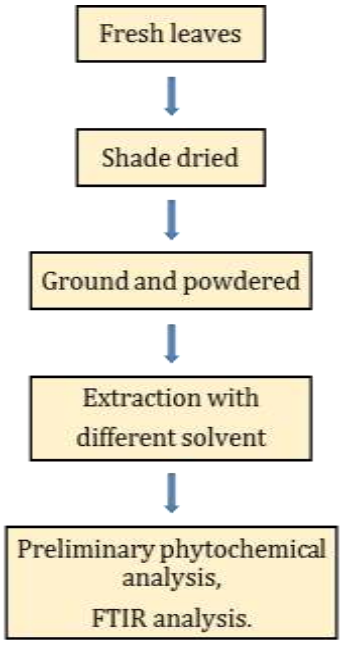

Fig. 2. Schematic representation of extraction processes

3.3.1 Test for alkaloids

a) Dragendorff test: To 2-3mL of each extract, add few drops of Dragendorff reagent. Formation of orange brown precipitate indicates the presence of alkaloids.

b) Mayer's test: To 2-3mL of each extract was added with few drops of Mayer‟s reagent. Formation of white precipitate indicates the presence of alkaloids.

3.3.2 Test for flavonoids

Shinoda test: To 2-3mL of each extract, few fragments of magnesium metal were added in a separate test tube followed by dropwise addition of conc. HCl. Formation of magenta colour indicated the presence of flavonoids (George *et al.,* 2015).

3.3.3 Test for glycosides

a) Keller-Kiliani Test: To 2mL of extract, glacial acetic acid, one drop of 5% FeCl3 and conc. H2SO4 was added. Reddish brown colour appears at junction of the two liquid layers and upper layer appears bluish green colour indicates the presence of glycosides. 5.2.3.4. Test for phenols

b) Ferric chloride test: When 0.5mL of FeCl3 solution was added to 2mL of test solution, formation of a dark violet colour indicated the presence of phenols.

3.3.4 Test for saponins

a) Foam test: 1mL of each extract was taken in separate test tubes and to this 5mL of distilled water was added. Then this mixture was shaken vigorously. A persistent froth that lasted for at least 15min indicates the presence of saponins (Kalawole *et al.,* 2006).

3.3.5 Test for steroids

a) Liebermann-Burchard Test: 2mL of each extract was mixed with chloroform. Added 1-2mL of acetic anhydride and 2 drops of conc. H2SO4 from the side of the test tube. Formation of brown ring at the interface of the two supernatant layers indicates the presence of steroids.

3.3.6 Test for tannins

a) Braemer's test: 2mL of each extract was diluted with distilled water followed by the addition of 2-3 drops of 5% ferric chloride solution. Indication of green-black or blueblack coloration showed the presence of tannins.

3.3.7 Test for terpenoids

a) Salkowski test: 2mL of chloroform and conc. H_2SO_4 were added to 1mL of each extract. Appearance of reddish-brown colour indicates the presence of terpenoids.

b) Copper acetate test: To 1mL of extract, few drops of copper acetate were added. Formation of green colour indicates the presence of terpenoids.

3.3.8 Test for Triterpenoids:

2 ml of test solution, added a piece of tin and 2 drops of thionyl chloride. The result was observed (Kolawole *et a*l., 2006).

3.3.9 Test for Phenols:

2 ml of aqueous extract is added to 2 ml of 2N Hcl and ammonia. The appearance of pink-red turns blue-violet indicates the presence of phenols (Harborne, 1973)

3.3.10 Test for Coumarins:

3 ml of 10% NaOH was added to 2 ml of aqueous extract formation of yellow colour indicates the presence of coumarins (Harborne, 1973).

3.3.11 Test for fixed oils and fats

a) Small quantity of the extracts was separately pressed between two filter papers oil stain on the paper indicates the presence of fixed oil.

b) The extract was diluted with 20 ml of distilled water and it was agitated on a graduated cylinder for 15 minutes. The presence of saponins was indicated by formation of 1 cm layer of foam.

3.3.12 Test for gums and mucilage's

About 10 ml of the extracts was added to 25 ml of absolute alcohol with stirring and filtered. The precipitate was dried in air and examined for its swelling properties and for the presence of carbohydrates

3.4.13 Test for volatile oil

0.1 ml of NaOH was added to 2ml of extract then add diluted Hcl shaken the formation of white precipitate indicate the presence of volatile oil.

3.3.14 Test for protein and amino acid

a) Ninhydrin test: Two drops of ninhydrin solution (10 mg of ninhydrin in 200 mL of acetone) were added to 2 mL of aqueous filtrate. A characteristic of purple colour indicates the presence of amino acid

b) Millon's test: To 2 mL of filtrate, few drops of Millon's reagent were added. A yellow precipitate indicates the presence of amino acid

c) Biuret test: 2ml of extract with few drops of 2% of copper sulphate solution, add 1 ml of ethanol followed by excess of potassium hydroxide pellets, formation of pink colour in the extract layer indicates the presence of protein.

3.3.15 Test for carbohydrates

a) Molisch's test To 2 mL of filtrate, two drops of alcoholic solution of α-naphthol were added, the mixture was shaken well and 1 mL of concentrated sulphuric acid was added slowly along the sides of the test tube and allowed to stand. Appearance of a violet colour ring indicates the presence of carbohydrates.

b) Barfoed's test To 1 mL of filtrate, 1 mL of Barfoed's reagent (copper acetate in glacial acetic acid) was added and heated on a boiling water bath for 2 min, formation of red precipitate indicates the presence of carbohydrates.

3.4. FTIR analysis

It is a valuable device for the identification and characterization of functional groups (chemical bonds) present in the compound. Besides, FTIR spectra are unique that they are like a molecular "fingerprint". (Rakhi *et al*., 2018) The drop forms a thin film between the cells. Solid samples can be milled with potassium bromide (KBr) and then compressed into a thin pellet using a hydraulic press, which was then used for the analysis (Rani, *et al*., 2021). The samples of *Vincetoxicum subramanii* ethanolic leaf extract was treated for FTIR spectroscopy IR-Affinity (Shimadzu, Japan). The samples were run at an infrared region between 1000 nm and 4000 nm and standard DLATGS detector was used at 2.8 mm/sec mirror speed.

4. **RESULT AND DISCUSSION**

4.1. Extractive Yield Percentage

The yield of sequential extracts (%) is shown in Table-1.

NAME OF THE SOLVENT USED	STEM	
	Colour of extract	Percentage yield (%w/w)
Hexane	Light Green	4.04%
Chloroform	Green	2.44%
Acetone	Dark green	1.96%
Ethanol	Dark yellowish Brown	7.36%
Water	Brown	3.57%

4.2. Phytochemical analysis

Phytochemical screening of the sequential extract of *Vincetoxicum subramanii* revealed the presence of various bioactive components of which phenolics, saponins, alkaloids, tannin, Glycosides, Proteins, Carbohydrates, and Amino acids are the most prominent components and the result of phytochemical test given in the Table 2.

Among these phytochemical tests, Alkaloids, were present in all solvent extracts. whereas most of the active compound are alkaloid, flavonoid, Glycosides, Tannin, Phenols are present in the ethanolic extract of plant material.

Table 2. Qualitative phytochemical analysis of the different extracts of *Vincetoxicum subramanii* stem

S. No	PHYTOCHEMICALS	STEM EXTRACT					
		HEX	CHL	ACE	ETH	WAT	HEX
1.	ALKALOID	+	++	++	+++	+	-
2.	FLAVANOID	-	+	+	+++	+	-
3.	STEROID	-	-	-	+	+	-
4.	TERPENOID	++	-	+	-	-	+
5.	TRITERPENOID	+	-	-	-	-	+
6.	TANNIN	-	++	++	+++	++	-
7.	PHENOL	-	+	+	++	+	-
8.	COUMARIN	-	-	-	-	-	-
9.	GLYCOSIDES	+	+	-	+++	+	+
10.	SAPONIN	-	-	-	-	+	-
11.	GUMS AND MUCILAGE	+++	+	-	-	-	+++
12.	VOLATILE OIL	+	+	-	-	-	-
13.	FIXED OIL	+	-	-	-	-	+
14.	CARBOHYDRATE	+	+	+	++	++	-
15.	PROTEIN	-	-	+	++	+	-
16.	AMINO ACID	-	+	+	++	++	-

(+++ abundant; ++ moderately present: +weakly present; -----absent, HEX-Hexane, CHL-Chloroform, ACE-Acetone, ETH-Ethanol, WAT-Water).

4.3. FTIR analysis

The FTIR spectrum was used to identify the functional groups of the active components present in the extract based on the peak values in the region of IR radiation. When the extracts were passed into the FTIR, the functional groups of the components were separated based on its peak ratio. Figure 3 and Table-3 reveals its functional groups present in Ethanolic stem extracts of *Vincetoxicum subramanii* and its peak are separated based on the IR absorption. The results are confirmed by the presence of the amine, alcohol, alkene, tertiary alcohol and halogen compounds

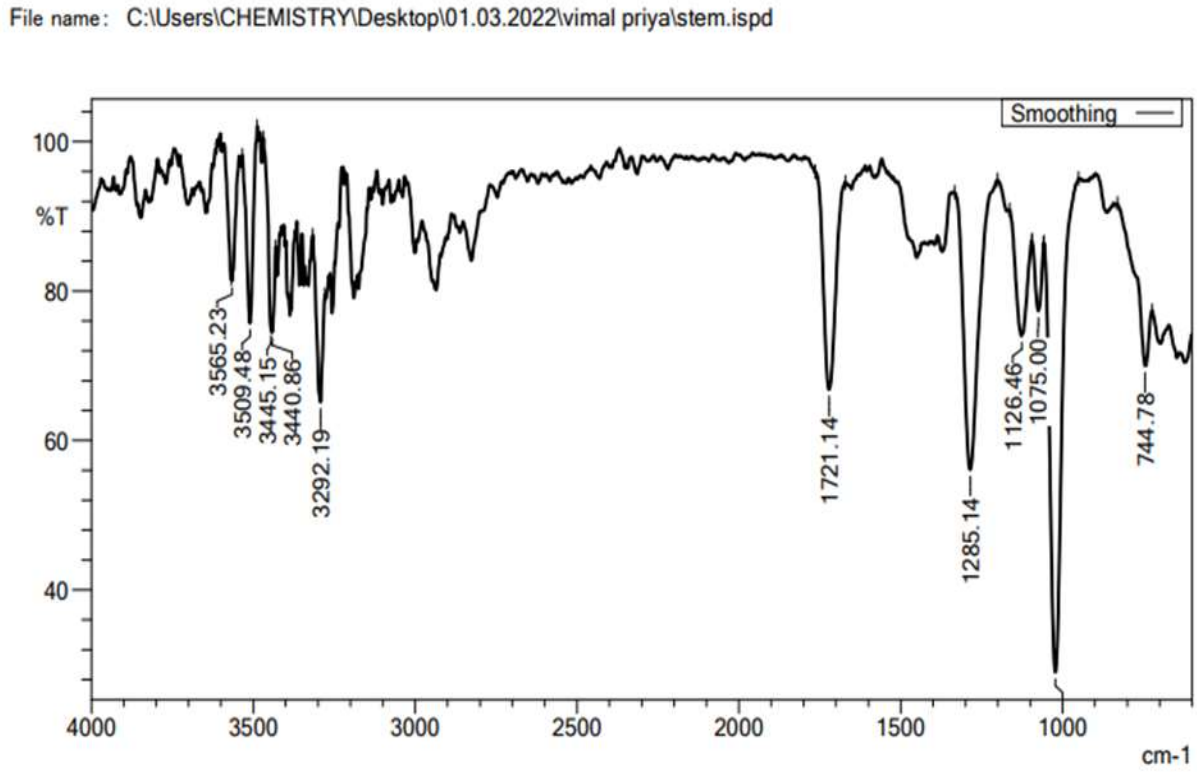

Fig. 3. FTIR spectrum of Ethanol extracts of *Vincetoxicum subramanii* stem

The FTIR analysis revealed the presence of polyphenols and flavonoids due to O-H stretching, terpens due to C-H group. The functional groups present in test plant are aldehydes, alkenes, amine, amides, alcohols, phenols, aromatics, carboxylic acids and anhydride, esters and lactones, ethers and organic halogen compounds. These were confirmed by FT-IR spectrophotometer study that predicted the presence of the groups: O-H, C-H, C-H and 'Oop" stretching it confirms the presence of Aromatic compounds. The presence of characteristic functional groups of carboxylic acids, anhydrides, alcohols, phenols, amines, amides, esters, ethers, sulphur derivatives, glycosides, nitrates, nitriles, iso nitriles, organic halogens and carbohydrate could be responsible for the various medicinal properties of *Vincetoxicum subramani.*

Table 3. Ethanolic stem extract FTIR interpretation of compounds

S. No	Standard (nm)	Wave number (cm^2)	Bond	Functional group	Phytocompound Identified
1	-	3565.23 3569.48	-	-	Unknown
2	3550-3200	3445.15 3440.86 3292.19	Single bond stretching	O-H stretching	Alcohol
3	2000-1650	1721.14	Triple bonds	C-H stretching	Aromatic compound
4	1310-1250	1285.14	Fingerprint region skeletal vibration	C-O-stretching	Aromatic ester
5	1205-1124	1126.46	Fingerprint region skeletal vibration	C-O-stretching	Tertiary alcohol
6	1085-1050	1075-00	Fingerprint region skeletal vibration	C-O-stretching	Primary alcohol
7.	900-675	744.78	Fingerprint region skeletal vibration	C-H 'Oop"	Aromatics

5. CONCLUSION

Researchers have spent tremendous amount of time and resource to find the importance of medicinal plants. Each and every plants contain special compounds which helpful in various pharmacological purposes. The investigation of our present study is a preliminary screening of *Vincetoxicum subramanii* as a rich source of secondary metabolites. The active compound detection of ethanolic extracts of stem was done under FTIR will act as Pharmacognostic marker to distinguish the medicinally important *Vincetoxicum subramanii* species this spectrocopic techinque is relatively simple, cost effective and can be use full to easily detect functional groups. The results of present study is a way to predict and compare the phytoconstituents present in this plant with other bioactive medicinally important plants. The ethanolic extract of *Vincetoxicum subramanii* contain significant amounts of phenolics and flavonoids. Phenolics and flavonoids are ubiquitously seen in most of the plant species and reported to possess a broad spectrum of biological properties. The plant shows the presence of many chemical constituents which are responsible for varied pharmacological and medicinal property. Further the bioactive compounds need to be isolated and the structure of the compounds can be determined by using advanced analytical techniques such as Mass and NMR Spectrophotometers.

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RESEARCH ARTICLE

MOLECULAR CHARACTERIZATION AND OPTIMIZATION OF *STREPTOMYCES ALBOGRISEOLOUS* NGP2 AND ITS PROSPECTS ON HEAVY METALS DEGRADATION

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ABSTRACT

The biosorbing ability of actinomycetes from the marine sediments of South Indian Coastal region, Chennai, Tamilnadu, India was examined and identified as *Streptomyces albogriseolous* NGP 2 (JX843531) by 16S rRNA gene sequence analysis. The isolate showed the potential metal removing capacity by incubating it with various concentrations of heavy metals such as Copper (Cu), Zinc (Zn), Chromium (Cr) and Cadmium (Cd). Simultaneously, various optimization properties such as pH, initial metal concentration and contact time were analyzed. The pH 7.0 was recorded as an optimum for the removal of Cr and Zn, for Cd and Cu it was recorded as 6.0. The biosorption of the heavy metals Cu and Zn by the strain was found to be highest and recorded as 92% and 90.6% respectively, while the lowest sorption was found for Cd (85 %) at 30 mg/l concentration. The effectiveness of the removal of heavy metals was best studied by Langmuir and Freundlich adsorption isotherm models. The regression Coefficients also significantly expressed in the removal process. The results revealed that the isolate has the potential capability for the removal of heavy metals.

Keywords: Actinomycetes, Biosorption, Langmuir and Freundlich, Optimization properties

1. INTRODUCTION

The waste water from the mines and metal refineries contains hazardous heavy metal ions which cause serious illness to the human as well as environment's health and wealth[7]. Based on the statistical report, India generates 62 million tonnes of waste per year and emits greenhouse gases at maximum limit[23]. Four major industries such as fertilizers, tannery, pesticide and chemical industries are responsible for causing more pollution. The discharged effluents after treatment also contains highest amount of heavy metals[22]. The heavy metals are arsenic, cadmium, chromium, copper, lead, nickel and zinc which interfere with our metabolic process and accumulate in body[14]. A number of conventional waste water treatment techniques were employed for the removal of pollution such as chemical coagulation, adsorption and activated sludge[6]. With this backdrop, the effective alternate method of removal mechanism achieved by microorganism as biosorbent[15]. Biosorption was done with various microorganisms such as bacteria, fungi, bio film, algae, genetically engineered microbe and immobilized microbial cell. This process highly depends on the optical structure of the cell wall which found as primary mechanism[25]. Among them actinomycetes specifically *Streptomyces* are of much more interest because of their ability to survive in heavy metal contaminated sites through the production of metal ion chelators such as Siderophores[18] also they have special feature of stability.

For the better understanding of absorption mechanism, Langmuir and Freundlich equations widely used. The Langmuir model suspects that without any interaction between adsorbed ions on a homogenous surface leads to the adsorption of metal ions. The adsorption proceeds until a complete monolayer formed[20]. The Freundlich model fully based on adsorption on a heterogeneous

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surface[9]. It has been broadly perceived and affirmed that its intra particle mass exchange rate which speaks to the bottleneck and in controlling the rate of whole sorption process. Finding of molecule size and its structures are hence extremely critical. Models have been produced to decide both the quantity of adsorption destinations required to tie each metal particle and the rate of adsorption, utilizing a batch reactor mass balance and the Langmuir hypothesis of adsorption to surfaces or continuous dynamic systems[18].

The aim of the present study was to investigate the biosorptive potential of *S. albogriseolous* NGP 2 and effects on various factors such as metal concentrations, contact time and pH through Langmuir equation.

2. MATERIALS AND METHODS

2.1. Collection and isolation of actinomycetes

The marine sediment was collected from the coastal region of marine, Chennai, Tamilnadu, India located at 13.05^0 N 80.28^0 E and 2-3 depth by using grab sampler. The collected sediment was serially diluted and spread over starch casein Agar (SCA) plates and incubated at 28 + 2^0 C for 7 days[3]. After incubation six actinomycetes were isolated and tested for heavy metal tolerance.

2.2. Selection of heavy metal tolerant strains

To determine metal tolerance actinomycete strains were aseptically streaked on SCA plates supplemented with 100-2000 µg/ml of copper sulphate ($CuSO_4.5H_2O$), zinc sulphate ($ZnSO_4$), potassium dichromate ($K_2\ Cr_2\ O_7$), cadmium sulphate ($CdSO_4$) and checked for growth after incubation at 28±2^0C for 72h with shaker (160r/min). After incubation the growth was detected by measuring the optical density at 600nm. The maximum tolerance level (MTL) was described as the highest concentration of individual metal supporting growth of actinomycetes.

2.3. Biochemical characterization of actinomycetes

Among six actinomycetes, strain NGP 2 showing the highest MTL values were selected and characterized morphologically and biochemically. Properties of the strain NGP 2 that included gram's reaction, methyl red test, voges – proskauer test, caseinase test, cellulase test, deaminase test, sugar fermentation, nitrate reduction test, gelatinase test were determined by the standard method given in Bergey's manual of determinative bacteriology[12].

2.4. 16 s rRNA identification

The partial sequencing of 16s rRNA gene chain of the strain NGP 2 was carried out by automated DNA sequence model ABI 3100 according to the protocol provided by the manufactures (ABI PRISM 3100 Genetic analyzer user's manual) using universal primers, oligo 1F (5'-GAG TTTGATCCTGGCTCAG - 3'). Later, nucleotide sequence data was deposited in the Gen-Bank. The online program BLASTn was used to find out the related sequences with known taxonomic information in the databank at NCBI website (http//www.ncbi.nlm.nih.gov/BLAST) to accurately identify the strain NGP 2[24].

2.5. Biomass production

Biomass of *S. albogriseolous* NGP 2 was produced by growing actinomycete culture in starch casein broth (pH 7.0) at 28± 2^0 C for 72 h. Cells were harvested by centrifugation at 6000 rpm for 20 min. Dry biomass prepared by vacuum oven drying at 90^0 C and then used for biosorption studies.

2.6. Metal solutions

A stock solution of $CuSO_4.5H_2O$, $ZnSO_4$, $K_2Cr_2O_7$, and $CdSO_4$ was prepared in fixed volume (100 mg/ml) of single metal ion solution in 100 ml conical flask by dissolving appropriate quantities of pure metal powders in 1% nitric acid.

2.7. Metal sorption

A batch equilibrium method was used to determine the sorption of copper, zinc, chromium and cadmium by *S. albogriseolous* NGP 2[13]. The strain was exposed to metal solution for 72h on an orbital shaker 160 rpm and centrifugation at 6000rpm for 15 min and the supernatant was analysed for residual metal concentration by flame atomic absorption spectrophotometer. Measurement of metal uptake, the amount of metal

bound by the biosorbent was calculated by $Q = V(Ci - Cf)/M$. Where, Q = Metal ion uptake capacity (mg/g), V = Solution volume (ml), Ci = Initial concentration of the metal in solution (mg/g), Cf = Final concentration of the metal in solution (mg/g), M = Dry weight of biosorbent (mg). Sorption models were chosen for comparison with experimental data.

The Langmuir model, $Q = Qax\,bCf / 1 + bCf$, in which Qmax = the maximum metal uptake under the given conditions, b = a constant related to the affinity between the biosorbent and sorbate.

The Freundlich model, $Q = kCf\,(1/n)$. K and n are Freundlich constants, which correlated to the maximum absorption capacity and adsorption intensity respectively.

2.8. Initial metal concentration

To examine the effect of initial metal concentration, the experiments were performed at different initial metal concentrations such as 30, 60, 90, 120 and 150 mg/l by using 100mg dried biomass of *S. albogriseolous* NGP2 incubated for 60 min on orbital shaking incubator at 160r/min [10].

2.9. Contact time on biosorption

To determine the equilibrium time required for biosorption was performed using 100 mg cell biomass from the initial metal concentration of each metal ion in 100 ml of metal solution. The metal solutions were taken at the desired intervals (from 0 to 60 min) and subsequently centrifuged at 6000 rpm for 10 min. The heavy metal concentration in the supernatant was analysed by flame atomic absorption spectroscopy[18]

2.10. pH on biosorption

In order to evaluate the impact of pH on biosorption, NGP2 strain was subjected to different pH (varying between 2 and 10) then allowed to contact with metal solutions of the corresponding pH 2,4,6,8 and 10. For contact time analysis, 10 ml of cell suspension (10 mg of dry cell biomass) was mixed with 100 ml aliquots of metal solutions in a 250 ml Erlenmerfer flask with appropriate controls. Flasks were incubated on an orbital shaker incubator (120 rpm) at 32±2°C. Samples of metal solutions were removed from each flask at different time intervals (0-72h) and were analysed for residual metal content.

3. RESULTS AND DISCUSSION

3.1. Screening and selection of heavy metal tolerant bacteria

In this study, total of six strains able to grow in the presence of toxic metals (Cu, Zn, Cr and Cd) on SCA medium. These strains grown in nutrient broth amended with varying concentrations of different heavy metals showed a variable tolerance level to the tested metals. Strain NGP2 was selected due to high degree of metal tolerance, ability to produce significantly higher amounts of biomass (Figure 1). Among various metals with different concentrations, strain NGP2 could survive at 1300, 1200, 800 and 600 mg/l of Cu, Zn, Cr and Cd respectively. In addition, varied growth behaviour of strain NGP2 was observed when it was grown in nutrient broth treated with fixed concentration at 100 mg/ml of each metal (Figure 2).

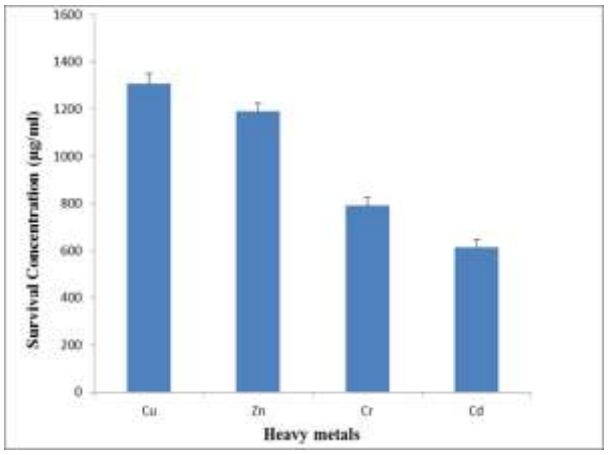

Fig. 1. Heavy metal tolerant pattern of strain NGP 2. *Streptomyces* was inoculated with different concentrations of heavy metals and analyzed its survival parameters

All the heavy metals were absorbed by the tested strain effectively. Cu and Zn were removed at 45h, whereas Cr and Cd were removed at 35 and 25h respectively. Similar to our study, 40 actinomycetes isolated from high metal content soils

in Iran, showed highly resistance to the heavy metals. Among them, 13 isolates were selected as high resistant actinomycetes that showed resistance to 140 mM $ZnCl_2$, 7 mM $CuSO_4$, 9.2 mM $CdCl_2$, and 60 mM $NiCl_2$[10]. The bioremediation efficiency against metals are as follows Fe>Cu>Ba>Cd (90%, 71%, 52% and 19% respectively) by the bacteria *Bacillus pumilus* isolated from water of Karnataka mangrove region[8].

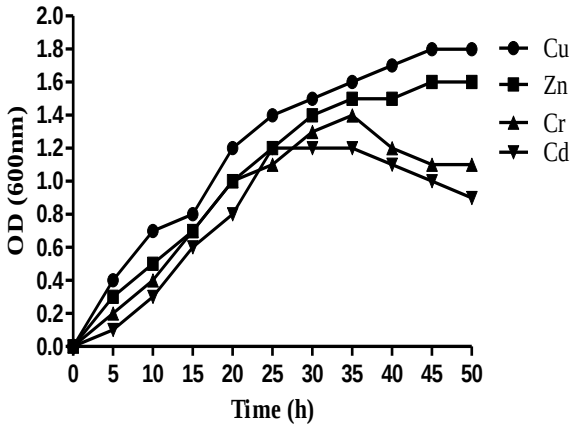

Fig. 2. Growth pattern of the strain NGP 2 at 100 mg/ml of metal.

3.2. Molecular identification of the strain NGP2

The selected strain was characterized and identified by using standard morphological, and biochemical tests (Table 1). On the basis of the characteristics observed for strain NGP2 and compared with those listed in Bergey's manual of determinative Bacteriology, presumptively identified as *Streptomyces Sp*[31]. For the further validation, the strain was subjected to 16srRNA gene sequence analysis[17]. The sequence was submitted to Gen-Bank (JX843531). A phylogenetic tree constructed by MEGA 4 software based on 16srRNA partial sequence. The metagenomics of actinomycetes based 16SrRNA and Genbank database and the results were showed that 17 bands were closely related with five genera of actinomycetes[16].

Table 1. Morphological and Biochemical characteristics of the strain NGP 2

Tests Performed	Characteristic Observed
Accession number	JX843531
Morphology	
Colony Color	White
Colony Shape	Cocci
Pigment	Nil
Biochemical Test	
Gram Staining	+ ve
Methyl Red Test	+ve
Voges-proskauer Test	+ve
Caesinase Test	+ve
Cellulase Test	+ve
Deaminase Test	-ve
Sugar Fermentation	+ve
Nitrate Reduction Test	-ve
Gelutinase Test	+ve

3.3. Biosorption profile

The Langmuir and Freundlich isotherms of heavy metals biosorption by the biomass of strain *S. albogriseolous* NGP 2 are presented in table 2.

The Langmuir and Freundlich absorption constants were evaluated from the isotherms with correlation coefficients (>0.98). Both models described a better absorption process as indicated by correlation coefficient (r^2)[35]. In the Langmuir isotherm *b* is a Langmuir constant related to energy of sorption. If value of *b* is higher than the affinity of biosorbent is enhanced for metal ions. In this study, the Langmuir constant *b* value was highest for Cu (0.143) and lowest for Cd (0.035). According to the *b* value observed here, the biomass could absorb the metal ions in the order: Cu > Cr > Zn > Cd.

Adsorption partition constant of metals were further determined by Freundlich isotherm where *K* and *n* are constants. *K* is the degree of adsorption. Conceptually, when *K* value is low it indicates minimal adsorption of heavy metals whereas the higher *K* value suggests greater sorption ability[36]. In the study, *K* value was highest for Cu (3.559) and it was lowest for Cd (2.322). The value of 1/*n* was lowest for Cu (0.693) but was highest for Cd (0.794) suggesting maximum

biosorption of Cu and least of Cd. All values favoured Freundlich isotherm and correspondingly the order of adsorption were similar to Langmuir isotherm. Activated Teff Straw (ATS) was studied for the biosorption of heavy metals such as Cr, Cd, Pb, Ni and Cu. The adsorption isotherm was checked with Langmuir and it could be fitted well. The R_T value was less than one, indicating that the adsorption of the metal ion onto ATS is favourable[5]. Similarly, the removal of nickel ions from aqueous solutions using carboxymethyl cellulose-graft-poly (acrylic acid) CMC-g-PAA hydrogel as an adsorbent was studied[33]. Isotherm for the adsorption of nickel on CMC-g-PAA hydrogel was developed and the equilibrium data fitted to the Langmuir and Freundlich isotherm models[2]. Batch adsorption experiment was carried out on the targeted metal ions Cu (II), Co (II) and the results were analyzed by the Langmuir and Freundlich equation at different concentrations (100–1000 mg/l) and the characteristic parameters for each adsorption isotherm were determined[29]. In another study, polypyrrole-based activated carbon was prepared by adsorption of lead (II) from aquous phase solution and analyzed initial ion concentration, pH, contact time, and adsorbent dose. The Freundlich isotherm equation (R2 = 0.9950) calculated maximum capacity, qm, determined from the Langmuir model was 50 mg/g[30].

Table 2. Langmuir and Freundlich isotherm parameter

Metal	Langmuir Parameters			Freundlich Parameter		
	Qmax	b	r^2	k	1/n	r^2
Cu	41.49	0.143	0.98	3.559	0.693	0.99
Zn	32.28	0.044	0.96	2.477	0.759	0.98
Cr	75.51	0.061	0.97	2.992	0.702	0.98
Cd	52.02	0.035	0.95	2.322	0.794	0.96

3.4. Effect of initial metal concentration, contact time and pH on biosorption

The effect of initial metal concentration on metal biosorption of *S. albogriseolus* NGP2 was evaluated under reaction condition, set at pH 6 and 30±2°C for equilibrium time half an hour as shown in figure 3. Here, it was observed that the rate of biosorption decreased with increased metal ion concentration[32]. The maximum biosorption of metal was recovered at a low initial metal ion concentration for example it was 92% for Cu at 30 mg/l, while it was 82.7% at 150 mg/l. A trend similar to Cu was also observed for other metals. At lower concentrations, all metal ions present in the solution, interact with the binding sites than at higher ion concentration. Similar results have been reported by others. *Gemella sp*., *Micrococcus sp*. and *Hafnia sp* isolated from Chittagong city, Bangladesh. Among them *Gemella sp*. and *Micrococcus sp*. showed resistance to Lead (Pb), chromium (Cr) and cadmium (Cd), where *Hafnia sp.* showed sensitivity to cadmium (Cd). Degrading potentiality was assessed using Atomic Absorption Spectrophotometer where *Gemella sp*. and *Micrococcus sp*. showed 55.16 ± 0.06% and 36.55 ± 0.01% reduction of Pb respectively. On the other hand, moderate degradation of Cd was shown by *Gemella sp*. (50.99 ± 0.01%) and Micrococcus sp. (38.64 ± 0.06%). The above results corroborate with other study that, 86 % removal of Cd from medium within 24h by *Enterobacter Cloacae*[11]. Another report suggests that, 29 % of Cd was removed by *E. Cloacae* bacteria isolated from tobacco[21]. Rhizophere microbes play a vital role in phytoremediation by siderphore production, acidification, releasing plant growth harmone and through redox changes[26]. Heavymetal uptake capacity was reported as 99% of Pb and Cr containing 10mg Pb and Cr in the medium, 77% of Cd was absorbed in the medium containing 10mg of Cd by *A. flavus* and *R. pusillus* [28]

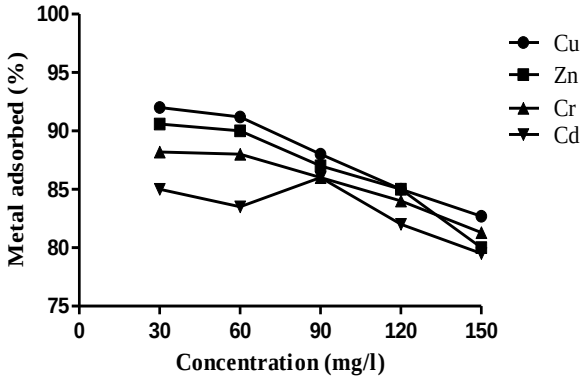

Fig. 3. Effect of initial metal ion concentration on biosorption of the strain NGP 2.

The contact time is one of the important factors of biosorption process. The biosorption of Cu, Zn, Cr and Cd by actinomycete biomass is shown in figure 4. In the experiment, the initial sorption rate was highest, after 30 min, the order of biosorption rate was Cu>Zn>Cr>Cd. Likewise, the heavy metals effect on *Rhodococcus opacus* biomass growth is accordingly, Cd > Ni > Pb > Cu > Zn > Fe. The total time for biodegradation increased from 144 to 216 h in the presence of Fe, Zn, Cu, or Pb, and it was up to 240 h in the presence of Cd or Ni[1]. It is reported in another work, the effect of contact time on the biosorption uptake capacity of Zn(II), Co(II) and Cd(II) ions onto *A. niger* at the initial concentration of 10 mg/l, biomass dosage of 2 g/l and at pH 5 [27]

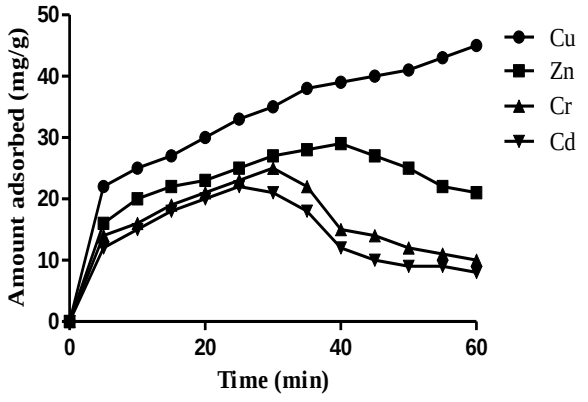

Fig. 4. Biosorption of heavy metals by the strain NGP 2 over the reaction time

pH plays vital role in the biosorption process. The experiment was carried out with varying pH to evaluate their effect on biosorption capacity of microbial biomass using a fixed concentration of Cu, Zn, Cr and Cd (Figure 5). The biosorption capacities for each metal ion increased with an increase in pH. The optimum pH for Cr and Zn removal was 7, while for Cd and Cu it was 6. According to the work of[4], the optimum pH for adsorption of Cr (VI) ranged from 1 to 3, using carboxymethyl cellulose-based hydrogel as adsorbent. Most of the living organisms have been shown to absorb heavy metals such as Cd and Cu at a low pH, due to their physiological properties[19].

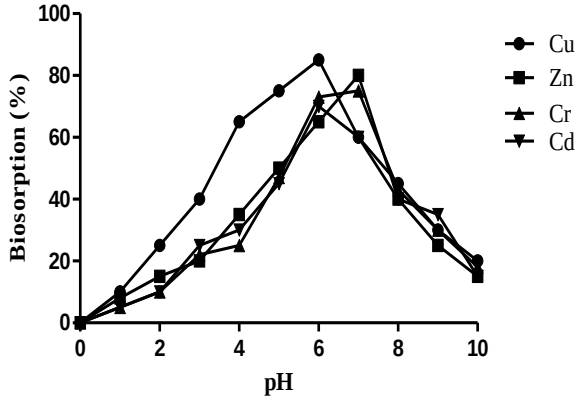

Fig. 5. Effect of pH on biosorption of the strain NGP 2 at different pH

4. Conclusion

The present research aimed at removal of heavy metals such as copper, zinc, chromium and cadmium at different concentration by *S.albogriseolus* NGP2. The optimum pH for chromium and zinc removal was pH 7, while for cadmium and copper it was 6 and the optimal contact time was 60 min for each metal by strain. Biosorption data fitted well with the Langmuir and Freundlich adsorption isotherm equations and indicated sufficient biosorption by the test strain at varying metal ion concentration. This study validates that the biomass of *S. albogriseolus* NGP2 could be used as an inexpensive and highly efficient reliable biosorbing bio-agent for effectively removing heavy metals.

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RESEARCH ARTICLE

SCREENING OF PHYTOCHEMICAL CONSTITUENTS AND QUANTITATIVE ESTIMATION OF TOTAL FLAVONOIDS AND PHENOLIC COMPOUNDS OF LEAFEXTRACTS OF *MITRACARPUS HIRTUS* (RUBIACEAE)

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ABSTRACT

Plants have the ability to synthesize mixtures of structurally diverse bio-active compound, with multiple and mutually potential therapeutic effects. The objective of the study is to cover the preliminary phytochemical screening of traditional medicinal plant *Mitracarpus hirtus* belonging to the family Rubiaceae. The preliminary screening revealed the presence of alkaloids, flavonoids, steroids, tannins, phenolics, glycosides, carbohydrates, proteins and amino acids. In quantitative estimation, among all the extracts, acetone extract exhibited the maximum amount of phenolics (40.26 mg GAE/g extract), and it depicted the maximum quantity of flavonoids (84.43 mg RE /g extract) which explains that the plant must have valuable medicinal properties and so it can be explored.

Keywords: *Mitracarpus hirtus,* Phytochemical analysis, Phenols, Rubiaceae.

1. INTRODUCTION

Origin of medicinal herbs. They have been using them for curative purposes successfully. The records are available in ancient texts. In India itself, there are more than 20000 medicinal plants grown all over the wild forests. Of these, some 60 genus are used immensely in medicinal preparation. Despite their demands today, they are not grown in controlled manner. Rather tribes use them as their livelihood in some belts where they are grown in the wild. Unlike India, in china, the spurts in demand for traditional medicine have made government to allow growth of these plants for further research and development. About 100 units have nearly 600 plant type, grown for their medicinal value. Herbal medicines are used in Ayurveda, Naturopathy and Homeopathy, tradition and Native American medicine (Rangari, 2002). The World Health Organization (WHO) estimates that about 80% of the population living in the developing countries relies almost exclusively on traditional medicine for their primary health care needs. In almost all the traditional medicine, the medicinal plant plays a major role and constitutes the backbone of traditional medicine (WHO, 1978). A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs. The plants that possess therapeutic properties or exert beneficial pharmacological effects on the animal body are generally designated as "Medicinal Plants". Although there are no apparent morphological characteristics in the medicinal plants growing with them, yet they possess some special qualities or virtues that make them medicinally important. It has now been established that the plants, which naturally synthesize and accumulate some secondary metabolites like alkaloids, glycosides, tannins, volatile oils, contain minerals and vitamins, possess medicinal properties (Adnan, 2002).

Mitracarpus hirtus is a generally erect, annual plant that can be simple or sometimes much branched. The relatively stout stems can be 30 - 60cm tall. The plant is sometimes gathered from

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the wild for local medicinal use. The dried leaves are said to heal old ulcers rapidly. The plant is an antidote for arrow poison (Ken Fern, 2021). Considering the medicinal activity of *M. hirtus* based on the aforesaid traditional information, the present study was focused on the phytochemical screening and quantitative estimation of secondary metabolites of the selected plant sample to add scientific conclusion to the traditional claims.

2. PLANT DESCRIPTION

Systematic Position

Kingdom: Plantae
Class: Dicotyledonae
Subclass: Gamopetale
Order: Rubiales
Family: Rubiaceae
Genus: Mitracarpus
Species: hirtus
Synonym: Mitracarpus villosus

Vernacularnames

Common name: Tropical girdle pod, Odia; Gothia gobi, Malayalam; Thaval

Fig. 1. Habit of *Mitracarpus villosus*

3. MATERIALS AND METHODS

3.1. Study area

The selected plant *Mitracarpus hirtus* was collected from Kozhikode District in the Northern part of Kerala. Keezhariyur is a small village situated in Kozhikode district, Kerala state, India. It comes under Keezhariyur Panchayath.

It is located 35 Km from District headquarters Kozhikode. The average annual rainfall of the state ranges from 101.6 to 362cm. The highest temperature recorded was 39.4°C in March and the lowest was 14◦C on December. The District has a generally humid climate with a very hot season extending from March to May. The rainy season is during the South West Monsoon, which sets in the first week of June and extend up to September.

3.2. Collection of Plant Material

The plant leaf of M.hirtus were collected during the month of December, 2021, Keezhariyur, Kozhikode district, Kerala. The authenticity of the selected plant materials were duly identified and confirmed by Botanical Survey of India, Coimbatore. Fresh and healthy plant leaf of *M. hirtus* was harvested shade dried and coarsely powdered for extraction.

3.3. Preparation of Plant Extracts

The powdered plant samples (40 g/200 ml) were extracted successively with hexane, acetone and ethanol using Soxhlet apparatus at 55-86℃ for 9-10 hr in order to extract the polar and nonpolar compounds. The powder was air dried and packed then used for each solvent extraction. The solvent of respective extracts were reduced under room temperature and stored at 5℃ for further use.

3.4. Qualitative screening of Phytochemical Compounds:

3.4.1. Extractive yield

The air dried leaves were exhaustively extracted with successive solvent extraction using soxhlet apparatus viz., petroleum ether, benzene, chloroform, ethyl acetate, ethanol and hot water was which indicates the presence of earthy materials in the sample. The water soluble ash is used to estimate the amount of inorganic compounds present in drugs (Thomas et al., 2008; Vaghasiya et al., 2008; Dave et al., 2010) Table 2. Physico-chemical analysis of Kedrostis foetidissima leaves performed as per Indian Pharmacopoeia

(Peach and Tracey, 1955). The extracts were filtered and concentrated to dryness under reduced pressure using rotary vacuum evaporator (RE 300; Yamato, Japan), Lyophilized (4KBTXL – 75; Vir Tis Benchtopk, New York, USA) to remove traces of water molecules and their extractive yield percentage wascalculated.

3.4.2. Qualitative phytochemical evaluation

Phytochemical screening for crude solvent extracts were carried out and their bioactive compounds were determined using standard methods (Brain and Turner, 1975; Trease and Evans, 1983; Harborne, 1984).

3.5. Quantitative phytochemical analysis

3.5.1. Determination of total flavonoids

The total flavonoid content of samples was determined by following the modified colorimetric method of Zhishen et al. (1999). 0.5 ml extract was mixed with 2 mL of distilled water and subsequently with 0.15 mL of 5% NaNO2 solution. After 6 min, 0.15 mL of 10% AlCl3 solution was added and allowed to stand for 6 min, then 2 mL of 4% NaOH solution was added to the mixture. Immediately distilled water was added to bring the final volume to 5 mL, and then the mixture was thoroughly mixed and allowed to stand for another 15 min. Absorbance of the mixture was recorded at 510 nm versus prepared water blank. Rutin was used as a standard compound for the quantification of total flavonoid. All the values were expressed as milligram of rutin equivalent (RE) per gram of extract.

3.5.2 Determination of total phenolics

The total phenolic content was determined according to the method described by Siddhuraju and Becker (2003). Aliquots of each extract were taken in test tubes and made up to the volume of 1 mL with distilled water. Then 0.5 mL of folinciocalteu phenol reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20%) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as gallic acid equivalents (GAE).

4. RESULT AND DISCUSSION

4.1. Extractive Yield Percentage

The yield of sequential extracts (%) is shown in Figure 2.

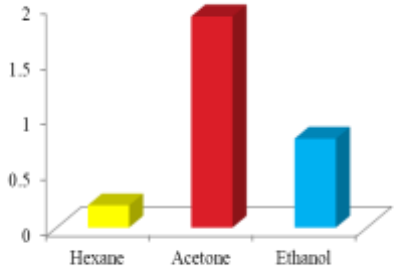

Fig. 2. Extractive Yield Percentage

4.2 Qualitative Phytochemical analysis

Phytochemical screening of the sequential extract of *M. hirtus* revealed the presence of various bioactive components of which phenolics, saponins, alkaloids, tannin, Glycosides, Proteins, Carbohydrates, and Amino acids are the most prominent components and the result of phytochemical test given in the Table 1.

Among these phytochemical tests, Alkaloids, were present in all solvent extracts. Whereas most of the active compound are alkaloid, flavonoid, Glycosides, Tannin, Phenols are present in the acetone extract of plant material.

Table 1. Qualitative phytochemical analysis of the different extracts of *Mitracarpus hirtus* leaf

S. No	Phytocons-tituents	Hex	Ace	EtOH
1	Carbohydrates	++	+	+
2	Proteins	_	_	_
3	Alkaloids	+	+	++
4	Glycosides	_	++	+
5	Saponin	-	+	+
6	Phenol	+	++	+
7	Flavonoids	+	++	+

(+++ abundant; ++ moderately present: +weakly present; -----absent,) Hex, Hexane; Ace, Acetone; EtOH, Ethanol

4.5. Quantitative Phytochemical analysis

Total flavonoids and phenolic content of leaves of *Mitracarpus hirtus* in rutin and gallic acid equivalents are presented in Table 2. The highest phenolic and flavonoid content was noted in the acetone leaf extract, 84.43 mgRE/g extract, and 40.26 mgGAE/g extract flavonoids and phenols were present in leaves respectively. The flavonoids are hydroxylated phenolic substances known to be synthesized by plants in response to microbial infection and they have been found to be antimicrobial substances against wide array of microorganisms *in vitro*. Their activity is probably due to their ability to complex with extracellular and soluble proteins and to complex with bacterial cell wall (Marjorie, 1996.). They are also effective antioxidants and show strong anticancer activities (Salah *et al.,* 1995; Del-Rio *et al.,* 1997; Okwu, 2004). The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (Singh *et al.,* 2007). They possesses biological properties such as anti-apoptosis, antiaging,anticarcinogen,antiinflammation,antiath erosclerosisand cardiovascular protection and improvement of endothelial function as well as inhibition of angiogenesis and cell proliferation activities (Han *et al.,* 2007). Several studies have described the antioxidant properties of medicinal plants which are rich in phenolic compounds (Brown and Rice-Evans, 1998; Krings and Berger, 2001).

Table 2. Total phenolic and flavonoid content of *M.hirtus* leaf extract

Extracts	Total phenolics (mg GAE/g extract)	Total flavanoids (mg RE/g extract
Hexane	12.31±0.3	10.008±0.036
Acetone	84.43±0.1	40.26±0.06
Ethanol	41.20±0.11	20.10±.03

(GAE - Gallic acid equivalent, RE – Rutin equivalent. Values are expressed as mean±SD (n=6).)

5. CONCLUSION

The results revealed the presence of medicinally important constituents such as flavonoids and phenol in the study plant. Further the plant could be considered for antioxidant, anticancer, immunomodulatory activities.

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RESEARCH ARTICLE

SURVEY AND DOCUMENTATION OF PLANT SPECIES IN NORTH COIMBATORE

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ABSTRACT

Due to the civilization, the core environmental changes has been observed in city where still documentation of cultivated and wild species has yet to be done for future studies over the seasonal changes and study of relationship between human and plants in recent scenario. Based on this fundamental concept, the study area has been chosen for the documentation of plant species in north Coimbatore city area to accomplish the project. The planned study area comprises of Periyanaicken palayam, Annur, Karamadai, Sarcarsamakulam. There are hundred plant species documented in this floristic study. Out of which 62 are domesticated plants as ornamental or other consumption purposes and 37 are wild plants around the residing area. This analysis indicated that the documented plant species comes under 40 taxonomic families. Highest species found in the family Fabaceae (15), Apocyanaceae (7), Solanaceae (5), Malvaceae (5), Acanthaceae (5). It is also revealed that the documented domesticated plant species are with the high number of Tree habits (40%) whereas documented wild plant species are with the high number of Herb habits (46%) than other habits. Among which most of the plants are used for domestic consumption, ornamentation and few are medicine. In this survey, no rare status plants have been observed and the area is completely civilized and the land area around the residence has been highly influenced by human beings. The wild species documented in this area are herbaceous weed plants blooming at every rainy season. This study concluded that the wild plants are highly destroyed for various purposes and lead to have only herbaceous weeds around us. Hence the cultivation of trees and protection management has to be initiated to increase the green cover of the study area to regain the misty, moderate climate as the identity of Coimbatore. It will definitely improve the wild fauna lives of the area and other ecological services from vegetation.

Keywords: North Coimbatore, Domesticated plants, Wild Plants, Documentation, Plant protection

1. INTRODUCTION

A flora is an inventory of the plants of a defined biogeographical region. The floristic studies are considered as the backbone of the assessment of phytodiversity, conservation management and sustainable utilization of bioresources of a region. Those are helpful in providing clues of changing floristic pattern, new invasions, current status, rare, endemic and threatened (RET) taxa in a phytogeographical area. A thorough taxonomic study of the flora and forest is essential to understand and assess the richness of their biodiversity. Quantitative inventories help to identify species that are in different stages of vulnerability [1] as well as the various factors that influence the existing vegetation in any region. Moreover in any resource management programs, continuous updating of data about any vegetation, flora and economically relevant plants of the region is an important component of bio-prospecting tools [2].

India possesses a rich biological diversity and incorporates two megadiversity centers. However, large concern exists on the conservation and sustainable utilization of these rich bioresources. The majority of rural communities living in

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mountain and hill regions use wild and non-cultivated edible plant species for food, medicine and other purposes [3, 4, 5]. Both in anthropology and ecology, a now classical distinction has been made between hunter gatherers and agriculturalists. The former were seen to rely regularly on non-cultivated managed plants, and the latter strictly on cultivated ones [6].

In recent years, various studies have shown that non-cultivated wild gathered plants play an important role in supplying seasonal food in rural Mediterranean communities [7] (Rivera et al. 2005). However, their availability, use, status and contribution to livelihood security are poorly documented, and they have been generally overlooked in recent agro-biodiversity conservation and management programmes [8]. Without flora and fauna, humans cannot exist. The flora generates and releases oxygen, which is needed by the fauna for respiratory purposes. In return, the fauna produces and releases carbon dioxide, which is needed by the flora for photosynthesis. It's a symbiotic kind of relationship. In the same line, humans cannot get by without both flora and fauna. The oxygen that we breathe in comes from the flora, and the carbon dioxide we exhale is vital for the flora. Besides, plants are an essential resource for human well-being. Ecology is the study of the relation and the interactions between organisms and their environment. It comprises the floral and faunal communities of an area. With changes in environmental conditions, structure, density and composition of plants, animals also undergo changes [9].

Crop wild relatives remain the largest reservoir of genetic diversity for crop improvement and have been utilized for major gene disease and pest resistance, and abiotic stress tolerance [10]. It is estimated that on the Earth there are between 300,000 and 500,000 species of higher plants, of which approximately 369,000 have been identified or described [11]. Many species are still unknown to science, while perhaps a third is at risk of extinction [12]. Approximately, 2,500 species have undergone some degree of domestication, and 250 species are considered to be fully domesticated, in the sense that their full lifecycle became dependent on human cultivation [13, 14].

Flora of southern Western Ghats regions of Coimbatore and Nilgiri mountains were botanically described by Gardener (1845), Lushington (1902), Fischer (1906, 1921) and Bladder (1908). The flora in Coimbatore city and its environs was studied by Chandrabose (1967), and Chandrabose and Nair (1988) published the Flora of Coimbatore. Some additions to the flora of Anamalai hills of Coimbatore district were compiled by Vajravelu and Joseph (1974) and no comprehensive floristic account of the floristic diversity of Coimbatore, particularly on the plants of the Madukkarai hills of Coimbatore and its environs [15].

Due to civilization, the core environmental changes have been observed in cities where documentation of cultivated and wild species has yet to be done for future studies over the seasonal changes and study of relationship between humans and plants in recent scenarios. Based on the above fundamentals, the following objectives have been framed to accomplish the project.

- ✓ To categorize the area for study to document the floristic composition.
- ✓ To identify and documentation of flowering plants available in the study period (November to April).
- ✓ To know the availability of the various plant species in the area.
- ✓ To identify the cultivation and wild plant species of the selected area
- ✓ To create awareness about the availability of plant species and beneficial information for the researchers.

2. MATERIALS AND METHODS

2.1. Study Area

Coimbatore North comprises of Periyanaickenpalayam, Annur, Karamadai, Sarcarsamakulam (S. S. Kulum). Coimbatore is located at11.0161°N 76.971°E. The city is located on the bank of Noyyal River surrounded by Western Ghats, at a distance of 490 kilometres (300 mi) south-west of Chennai, 190 kilometres (120 mi) south of Mysore, 330 kilometres (210 mi) south of Bangalore. It is located in the western part of the

state in the Kongu Nadu region. The annual rainfall throughout the year is 616.7mm. A project plan was framed on the theme of beneficial and ornamental flowering wild and cultivated available plants in Coimbatore. Local community blended flora was an important part of this study. The study area map is represented in (Figure -1).*2.2 Methods of Data Collection*

In this floristic study, the flowering vascular plants of cultivated and wild species have been documented during November 2021 to April 2022 in the North Coimbatore city area. Four visits of each zone of the study area were made and plants collected with different flowering seasons are considered for more information. The photos collected from the area have been considered for the identification of the same with the help of taxonomists. The identified plant species are traced for their family and common name. The collected information has been tabulated and photographs are documented.

Fig. 1. Study area of North Coimbatore, Tamil Nadu, India

The data is analyzed for the availability of the species for their composition in north Coimbatore, family dispersion, and habit status over the cultivated and wild plant species of the study area. Before starting the field work, preliminary information about the geographical area of study, its physiological features, climatic seasons, etc. were collected. The photographs of the plant are represented in the plate 1 to 4.

3. RESULTS AND DISCUSSION

The survey results provided that there are 102 plant species of wild and cultivated plant species in study area (Table 1 & 2). The observed plants have been identified and photographs are hoarded. The documented plant species common name, local name, botanical name, family have been tabulated (Table 3, 4 & 5). The plant comes under 41 families whereas the habit is maximally recorded as Trees for cultivated and Herbs in wild categories.

In a previous study, 300 plant species belonging to 206 genera and 72 families have been recorded from the area under study. The monocots were represented by 59 species belonging to 35 genera and 7 families, and dicots contributing 241 species belonging to 169 genera and 65 families. Based on habit classification of the enumerated plants, the majority of species were herbs (176 species) followed by climbers (53 species), trees (39 species) and shrubs (32 species) [16].

In another study, the total area of Karunya university campus constitutes about 0.001770% of the total area of the Western Ghats. If a small part of the Western Ghats is so diverse, then one can imagine the biodiversity of the whole Western Ghats. Identified different trees from 53 genera belonging to 27 families and ornamental plants from 58 genera belonging to 32 families and have studied their properties and uses [17].

Synthesis of ethnomedical uses and modern biological knowledge has been done on 40 medicinal plants used by women in hamlets in and around Anaikatty hills of Coimbatore District, Tamil Nadu. Women in these areas possess a rich knowledge of medicinal plants and still continue the medical tradition of using plants as medicine for themselves, their families and others around them [18].

A previous study reported that 30 plant species belong to 26 genera and 21 families. Among plant families Arecaceae and Fabaceae are dominant

with 3 species followed by Moraceae, Poaceae, Apocynaceae, Typhaceae and Solanaceae with 2 species each and rest of the families with 01 species each respectively [19]. In the present study, though the information is not collected from the indigenous people but the urban flora distribution has been evaluated and represented.

Habit

Habit of cultivated plant species recorded as Herb 11, shrub 22, climbers 7, and tree 26 of the plant specimens. It indicates the cultivation of trees for various purposes in the study area. It needs to improve further to get all the ecological services.

The hamlets lying adjacent to the Periyanayakkanpalayam forest range have been evaluated in a previous survey. The floral elements in the tribal hamlets are dominated by wild shrubs and trees whereas in the harijan hamlet they are dominated by herbaceous plants and cultivated crops. Some ethnobotanical studies have been conducted on the use of medicinal plants by the Irula tribes in Coimbatore and Anaikatty [20, 21].

Habit of wild plant species recorded in present survey as Herb 16, shrub 08, climbers 1, and tree 09 of the plant specimens. It indicates that the high anthropogenic influence causes the herb domination in and around the residence of study area.

Table 1. List of Domesticated Plant species in North Coimbatore

S. No.	Scientific Name	Family	Habit	Common Name
1.	*Abelmoschus esculentus L.*	Malvaceae	Herb	Ladies Finger
2.	*Alangium salviifoliunm* (L.F)	Comaceae	Tree	Alanji
3.	*Aloe barbadensis L.*	Asphodelaceae	Herb	Aloe Vera
4.	*Amaranthus spinosus L.*	Amaranthaceae	Herb	Spiny Amaranth
5.	*Arachis hypogaea L.*	Fabaceae	Herb	Groundnut
6.	*Areca catechu*	Arecaceae	Tree	Areca palm
7.	*Azadirachta Indica*	Meliaceae	Tree	Neem
8.	*Bougainvillea spectabilis* L.	Myctaginaceae	Small Tree	Great Bougainvillea
9.	*Caesalpinia pulcherrima L.*	Fabaceae	Tree	Peacock Flower
10.	*Canna Indica L.*	Cannaceae	Herb	African Arrowroot
11.	*Cassia fistula L.*	Fabaceae	Tree	Golden Shower Tree
12.	*Celosia spicata L.*	Amaranthaceae	Shrub	Silver Cockscomb
13.	*Citrus cavaleriei (H.Lev)*	Rutaceae	Tree	Ichang Papeda
14.	*Citrus limon L.*	Rutaceae	Tree	Lemon
15.	*Clitoria ternatea* L.	Fabaceae	Climber	Butterfly Pea
16.	*Cocos nucifera* L.	Arecaceae	Tree	Coconut Tree
17.	*Coriandrum sativam L.*	Apiaceae	Herb	Coriander
18.	*Crossandra infundibuliformis L.*	Acanthaceae	Shrub	Firecracker Flower
19.	*Cucurbita maxima* Ducheshe	Cucurbitaceae	Climber	Pumpkin
20.	*Epipremnum aureum L.*	Araceae	Climber	Golden Pothos
21.	*Ficus carica L.*	Moraceae	Tree	Fig
22.	*Gossypium arboreum L.*	Malvaceae	Shrub	Cottom

23.	*Hibiscus rosa sinensis L.*	Malvaceae	Shrub	China-Rose
24.	*Ixora chinensis (Lem)*	Rubiaceae	Shrub	Chinese Ixora
25.	*Jasminum auriculatum(vahl.)*	Oleaceae	Shrub	Jasmine
26.	*Jasminum grandiflorum L.*	Oleaceae	Shrub	Spanish Jasmine
27.	*Jasminum multiflorum (Burm.f.)*	Oleaceae	Climber	Star Jasmine
28.	*Justica adathoda L.*	Acanthaceae	Shrub	Malabar Nut
29.	*Lagenaria siceraria (molina)*	Cucurbitaceae	Climber	Bottle Guard
30.	*Lawsonia inermis L.*	Lythraceae	Shrub	Henna
31.	*Mangifera indica L.*	Anacardiaceae	Tree	Mango
32.	*Mangnolia champaca* (L.) Figlar.	Magnoliaceae	Tree	Champak
33.	*Mimusops elengi L.*	Sapotaceae	Tree	Spanish Cherry
34.	*Mirabilis jalapa* L.	Myctaginaceae	Herb	Four-O-Clock
35.	*Momordica charantia* L.	Moringaceae	Climber	Bitter-Melon
36.	*Moringa oleifera Lam*	Moringaceae	Tree	Drum Stick
37.	*Murraya koenigii L.*	Rutaceae	Tree	Curry Leaves
38.	*Musa acuminata L.*	Musaceae	Tree	Banana
39.	*Nerium oleander L.*	Apocynaceae	Shrub	Nerium
40.	*Ocimum tenuiflorum L.*	Lamiaceae	Herb	Sacred Basil
41.	*Passiflora foetida L.*	Passifloraceae	Climber	Passion Flower
42.	*Phyllanthus emblica L.*	Phyllanthaceae	Tree	Amla
43.	*Plumeria pudica (jacq)*	Apocynaceae	Shrub	Bridal Bouquet
44.	*Psidium guajava L.*	Myrtaceae	Small tree	Guava
45.	*Punica granatum L.*	Lythraceae	Shrub	Pomegranate
46.	*Ricinus communis L.*	Euphorbiaceae	Shrub	Castor
47.	*Rosa damascena L.*	Rosaceae	Shrub	Rose
48.	*Senna auriculata L.*	Fabaceae	Shrub	Avaram Senna
49.	*Solanum lycopersicum L.*	Solanaceae	Shrub	Tomato
50.	*Solanum melongena L.*	Solanaceae	Shrub	Brinjal
51.	*Solanum torvum L.*	Solanaceae	Shrub	Turkey Berry
52.	*Solanun nigrum L.*	Solanaceae	Shrub	Black Nightshade
53.	*Sorghum bicolor L.*	Poaceae	Herb	Great Millet
54.	*Syzygium cumini L.*	Myrtaceae	Tree	Java Plum
55.	*Tabernaemontana corymbosa*	Apocynaceae	Shrub	Great Rosebay
56.	*Tabernaemontana divaricata R.*	Apocynaceae	Shrub	Pinhweel Flower
57.	*Tarmarindus indica L.*	Fabaceae	Tree	Tamarind
58.	*Tecoma stans L.*	Bignoniaceae	Shrub	Yellow Bells
59.	*Terminalia arjuna L.*	Combretaceae	Tree	Arjun Tree
60.	*Terminalia catappa L.*	Combretaceae	Tree	Tropical Almond
61.	*Vachellia nilotica L.*	Fabaceae	Tree	Gum Arabic
62.	*Vitex negundo L.*	Verbanaceae	Small tree	Chinese Chaste Tree

Table 2. List of Wild Plant species in North Coimbatore

S. No	Scientific Name	Family	Habit	Common Name
1.	*Abutilon hirtum (Lam)*	Malvaceae	Herb	Indian mallow
2.	*Abutilon indicum* L.	Malvaceae	Shrub	Monkey Bush
3.	*Alangium salviifolium L. F*	Comaceae	Tree	Hill sack tree
4.	*Albizia julibrissin L.*	Fabaceae	Tree	Silk tree
5.	*Barleria cristata L.*	Acanthaceae	Shrub	Philippine violet
6.	Blumea lacera L.	Asteraceae	Herb	Lettuce-Leaf Blumea
7.	*Calotropis gigantea (Linn.) Aiton f.*	Apocynaceae	Shrub	Crown flower
8.	*Calotropis procera L.*	Apocynaceae	Shrub	Giant milkweed
9.	*Catharanthus roseus L.*	Apocynaceae	Herb	Cape periwinkle
10.	*Cleome gynandra* L.	Cleomaceae	Herb	Spider Flower
11.	*Cleome rutidosperma DC.*	Cleomaceae	Herb	Fringed spider flower
12.	*Cleome viscosa* L.	Cleomaceae	Herb	Asian Spider Flower
13.	*Coccinia grandis (L.) Voigt.*	Cucurbitaceae	Climber	Ivy gourd
14.	*Commelina benghalensis L.*	Commelinaceae	Herb	Benghal dayflower
15.	*Cynodon dactylon L.*	Poaceae	Herb	Bermuda grass
16.	*Delonix regia (Hook.) Raf.*	Fabaceae	Tree	Royal Poinciana
17.	*Duranta erecta L.*	Verbenaceae	Shrub	Golden dewdrop
18.	*Evolvulus alsinoides L.*	Convolvulaceae	Herb	Dwarf morning-glory
19.	*Ficus benghalensis L.*	Moraceae	Tree	Banyan
20.	*Ficus religiosa L.*	Moraceae	Tree	Arasamaram
21.	*Ipomoea obscura L. Ker Gawl.*	Convolvulaceae	Herb	Obscure morning glory
22.	*Lantana camara L.*	Verbanaceae	Shrub	Lantana
23.	*Leucas aspera L.*	Laminaceae	Herb	Tamba
24.	*Mesosphaerum suaveolens (L.) Kuntze*	Lamiaceae	Herb	Mint weed
25.	*Millettia pinnata L.*	Fabaceae	Tree	Indian beech
26.	*Millingtonia hortensis L.f.*	Bignoniaceae	Tree	Indian cork tree
27.	*Muntingia calabura (L.)*	Muntingiaceae	Tree	Jamaica tree
28.	*Oldenlandia corymbosa L.*	Rubiaceae	Herb	Old World Diamond-Flower
29.	*Peltophorum pterocarpum (DC.) Backer ex K. Heyne*	Fabaceae	Tree	Copper pod
30.	*Perityle emoryi*	Asteraceae	Herb	Emorya rocks rockdaisy
31.	*Phyllanthus amarus* Schum. & Thonn.	Phyllanthaceae	Herb	Child Pick-A-Back
32.	*Pinus densiflora L.*	Pinaceae	Tree	Japanese red pine
33.	*Prosopis juliflora (S.w)*	Fabaceae	Shrub	Mesquite
34.	*Ruellia tuberosa L.*	Acanthaceae	Herb	Cracker plant
35.	*Solanum surattense Burm. f.*	Solanaceae	Shrub	Wild Eggplant
36.	*Thunbergia erecta* (Benth.) T. Anderson	Acanthaceae	Shrub	Bush Clock Vine
37.	*Tridax procumbens L.*	Asteraceae	Herb	Coat buttons

Table 3. Number of Families From domesticated and Wild Plants

S. No	Family	No of species
1.	Acanthaceae	5
2.	Amaranthaceae	2
3.	Anacardiaceae	1
4.	Apiaceae	1
5.	Apocynaceae	7
6.	Araceae	1
7.	Arecaceae	2
8.	Asphodelaceae	1
9.	Asteraceae	3
10.	Bignoniaceae	2
11.	Cannaceae	1
12.	Cleomaceae	3
13.	Comaceae	2
14.	Combretaceae	2
15.	Convolvulaceae	2
16.	Cucurbitaceae	3
17.	Euphorbiaceae	1
18.	Fabaceae	12
19.	Lamiaceae	2
20.	Lythraceae	2
21.	Lythraceae	3
22.	Magnoliaceae	1
23.	Malvaceae	5
24.	Meliaceae	1
25.	Moraceae	3
26.	Moringaceae	2
27.	Muntingiaceae	1
28.	Musaceae	1
29.	Myctaginaceae	2
30.	Myrtaceae	2
31.	Oleaceae	3
32.	Passifloraceae	1
33.	Phyllanthaceae	2
34.	Pinaceae	1
35.	Poaceae	2
36.	Rosaceae	1
37.	Rubiaceae	2
38.	Rutaceae	3
39.	Sapotaceae	1
40.	Solanaceae	5
41.	Verbanaceae	2

Table 4. Habit of Cultivated Plants

S. No.	Habit	Number of Species	% of Habit Distribution
1	Herb	9	14.51
2	Shrub	21	33.87
3	Climber	7	11.29
4	Tree	25	40.32

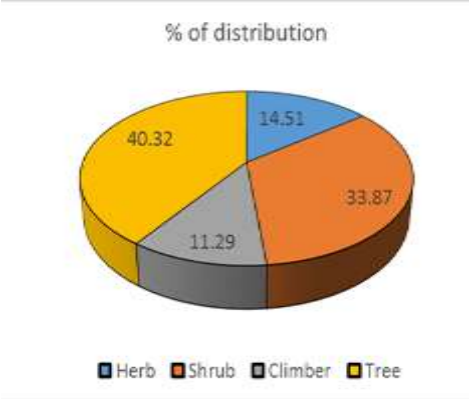

Fig. 2. Habit Distribution of Cultivated Plant Species

Table 5. Habit of Wild Plants

S. No.	Habit	Number of Species	% of Habit Distribution
1	Herb	17	45.94
2	Shrub	9	24.32
3	Climber	1	2.7
4	Tree	10	27.02

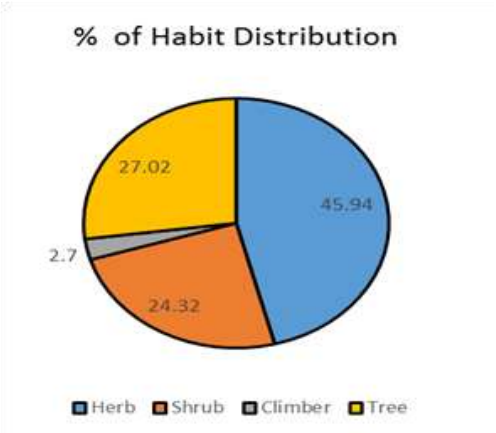

Fig. 3. Habit Distribution of Wild Plants Species

Many trees were very old and appear to be carefully conserved by the peoples looking to the benefits such as small timber for construction and agricultural purposes, fuel wood, fruits, fodder and other benefits rendered by trees [21]. In present study, domesticated for their commercial benefits or consumption benefits either may be for food or ornamental or shade due to their large canopy. The species like *Tarmarindus indica, Ficus carica, Mimusops elengi, Alangium salviifoliunm, Terminalia catappa, Cassia fistula* are cultivated for shade particularly since those trees are having large enough canopy.

4. CONCLUSION

There is not much awareness acquired by the local people about the importance and conservation of these wild plants, even though they play a significant role in our day-to-day life. The remarkable adaptation of plants, various disturbances including their ability to accumulate toxins in their environment suggest an important plant species in conservation. In this survey, no rare status plants have been observed in this study area. Hence it is indicating that the area is completely civilized and the land area around the residence has been highly influenced by human beings. The wild species documented in this area are herbaceous weed plants blooming at every rainy season.

This study suggests that the wild plants are highly destroyed for various purposes and lead to have only herbaceous weeds around us. The most benefited trees like *Azadirachta indica*, *Phyllanthus emblica, Psidium guajava, Moringa oleifera, Murraya koenigii* are domesticated for their commercial benefits or consumption benefits either may be for food or ornamental or shade due to their large canopy. Hence the cultivation of trees, greening of bare lands and protection management has to be initiated to increase the green cover of the study area – north Coimbatore to regain misty, moderate climate as the identity of Coimbatore. It will definitely improve the wild fauna lives of the area and other ecological services like rainfall, pure air and beauty of nature to enjoy.

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RESEARCH ARTICLE

CHANGES IN POLYPHENOLIC CONTENTS AND ANTIOXIDANT POTENTIAL OF THE LEAFY VEGETABLE, TALINUM PORTULACIFOLIUM BY MOIST HEAT COOKING

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ABSTRACT

In general, most of green leafy vegetables undergo a cooking process prior to consumption. This present study was carried out to investigate the effect of moist heat cooking treatments of the green leafy vegetable, *Talinum portulacifolium* on the total phenolic content and antioxidant potential using phosphomolybdenum reduction, DPPH, and ABTS radical scavenging activities. The total phenol and tannin contents were found maximum in the raw samples of the plant 78.25 and 33.84 mg GAE/g extract, respectively. The boiling and frying treatments significantly reduced the total phenolic and tannin contents in *T. portulacifolium*. In the antioxidant study, the raw sample of ethanol extract exhibited a strong antioxidant potential based on ABTS, DPPH, radical scavenging and phosphomolybdenum reduction assays. Further, the raw and processed powders were subjected to FTIR analysis and the functional groups of the components were separated based on their peaks. It could be concluded that boiling and frying treatments have a determining effect on the levels of phenolic content and antioxidant capacities of vegetables.

Keywords: leafy vegetable, moist heat cooking, *Talinum portulacifolium*, antioxidant, phenolics

1. INTRODUCTION

Green leafy vegetables are an important source of dietary micronutrients and antioxidants in the human diet. They are a rich source of carotene, ascorbic acid, riboflavin, folic acids, and minerals like calcium, iron, and phosphorus [1]. Epidemiological studies have shown that the intake of vegetables can protect humans against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species. Leafy vegetables are also rich in compounds having antidiabetics, anti–histaminic, anti–carcinogenic, and hypolipidemic properties against cardiovascular disease, aging, obesity, hypertension, and insomnia. Several in vitro, pre-clinical and clinical investigations have revealed an inverse relationship between high consumption of vegetables and a lower incidence of chronic diseases [2].

Most of the green leafy vegetables undergo a cooking process prior to consumption Evidence is emerging that in vivo bioavailability of many protective compounds is enhanced when vegetables are cooked [3]. It is known that cooking induces significant changes in chemical composition, influencing the concentration and bioavailability of bioactive compounds in vegetables. In addition, cooking can make food microbiologically safer to eat as well as to improve the edibility of the food. However, it can also result with some undesired consequences such as the losses of nutrients and the formation of toxic compounds with negative effects on flavor, texture, or color [4,5]. Numerous studies have been focused on the effect of cooking methods on dietary phytochemicals [6-8].

Talinum portulacifolium (Forssk.) Asch. ex Schweinf (Portulacaceae) is a succulent subshrub, up to 3 ft tall, growing from tuberous roots. Leaves are elliptic to obovate, up to 12 cm long, much smaller below the inflorescence, slightly fleshy. In traditional Chinese medicine, this plant is used to improve digestion, moistens the lungs and promotes breast milk. This plant is useful for treating headaches, aphrodisiac, pneumonia, diarrhea, polyuria, irregular menstruation, vaginal discharge and little milk [9]. The juice from the leaves is also

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used to smooth expenditures, treat ulcers, and increased appetite. It contains high antioxidants level such as ginsenosides, phenol acids, flavonoids, saponins and tannins [10].

The present study aimed to investigate the influence of moist heat cooking methods on the total phenolic content and antioxidant potentials of ethanol extract of *T. portulacifolium.*

2. MATERIALS AND METHODS

2.1. Plant material

The leaves of *T. portulacifolium* were purchased from local farms at Calicut district in Kerala (Figure 1).

Fig. 1. The leafy vegetable, *Talinum portulacifolium* used in this study

2.2. Preparation of samples

The leaves of *T. portulacifolium* were washed thoroughly with tap water and drained to remove excess water, then chopped into small pieces. The chopped leaves were divided into three parts: Raw (uncooked served as a control), boiled (100 g of vegetables were boiled in 500 mL of water in a stainless steel pan), and fried (100 g of vegetables were cooked in a frying pan with 100 mL of water). The cooking treatment was carried out under sim conditions using a gas stove for 30 minutes. After cooking treatment, all samples were shade dried and powdered.

2.3. Total phenolic and tannin content analysis

The total phenolic content was determined according to the Folin-Ciocalteu method described by Siddhuraju and Becker [11]. Tannins in the extracts were estimated after treatment with polyvinyl polypyrrolidone (PVPP). The analysis was performed in triplicate and the results were expressed as the gallic acid equivalents (GAE).

2.4. In vitro antioxidant activity

2.4.1. Phosphomolybdenum assay

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex according to the method of Prieto et al. [12]. An aliquot of 100 ml of sample solution was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in a 4 ml vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported are mean values expressed as g of ascorbic acid equivalents/100 g extract (ascorbic acid equivalent antioxidant activity).

2.4.2. Free radical scavenging activity on DPPH•

The antioxidant activity of the extracts was determined in terms of hydrogen donating or radical scavenging ability using the stable radical DPPH according to the method of Blois [13]. Ethanol extracts at various concentrations were taken and the volume was adjusted to 100 ml with methanol. 5 ml of a 0.1 mM methanolic solution of DPPH was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27 8C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula:

% DPPH radical scavenging activity = (Control OD - Sample OD/Control OD) x 100.

IC_{50} values of the extract i.e., the concentration of extract necessary to decrease the initial concentration of DPPH by 50% was calculated.

2.4.3. ABTS•+ radical scavenging activity

The assay was performed by a slightly modified protocol of Re et al. [14]. ABTS solution (7 mM) was reacted with potassium persulphate (2.45 mM) solution and kept for 12–16 h in the dark, to produce a dark-colored solution containing ABTS

radical cations. Prior to the assay, this solution was diluted in ethanol (about 1:89, v/v) and equilibrated at 30 °C to give an absorbance at 734 nm of 0.700 ± 0.02. Different concentrations of the sample were prepared. About 0.3 ml of the sample was mixed with 3 ml of ABTS working standard. Absorbance was measured at 734 nm exactly 30 min after the initial mixing.

% ABTS radical scavenging activity = (Control OD - Sample OD/Control OD) x 100. IC_{50} values of the extract i.e., concentration of extract necessary to decrease the initial concentration of ABTS by 50% was calculated.

2.5. FTIR spectrometric analysis

The infrared spectrum of raw and processed powders from *T. portulacifolium* leaves was determined using an FT-IR (Model/Make: IFS 25, Bruker, Germany) with PC-based software controlled instrument operation and data processing. The data of infrared transmittance was collected over a wave number ranging from 4000 cm^{-1} to 500 cm^{-1}. The spectral data were compared with a reference to identify the functional groups existing in the sample.

3. RESULTS AND DISCUSSION

3.1. Recovery percent, total phenolic and tannin contents

Plant phenolics present in the vegetables have received considerable attention because of their potential biological activity. These activities might be related to their antioxidant activity. Therefore, it is necessary to determine the total phenolic content and antioxidant capacity and to make comparisons among vegetables [15]. The yield percent, total phenolic, and tannin contents of the extracts obtained from the leaves of *T. portulacifolium* are shown in Table 1. The maximum extract yield was obtained in the ethanol extract of raw sample *T. portulacifolium* (5.40%). Variations in phenolic and tannin contents were observed in extracts from raw and processed samples. The extractable total phenolics (78.25 mg GAE/g extract) and tannins (33.84 mg GAE/g extract) were found to be higher in the ethanol extract of the raw sample of *T. portulacifolium*. The boiling and frying treatments significantly reduced the total phenolic and tannin content in *T. portulacifolium* when compared with raw samples.

Table 1. Extract yield percentage, total phenolic and tannin contents of raw, boiled and fried samples of *Talinum portulacifolium*

Sample	Extract yield (%)	Total phenolics (mg GAE/g extract)	Tannins (mg GAE/g extract)
SD	5.40	70.74 ± 4.14	33.84 ± 2.79
SB	3.92	21.23 ± 5.21	10.33 ± 0.97
SC	4.64	55.22 ± 4.81	26.52 ± 1.73

SD, raw sample of *Talinum portulacifolium*; SB, boiled sample of *Talinum portulacifolium;* SC, fried sample of *Talinum portulacifolium*.

Total phenolic and tannin contents are expressed as gallic acid equivalent (GAE). Values are mean of three replicate determinations (n =3) ± standard deviation.

Mao et al. [16] observed that the phenolic content and antioxidant capacity were higher after ethanol extraction than in a water extract. It was evident that the loss of total phenolic content increased with prolonged boiling time. In the cases of Thai basil leaf and sweet potato leaf, there was a significant increase in TPC in their extracts at the initial stage of boiling for 1 minute and 5 minutes, respectively. Subsequently, the TPC decreased as the boiling time increased. The initial increase in TPC might have been due to the liberation of phenolics from the intracellular proteins, changes in plant cell structure, matrix modifications, or the inactivation of the polyphenol oxidase [17]. By contrast, as suggested by Migilo et al. [18], water cooking has a detrimental effect on polyphenols in vegetables, resulting in a complete loss of phenolic compounds, likely due to diffusion into the boiling water. In addition, Wachtel-Galor et al. [19] found that with a boiling time of 5-10 minutes, the TPC in vegetable extracts decreased whereas it increased in the cooking water.

3.2. Antioxidant activity

3.2.1. Phosphomolybdenum reduction assay

In the presence of the extracts, Mo (VI) is reduced to Mo (V) and forms a green-colored phosphomolybdenum V complex, which shows maximum absorbance at 695 nm. From the results obtained, it can be seen that ethanol extracts of raw and processed samples exhibited significant antioxidant activity (Table 2). The ethanol extract of the raw sample of *T. portulacifolium* leaves (302.2 mg AAE/g extract) showed the highest phosphomolybdenum reduction activity. The results showed that boiling and frying treatments have a detrimental effect on phosphomolybdenum reduction antioxidant capacity and phenolic concentration of this leafy vegetable.

The differential response of extracts in various antioxidant assay systems may be explained by the fact that the transfer of electrons/hydrogen from antioxidants occur at different redox potential in various assay systems and the transfer also depends on the structure of the antioxidants [20]. The variations in antioxidant activity may be due to phenolic and tannin contents. Dasgupta and De [21] investigated the total antioxidant activity of eleven edible leafy vegetables of India in different systems of the assay. The extracts were found to have different levels of antioxidant properties.

Table 2. Phosphomolybdenum reduction activity of raw, boiled and fried samples of *Talinum portulacifolium*

Sample	Phosphomolybdenum assay (mg AAE/ g extract)
SD	302.2 ± 24.1
SB	212.2 ± 12.7
SC	256.1 ± 14.5

SD, raw sample of *Talinum portulacifolium*; SB, boiled sample of *Talinum portulacifolium;* SC, fried sample of *Talinum portulacifolium*.
Values are mean of three replicate determinations (n =3) ± standard deviation.

3.2.2. DPPH radical scavenging assay

DPPH assay is one of the most widely used methods for screening the antioxidant activity of plant extracts. DPPH is a stable nitrogen-centered free radical, which produces a violet color in methanol solution. DPPH radicals react with suitable reducing agents, during which the electrons become paired off and the solution loses color stoichiometrically depending on the number of electrons taken up [13]. The results obtained clearly indicate the potential of the plant extracts in scavenging free radicals (Table 3). The ethanol extracts of the raw and processed samples of *T. portulacifolium* leaves exhibited an inhibition ranging from 21.99 to 52.55% at 400 µg/ml.

Table 3. DPPH$^{\bullet}$ radical scavenging activity of raw, boiled and fried samples of *Talinum portulacifolium*

Sample	% of activity			
	100 µg/ml	200 µg/ml	300 µg/ml	400 µg/ml
SD	27.48 ± 0.95	36.89 ± 0.35	43.00 ± 1.85	52.55 ± 1.51
SB	4.31 ± 0.47	11.97 ± 2.44	17.08 ± 1.02	21.99 ± 0.10
SC	10.90 ± 1.04	21.87 ± 0.88	27.32 ± 1.00	38.44 ± 1.66

SD, raw sample of *Talinum portulacifolium*; SB, boiled sample of *Talinum portulacifolium;* SC, fried sample of *Talinum portulacifolium*.
Values are mean of three replicate determinations (n =3) ± standard deviation.

As presented in Figure 2, the scavenging abilities of ethanol extracts of raw and processed samples of *T. portulacifolium* were concentration-dependent and expressed as IC_{50} values. All the extracts exhibited appreciable DPPH radical scavenging activity. When compared to processed samples, raw samples of *T.*

portulacifolium showed higher DPPH radical scavenging activity with the IC_{50} value of 340.5 µg/ml, respectively. The lowest DPPH radical scavenging activity was found in the ethanol extract obtained from the boiled sample of *T. portulacifolium* with the IC_{50} value of 896 µg/ml.

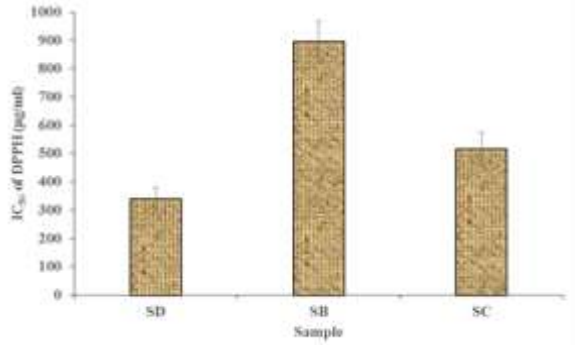

Fig. 2. IC_{50} of DPPH• radical scavenging activity of raw, boiled and fried samples of *Talinum portulacifolium.* SD, raw sample of *Talinum portulacifolium*; SB, boiled sample of *Talinum portulacifolium;* SC, fried sample of *Talinum portulacifolium*. Values are mean of three replicate determinations (n =3) ± standard deviation.

Antioxidants with DPPH radical scavenging activity could donate hydrogen to free radicals, particularly to the lipid peroxides or hydroperoxide radicals that are the major propagators of the chain autoxidation of lipids, and form nonradical species, resulting in the inhibition of propagating phase of lipid peroxidation [22]. These results suggested that phenolic compounds were the major contributor to the DPPH radical scavenging activity.

3.2.3 ABTS radical scavenging assay

ABTS assay is based on the inhibition of the absorbance of the radical action ABTS+, which has a characteristic long-wavelength absorption spectrum [23]. The results obtained clearly imply that the ethanol extracts of raw and processed samples inhibit the radicals or scavenge the radical in a concentration-dependent manner. The ethanol extracts of the raw and processed samples of *T. portulacifolium* leaves exhibited an inhibition ranging from 66.86 to 87.69% at 300 µg/ml (Table 4). ABTS radical scavenging effect raw and processed samples of *T. portulacifolium* leaves was in this order: SD> SC> SB (Figure 3). Similar to DPPH radical scavenging activity, the raw sample of *T. portulacifolium* exhibited the highest ABTS radical scavenging activity than processed extracts. The lowest ABTS radical scavenging activity was found in the ethanol extract obtained from the boiled sample of *T. portulacifolium* with the IC_{50} value of 239.2 µg/ml.

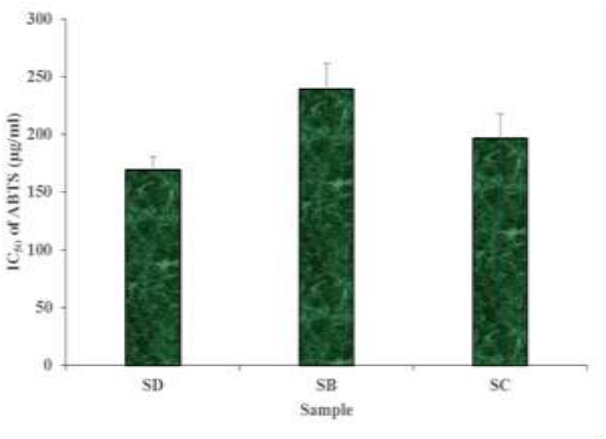

Fig. 3. IC_{50} of ABTS radical scavenging activity of raw, boiled and fried samples of Talinum portulacifolium. SD, raw sample of *Talinum portulacifolium*; SB, boiled sample of *Talinum portulacifolium;* SC, fried sample of *Talinum portulacifolium*. Values are mean of three replicate determinations (n =3) ± standard deviation.

Rice-Evans et al. [24] reported that phenolic compounds play a major role in scavenging the free radicals. Hagerman et al. [25] have made a similar observation that the high molecular weight phenolics have more ability to quench free radicals ($ABTS^{\bullet+}$). ABTS radical scavenging activity of these vegetable extracts indicated that the mechanism of antioxidant action of this fraction was as a hydrogen donor and it could terminate the oxidation process by converting free radicals to stable forms [26]. The findings of the present study revealed that boiled or fried leafy vegetables have lower antioxidant capacity than raw ones. Several studies reported that the total phenolic content was decreased after boiling, steaming, microwaving, baking, and frying [27,28]. This decrease may be due to water-soluble phenols leaching into the cooking water and the

structural changes of phenolics that occurs during heat processing [29]. Samples are not in contact with water and the inactivation of oxidative enzymes has prevented the disruption of phenolic biosynthesis during these cooking methods [30]. Tian et al. [28] suggested that steaming and microwaving methods with lower temperatures may be better for the retention of total phenolic contents.

Table 4. ABTS radical scavening activity of raw, boiled and fried samples of *Talinum portulacifolium*

Sample	% of activity		
	100 µg/ml	**200 µg/ml**	**300 µg/ml**
SD	33.85 ± 1.19	67.45 ± 0.86	87.69 ± 0.77
SB	17.93 ± 2.42	39.07 ± 1.14	66.86 ± 1.98
SC	29.03 ± 0.65	53.38 ± 1.70	72.82 ± 0.37

SD, raw sample of *Talinum portulacifolium*; SB, boiled sample of *Talinum portulacifolium;* SC, fried sample of *Talinum portulacifolium*.
Values are mean of three replicate determinations (n =3) ± standard deviation.

3.3. FTIR analysis

The raw and processed powders of *T. portulacifolium* leaves were subjected to FTIR analysis and the functional groups of the components were separated based on their peaks.

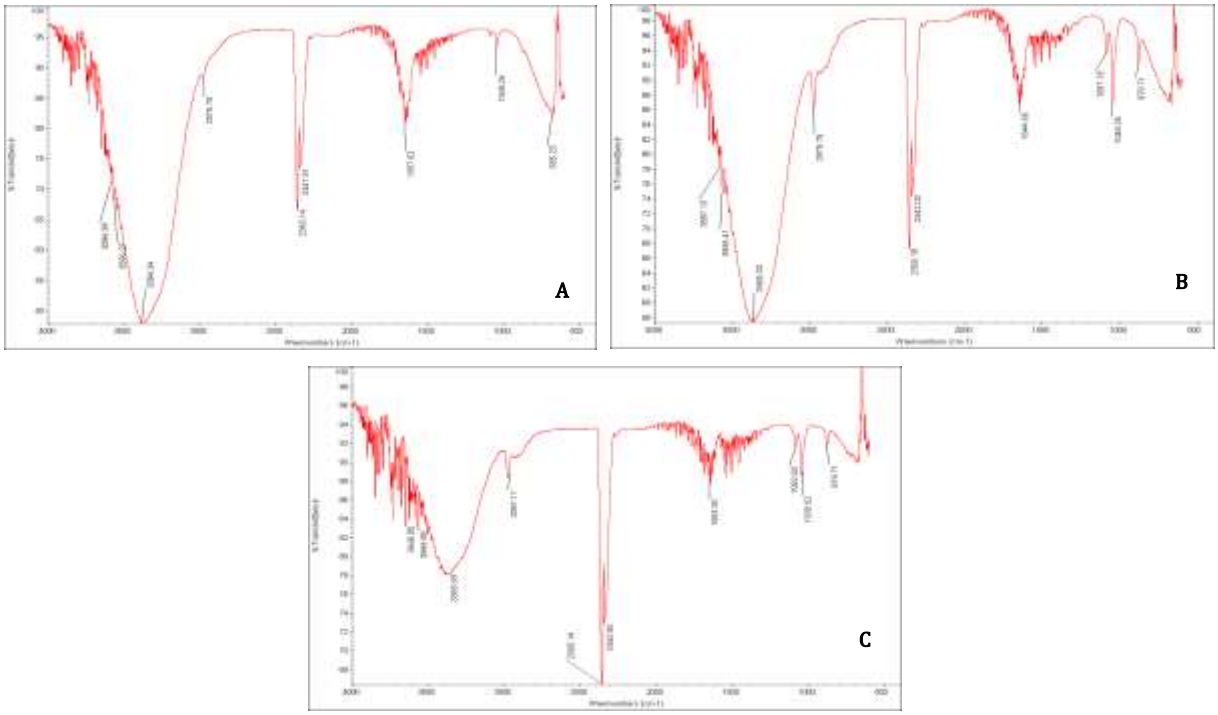

Fig. 4. FTIR analysis of raw (A), boiled (B) and fried (C) powders of *Talinum portulacifolium*

The broad band at 3362 - 3366 cm^{-1} was attributed to O-H stretching vibration of hydrogen-bonded hydroxyl groups in the sample, and the band at 2975 cm^{-1} was attributed to C-H stretching vibration of methylene or methyl groups [31]. The bands at 2360 were attributed to the stretching vibration of N-H/C-O [32]. The band at 1644 -1653 cm^{-1} was due to C=O stretching vibration or the HO-H vibration [33,34]. The bands at 1087 cm^{-1} and 1048 cm^{-1} were attributed to the stretching vibration of the pyran ring [35]. The band in the range of 1000-1200 cm^{-1} was also assigned to the bending or stretching vibration of C-O groups. In addition, the band at 879 cm^{-1} was attributed to the stretching vibration of aromatic out-of-plane-rings with 2 neighboring C-H groups (Figure 4). FTIR analysis for five selected green leafy vegetables such as *Hibiscus cannabinus*, *H. sabdariffa*, *Basella alba, B. rubra* and *Rumex vesicarius* confirmed the presence of free alcohol, intermolecular bonded alcohol, intramolecular bonded alcohol, alkane, aromatic compounds, imine or oxime or ketone or alkene, phenol, and amine stretching [31].

4. CONCLUSIONS

The findings of the present study revealed that boiled or fried leafy vegetables possess lower antioxidant capacity than raw ones. The decrease in antioxidant activity may be due to water-soluble phenolic compounds leaching into the cooking water and the structural changes of phenolic compounds that occur during heat processing. Further studies are warranted in relation to other cooking practices like steam cooking, microwave cooking, etc. in order to select the best cooking practice for the improvement of the antioxidant potential of leafy vegetables.

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RESEARCH ARTICLE

WEARABLE E-TEXTILE INTEGRATED SENSORS FOR DETECTING HYPERKALEMIA AND HYPOKALEMIA

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ABSTRACT

Recent evolutions in wearable and personalized healthcare devices have led to the emergence of real-time monitoring of the physiological and metabolic status of human beings. Detection of disorders related to potassium levels including hyperkalemia and hypokalemia is very much challenging in the current scenario. Potassium imbalance leads to massive change in electrical activity of the body especially disturbs the function of the brain, heart, nerves, bone and muscles in hospitalized patients. Potassium loss is a major concern in disorders such as hyperhidrosis and Cystic Fibrosis. Hence the real-time monitoring of potassium levels in the hospitalized patients may provide information related to latent cardiac problems and also assist in the diagnosis of apparent life-threatening diseases. Comprehensively, the real-time monitoring of potassium concentrations may shed light on the electrolyte loss, dehydration status and also the other associated diseases. In this study, the correlation between the electrocardiogram (ECG) signals and the potassium levels can diagnose the potassium imbalance in a non-invasive manner. The novelty of our work is to develop a wearable E-textile integrated sensor for the detection of hyperkalemia and hypokalemia through ECG signal. The main advantage of this product is wearable and portable, and also ensures the patient safety and user friendly particularly for elderly and disabled patients and also for children.

Keywords: Hyperkalemia, Hypokalemia, ECG.

1. INTRODUCTION

Unexpected cardiac deaths have been raised due to the impact of hyperkalemia and hypokalemia in clinical sectors (C. S. Lin et al., 2020). This dyskalemias has been occurred during the correction of potassium (K+) ions. To track these ions, point-of-care blood tests have been recommended for quick analysis of electrolyte levels in blood and plasma (Dylewski & Linas, 2018; Gavala & Myrianthefs, 2017). Potassium is one of the pivotal electrolytes like sodium, chlorine and plays a significant role in the generation of bio-potential signals of our body which helps to maintain the function of brain, heart, nerves, bone and muscles (Aburto et al., 2013). Potassium is the third most abundant minerals in the body and also regulates the water balance and the acid-base balance in the blood and tissues (Stone, Martyn, & Weaver, 2016). Potassium is highly reactive in water which produces the positive charged ion (K+) and assists in the maintenance of intracellular fluids and trans-membrane electrochemical gradient. Potassium has no effective way to be conserved like sodium in our body for future uses. Even when a potassium shortage exists, the kidneys continue to excrete it. Because the human body relies on potassium balance for a regularly functioning of the heart and nervous system, it is essential to strive for potassium's balance. Insufficient potassium causes a change in electrical activity of the body (Kuntjoro, Teo, & Poh, 2012).

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Bioelectric potential or bio-potential are produced as a result of the electrochemical activity of the excitable cells, which are components of the nervous, muscular or glandular tissues. It is mainly occurred due to trans-membrane electrochemical exchange with the action of sodium, potassium and chlorine. A typical potassium level for adult falls between 3.5 and 5.0 milli equivalent per liter (mEq/L). Hyperkalemia is a condition due to high potassium minerals in human blood usually greater than 5.0 mEq/L and 5.5 mEq/L; the range in infants and children respectively. Levels higher than 7 mEq/L can lead to significant hemodynamic and neurologic consequences, whereas potassium levels exceeding 8.5 mEq/L can cause respiratory paralysis, arrhythmias or cardiac arrest (Alfonzo, Isles, Geddes, & Deighan, 2006). For example, hypokalemia and hyperkalemia are paucisymptomatic, and generally found in cardiac or renal disease patients. Therefore, in this study, we developed the emerging non-invasive and blood-free potassium tracking method for the advance clinical purposes.

2. MATERIALS AND METHODS

2.1. Basic components

The basic components including Node Micro-Controller Unit (NODE-MCU), Electrocardiogram (ECG) Sensor, Arduino IDE and connecting wires are used in this system. In this block diagram, ECG sensor is connected to core controller which accesses the sensor values and processes them to transfer the data through internet. NODE-MCU ESP8266 is used as a core controller. The obtained ECG signal can be viewed on the internet Wi-Fi system. Figure 1 shows the block diagram of the proposed wearable E-integrated sensor.

2.2. Internet of Things based ECG monitoring system

The ECG electrode (dry) is attached to the textile in three different positions as shown in the figure 2. The three electrodes were connected to the heart rate sensor and microcontroller which will acquire the ECG signal. Microcontroller signal could be received in the computer containing Arduino Integrated Development Environment (Arduino IDE) software via the Internet of Things (IoT). The signals were analyzed in smart phone application named blynk. The IoT-based ECG monitoring system is implemented using the advanced techniques of mobile sensing, cloud computing and Web. The ECG monitoring node is responsible for collecting ECG data from the human skin and then sending these data to the access point via a wireless channel.

2.3. Implementation

E-Textile integrated sensors are connected with the electronic textile including electrodes, sensor, and microcontroller, which are used to detect the ECG signal from the human body. Obtained ECG signals (Figure 3) can be viewed through phone application blynk. The data can be transferred from microcontroller to this application. A guardian or doctor can log in to this web portal to access the potassium levels in the blood serum. Through this E-Textile integrated sensor, the early detection of potassium homeostasis can be diagnosed from the obtained ECG signal in a non-invasive manner. Potassium is an important component for electrical activity of the human body. Variations in the potassium levels in the blood serum may lead to medical conditions called hyperkalemia and hypokalemia. A low potassium level called hypokalemia has many causes but usually results from vomiting, diarrhea, adrenal gland disorders or use of diuretics. Hypokalemia can make muscles feel weak, cramp, twitch or even become paralyzed and abnormal heart rhythms. A high potassium level called hyperkalemia can cause life-threatening heart rhythm changes, or cardiac arrhythmias. It can also cause paralysis and weakness. The novelty of our study is to develop a wearable E-textile integrated sensor for the detection of hyperkalemia and hypokalemia through ECG signal. The main advantage of this product is wearable and portable, and also ensures the patient safety and user friendly. It can be easily used for elderly and disabled patients and also for children.

3. RESULTS AND DISCUSSION

In general, the ranges of the serum potassium were clinically observed in blood as 3.5 -

5.0 mEq/L for adults, and greater than 5.0 mEq/L and 5.5 mEq/L; the range in infants and children. Similarly, < 3.5, < 3.0 and < 2.5 mmol/L ranges were observed in case of normal, moderate and severe hypokalaemia conditions (Palaka, Grandy, Darlington, McEwan, & van Doornewaard, 2020).The changes in the ECG signal can be used for indirectly predicting the serum potassium level in the hospitalized patients. The peaked T waves have been obtained when the serum potassium level is increased. Further increase in serum potassium level leads to the ECG report with wide range of PR interval, wide QRS duration and peaked T wave (Chew & Lim, 2005). At severe hyperkalemic condition, the ECG presentation will be like loss of P wave and sinusoidal wave (Galloway et al., 2019). At moderate hypokalemic condition, the ECG presentation looks like flattening and inversion of T waves. During severe hypokalemia, the ECG changes include prolonged Q-T interval, visible U wave and mild ST depression. Ultimately, the obtained ECG can be used to predict the potassium levels at various conditions like hyperkalemia and hypokalemia during remote monitoring without performing the blood test in an unobtrusive manner (Attia et al., 2016).

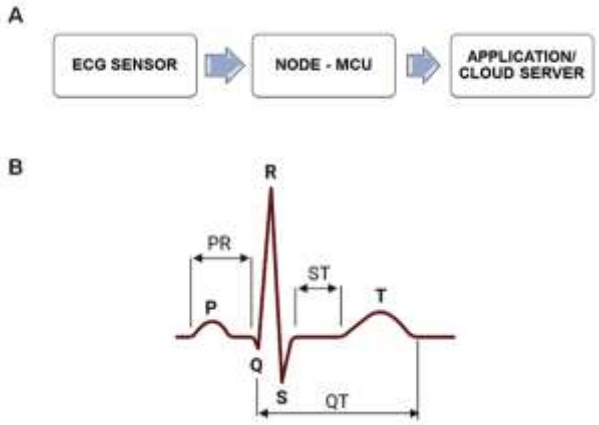

Fig. 1. Block Diagram of the proposed wearable E-integrated sensor (A) and normal ECG Waves (B).

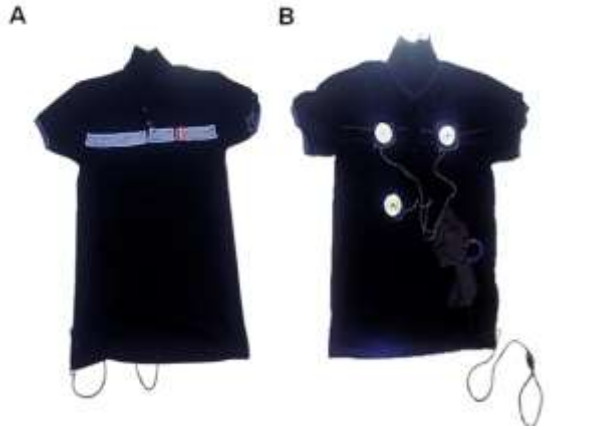

Fig. 2. Positioning of Electrodes in the textile outside view (A) and inside view (B).

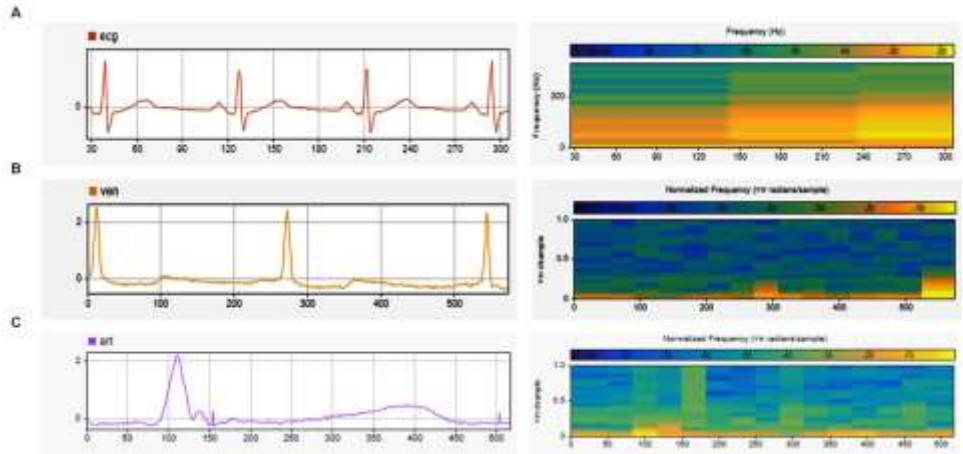

Fig. 3. Comparative analysis of Normal ECG wave (A) ventricle wave (B) and atrium wave (C) and their frequency.

ECG wave pattern depends on the level of serum potassium. When the serum potassium level is 5.5 to 6.5 mEq/L, the ECG will show tall, peaked T-waves. When the serum potassium level is 6.5 to 7.5 mEq/L, ECG will show loss of P-waves or prolonged PR interval (Kim, Oh, & Jeong, 2005).When the serum potassium level is greater than 9 mEq/L, the ECG will show widening of the QRS complex for greater than 0.12 seconds and leading to cardiac arrhythmia associated with severe hyperkalemia (Walter & Bachli, 2002).Potassium Loss Syndrome is a condition when the serum potassium isas below 3.5 mEq/l (Rastegar & Soleimani, 2001). 20% of hospitalized patients are susceptible to hypokalaemia. When the serum potassium level is < 3.0 mmol/L, this condition is called as moderate hypokalaemia, and when the serum potassium level is < 2.5 mmol/L, the condition is known as severe hypokalaemia. In mild hypokalemia, ECG changes include flattening and inversion of T waves, and in more severe hypokalemia condition, the Q-T interval prolongation and visible U wave and mild ST depression occurs. Severe hypokalemia can result in arrhythmias and ventricular tachycardia (Kishimoto, Tamaru, & Kuwahara, 2014).

The proposed work represents the detection of serum potassium levels using electrocardiography. Real-time monitoring and detection of electrolyte imbalance is essential for the management of metabolic disorders. Kwon et al demonstrated an artificial intelligence based model using ECG presentations for the detection of electrolyte imbalance including hypokalemia, hyperkalemia, hypercalcemia, hypocalcemia, hypernatremia and hyponatremia. In this report, the artificial intelligence based model is employed to promptly visualize the important ECG regions for detecting imbalances of each electrolytes, and variations in the P wave, QRS complex, or T wave helped to detect the electrolyte imbalances (Kwon et al., 2021). Deep-learning based model, ECG12Net guides physicians to expeditiously recognize severe hypokalemia and hyperkalemia and thereby possibly reducing cardiac events. In this study, six clinicians-three emergency physicians and three cardiologists were participated in human-machine competition and reported that the specificity, sensitivity performance of ECG12Net has better results compared with that of the physicians for detecting dyskalemias based on ECG signals (C. S. Lin et al., 2020).

Wang et al developed and validated a deep learning model (DLM) for non-invasively screening hypokalemia condition in hospitalized emergency patients and reported that artificial intelligence can be used as promising tool for the detection of hypokalemia after ECG examination. The Convolutional Neural Network based DLM has high accuracy and rapid detection capability and reliable detection of serum potassium level compared with the other traditional methods, and thereby improvising the clinical outcome of the hospitalized patients (Wang et al., 2021). Recent studies suggested that AI assisted ECG can be not only used for early detection of electrolyte imbalance but also employed for prediction of adverse outcomes of hospitalized patients. Lin et al also determined that this point of care non-invasive AI assisted ECG has improved clinical accuracy, specificity and sensitivity compared with that of the invasive blood tests (C. Lin et al., 2022).

The evolution of e-textile technologies is expansively used for various medical applications especially for real-time health monitoring of physiological parameters. The design and development of wearable e-textile based integrated sensor is used for detection of serum potassium level using the obtained ECG signal. In this study, the ECG sensors were completely integrated with clothing which can be used for continuously monitoring the ECG signal. The obtained ECG signal can be sent to the physicians and clinicians for indirectly calculating the serum potassium level. It is also helpful to detect the heart related abnormalities like hyperkalemia and hypokalemia in our body. The main aim of our project is to propose a method to remotely record the ECG of a patient and thereby detecting the serum potassium levels by correlating it with normal ECG waves. This ECG based authentication of serum potassium level has many advantages over the conventional ECG techniques. The main advantage of our system is portable, wearable and comfortable, reduces anxiety level

among patient, and ensures patient safety and no need of technicians to obtain the ECG signal.

6. CONCLUSION

The wearable e-textile integrated sensors for ECG measurement were constructed and the obtained ECG signals were indirectly correlated with serum potassium levels. The obtained ECG can be analyzed to detect the conditions like hyperkalemia and hypokalemia which are life threatening conditions in hospitalized elder and disabled patients. Based on our results, we conclude that the changes in ECG presentation can be strongly correlated with the serum potassium level for detecting potassium homeostatic conditions. However, the correlation between the ECG presentation and serum potassium level might be refined and improved towards the accuracy of estimation of serum potassium levels in the larger population. The unobtrusive and non-invasive E-textiles based potassium level prediction strategy can be implemented with more advancements and sustainability in the future.

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RESEARCH ARTICLE

ANTIBACTERIAL STUDY OF ZnO AND $Zn_{0.5}Mg_{0.5}O$ NANOPARTICLES SYNTHESIZED BY CO-PRECIPITATION METHOD

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ABSTRACT

In the present study, zinc oxide (ZnO) and magnesium doped zinc oxide ($Zn_{0.5}Mg_{0.5}O$) nanoparticles were synthesized by simple and cost-effective co-precipitation method. The synthesized materials were characterized by X-ray Diffraction (XRD), Fourier Transform Infrared (FTIR) spectroscopy, Field Emission Scanning Electron Microscopy (FESEM) with Energy dispersive X-ray spectrum (EDAX). Finally, the effect of magnesium (Mg) doping on the structural, morphological and anti-bacterial property of ZnO nanoparticles was analyzed. From the XRD results, it was found that there was a formation of hexagonal structured ZnO and the average crystallite size of ZnO and Zn0.5Mg0.5O was calculated to be 71 nm and 36 nm respectively. The FTIR analysis confirmed the existence of possible functional groups in ZnO and $Zn_{0.5}Mg_{0.5}O$. There was formation of almost spherical shaped particles as evident from FESEM images and agglomeration of particles was observed upon doping Mg into ZnO. The EDAX spectra of the prepared nanoparticles provided the composition of Zn, O in ZnO and Zn, Mg, O in $Zn_{0.5}Mg_{0.5}O$. No other elements have been found in the EDAX spectra of ZnO and $Zn_{0.5}Mg_{0.5}O$ that confirmed the formation of pure materials. Finally the anti-bacterial study demonstrated that ZnO and $Zn_{0.5}Mg_{0.5}O$ was effective in inhibiting *E. coli* bacteria.

Keywords: Zinc oxide; Magnesium; Dopant; Anti-bacterial; Co-precipitation.

1. INTRODUCTION

The bacterial infections are increasing day by day worldwide. The development of antibiotic resistance of bacteria is one of the major problems in the health sector. Nanotechnology plays a crucial role in developing efficient antibiotics [1]. Antibacterial as well as antiviral activity of a molecule is completely associated with the compounds that destroys the bacteria and virus or slow down their rate of growth, without affecting the nearby tissues. Antibacterial agents are of two types namely organic and inorganic. Organic antibacterial materials are less stable especially in high temperature. But inorganic materials like metals and metal oxides can withstand extreme conditions [2, 3]. ZnO is an inorganic compound appears as a white powder which is insoluble in water, inexpensive and can be easily produced. ZnO has been found to possess good antibacterial property [4]. ZnO nanoparticles possess optical, electrical and photocatalytic property [5,6]. In recent years, ZnO nanoparticles have worldwide usage that has driven attention towards research in biomedical applications focusing on cancer cell imaging [7]. Additionally, it is used as sun screen cream, rubber production and water repellent clothing [8-10]. In the present work, zinc oxide (ZnO) and magnesium doped zinc oxide ($Zn_{0.5}Mg_{0.5}O$) nanoparticles were synthesized by simple, cost-effective co-precipitation method. The synthesized materials were characterized by X-ray Diffraction (XRD), Fourier Transform Infrared (FTIR) spectroscopy, Field Emission Scanning Electron Microscopy (FESEM) with Energy dispersive X-ray spectrum (EDAX). Finally, the effect of magnesium doping on the structural,

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morphological and anti-bacterial property of ZnO nanoparticles were examined. Doping of Mg (Magnesium) into ZnO is expected to modify the properties like crystallite size, surface to volume ratio, antibacterial properties etc [11].

2. MATERIALS AND METHODS

All the materials were purchased and used without further purification.

2.1. Synthesis of zinc oxide (ZnO) nanoparticles by co-precipitation method

For the preparation of ZnO, stoichiometric amount of the starting precursors such as zinc nitrate hexahydrate $(ZnNO_3)_2.6H_2O$ and sodium hydroxide (NaOH) were taken. Initially, zinc nitrate hexahydrate was dissolved in 30 ml of distilled water. It was stirred for around 15 minutes. Meanwhile, sodium hydroxide was dissolved in 30 ml of water. After 15 minutes sodium hydroxide solution was added drop by drop to the aqueous zinc nitrate hexahydrate solution. It was then stirred for another 30 minutes. No specific temperature condition is applied for stirring. The precipitate was formed. The precipitate was centrifuged at 2000 rpm for 10 minutes. This centrifugation was done several times in order to remove the impurities. Then the remaining precipitate was dried at 100^oC in the hot air oven for 2 hours. This dried sample was then placed in a muffle furnace at 500^oC for 3 hours. The obtained material was ground using a mortar and pestle. Thus, ZnO nanoparticles were obtained by co-precipitation method. The obtained ZnO nanoparticles were used for further characterization.

2.2 Synthesis of Mg doped ZnO ($Zn_{0.5}Mg_{0.5}O$) nanoparticles by co-precipitation method

The synthesis of Mg doped nanoparticles ($Zn_{0.5}Mg_{0.5}O$) by co-precipitation method involves the same procedure as ZnO synthesis. But here, in addition to $(ZnNO_3)_2.6H_2O$ and (NaOH), magnesium nitrate hexahydrate $(Mg(NO_3)_2.6H_2O)$ is taken in stoichiometric amount and the same synthesis procedure is followed. For the preparation of $Zn_{0.5}Mg_{0.5}O$, $(ZnNO_3)_2.6H_2O$ and $(Mg(NO_3)_2.6H_2O)$ were dissolved in distilled water separately. These aqueous solutions are mixed and stirred for around 15 minutes. After that, the aqueous sodium hydroxide was added into the above mixture and then again stirred for another 30 minutes. The precipitate was centrifuged, dried and calcined at 500^oC for 3 hours to obtain $Zn_{0.5}Mg_{0.5}O$.

The XRD was performed using X'Pert Pro PANalytical with CuKα radiation (λ=1.5405Å) at 30 mA and 40kV. The fourier transform infrared (FTIR) spectroscopy was performed using SHIMADZU (miracle 10) in the wave number range of 4000-400 cm^{-1}. The field emission scanning electron microscopic (FESEM) images coupled with Energy dispersive X-ray spectrum (EDAX) was recorded using Zeiss FESEM SIGMA VP 03-04 model.

The preparation of the bacterial inoculums for anti-bacterial study is given. At first, the stock cultures were maintained at 4°C on slopes of nutrient agar and potato dextrose agar. The active culture for experiments were prepared by transferring a loop full of cells from stock cultures to test tubes (50ml nutrient broth bacterial cultures) were incubated with agitation for 24hours (37°C) on shaking incubator for 3-5 days. Each suspension of test organism was consequently stroke out on nutrient agar media and potato dextrose agar. These stock cultures were kept at 4°C. For use in experiments, a loop of each test organism was transferred into 50ml nutrient broth and incubated separately at 37°C for 18-20 hours for bacterial culture.

Antibacterial activity was performed by agar diffusion method. The stock culture of *E. coli* bacteria were received by inoculating in nutrient broth media and grown at 37 % for 18 hours. The agar plates of the above media were prepared. Each plate was inoculated with 18 hours old cultures the bacteria were swab in the sterile plates. The extracts were taken in the ratio of 25µl, 50 µl, 75 µl, 100 µl. All the plates were incubated at 37^oC for 24 hours and the diameter of inhibition zone was noted in Cm.

3. RESULTS AND DISCUSSION

3.1 X-ray Diffraction

The XRD pattern of ZnO and $Zn_{0.5}Mg_{0.5}O$ is shown in figure 1. The XRD peaks are sharp that confirm the crystalline nature of both pure and Mg doped ZnO. The diffraction peaks observed at 2θ of 31.8236^o, 34.5707^o, 36.3051^o, 47.5819^o, 56.6296^o, 63.0795^o, 67.9971^o and 69.1325^o corresponds to (100), (002), (101), (102), (110), (103), (112) and (201) planes that corresponds to hexagonal structure of ZnO [12]. The observed peaks are matched with JCPDS card number 79-0205. The crystallite size of the synthesized nanomaterials is calculated using Scherer's formula. The average crystallite size of pure ZnO and Mg doped ZnO is found to be 71 nm and 36 nm respectively. It is evident from the XRD pattern that upon dopant

addition, the crystallite size is decreased. The well-known fact is that upon decrease in crystallite size, the surface area is enhanced. The improved surface area upon Mg doping will be beneficial for various applications including anti-bacterial activity.

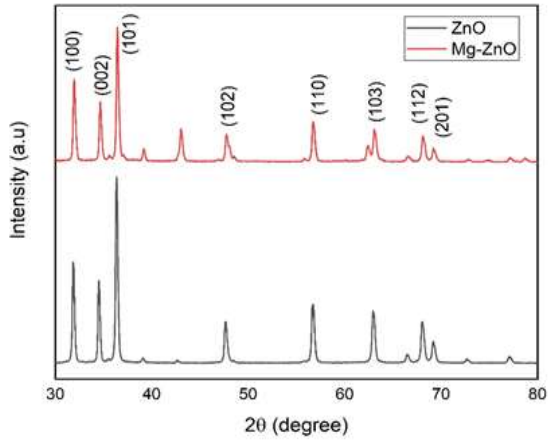

Fig. 1. XRD pattern of ZnO and $Zn_{0.5}Mg_{0.5}O$ (Mg-ZnO) nanoparticles

3.2 Fourier Transform Infrared Spectroscopy:

FTIR spectroscopy is a characterization technique used for the detection of functional groups present in the compounds. The FTIR spectra of ZnO and $Zn_{0.5}Mg_{0.5}O$ are provided in Figure 2.

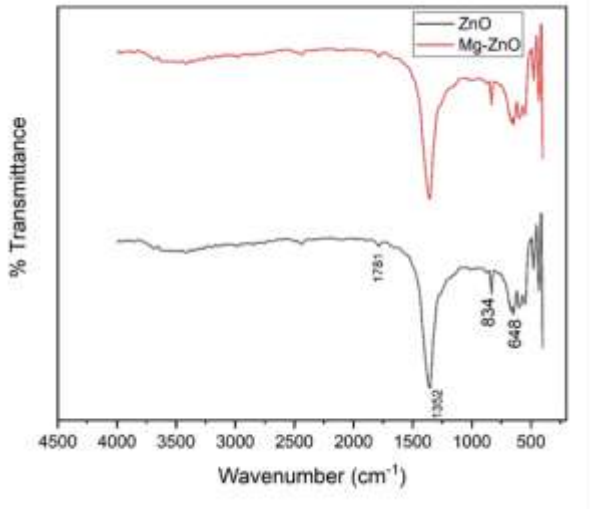

Fig. 2. FTIR spectra of ZnO and $Zn_{0.5}Mg_{0.5}O$ (Mg-ZnO)

The synthesized ZnO showed main bands around 490 cm^{-1} that attributes to Zn-O stretching peak [13]. A slight shift in the band in $Zn_{0.5}Mg_{0.5}O$ illustrates that Mg was successfully incorporated into ZnO lattice. The C-C-O symmetric stretching was observed 834 cm^{-1} [14]. The broad band at 1352cm^{-1} is due to the presence of phyto-active compound bound to the surface of ZnO nanoparticles [15]. The FTIR analysis confirmed the presence of functional groups in ZnO and Mg doped ZnO.

3.3. Field Emission Scanning Electron Microscopy:

The morphological analysis of ZnO nanoparticles was carried out using field emission scanning electron microscopy (FESEM). Figure 3 (a,b) shows the FESEM image of ZnO in different magnifications. There was formation of almost spherical shaped particles. The size of the particles is in the range of 50-100 nm. The elemental compositional analysis of ZnO was carried out using the energy dispersive X-ray analysis and the corresponding EDAX spectra in provided in figure 3 (c). It is apparent from the EDAX spectrum that the prepared nanoparticles are the composition of Zn and O alone and no other elements have been found in the EDAX spectrum. The observed intense peaks correspond to the composition of Zn and O and rest of the peaks arises by carbon tape. The weight percentage of ZnO was found to be 31.81% of O and 68.19% of Zn respectively.

Figure 4 (a,b) shows the FESEM image of $Zn_{0.5}Mg_{0.5}O$ in different magnifications. There was agglomeration of spherical particles observed after the addition of magnesium. The elemental compositional analysis of $Zn_{0.5}Mg_{0.5}O$ carried out using the energy dispersive X-ray analysis showed that the prepared nanoparticles possess only Zn, Mg and O and no other elements have been found in the EDAX spectrum. The observed peaks correspond to the composition of Zn, Mg and O, the weight percentage of ZnO was found to be 43.61% of O, 19.99% of Mg and 36.40% of Zn respectively. The EDAX spectrum conform the successful doping of Mg in pure ZnO.

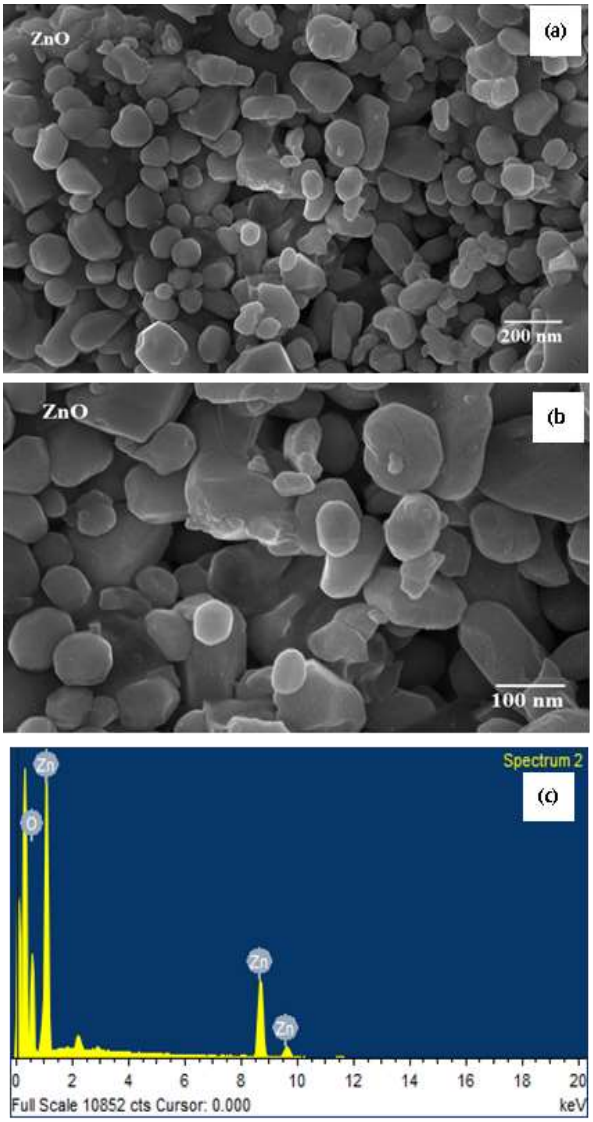

Fig. 3 (a, b) FESEM image (c) EDAX spectrum of ZnO

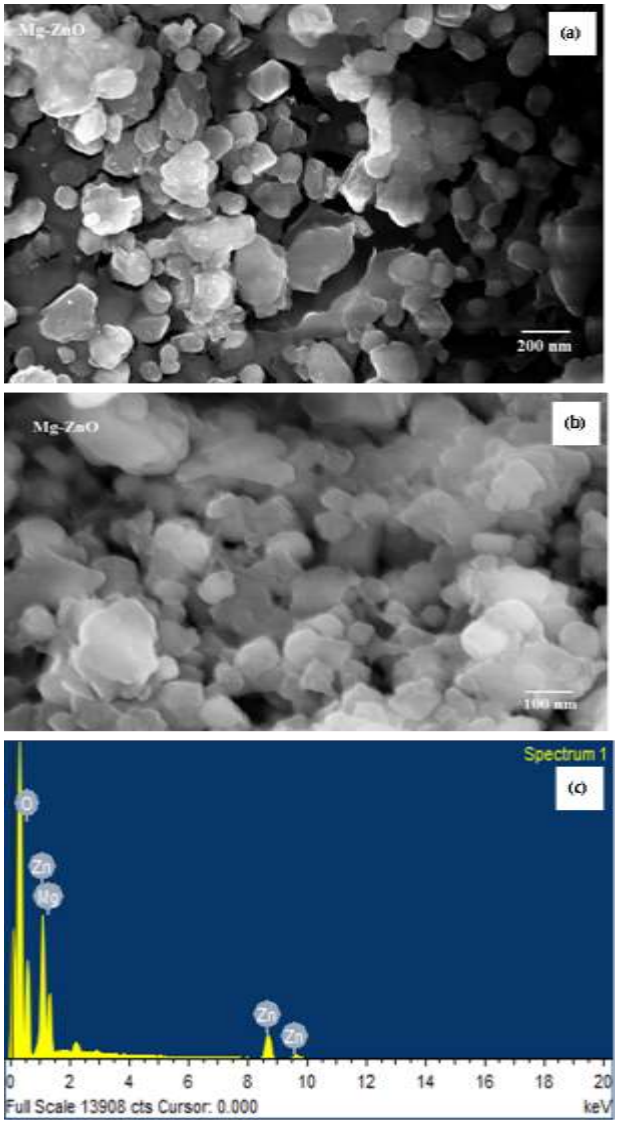

Fig. 4 (a, b) FESEM image (c) EDAX spectrum of $Zn_{0.5}Mg_{0.5}O$ (Mg-ZnO)

3.4 Antibacterial study

Figure 5 (a) and (b) shows the antibacterial activity of ZnO and Mg doped ZnO against *E. coli* bacteria respectively. From the figure we infer that zone of inhibition to the bacteria is present for both the synthesized nanomaterials and hence we can confirm that the synthesized material inhibits the growth of *E. coli* bacteria. The zone of inhibition is measured for different concentrations of the extract and it is tabulated below.

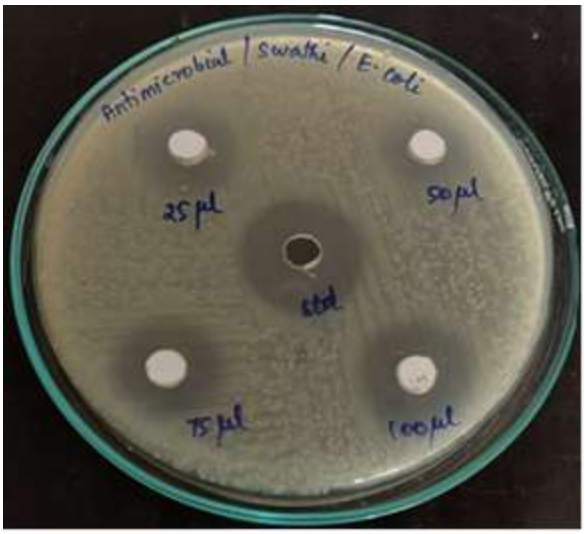

Fig. 5 (a) Antibacterial activity of ZnO nanoparticle against *E. coli*

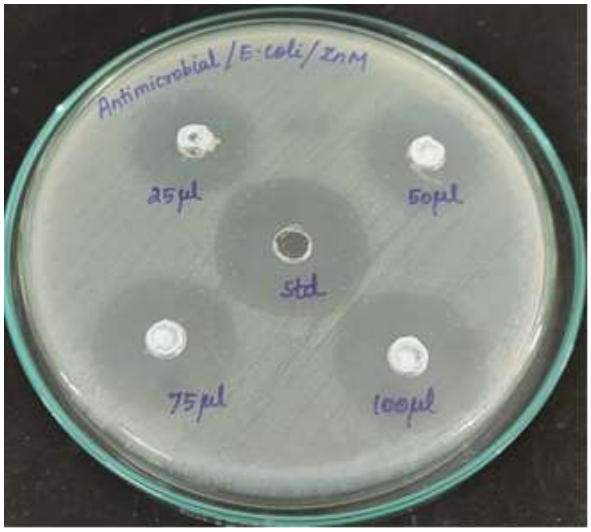

Fig. 5 (b) Antibacterial test of $Zn_{0.5}Mg_{0.5}O$ (Mg-ZnO) nanoparticle against *E. coli*

Concentration	Zone of inhibition
25 µl	0.6 cm
50 µl	0.7 cm
75 µl	1.0 cm
100 µl	1.5 cm
Standard (Chloramphenicol)	1.7 cm

Concentration	Zone of inhibition
25 µl	0.5 cm
50 µl	0.6 cm
75 µl	0.9 cm
100 µl	1.2 cm
Standard (Chloramphenicol)	1.5 cm

From the above table the zone of inhibition, it is evident that ZnO and $Zn_{0.5}Mg_{0.5}O$ are proven to have better antibacterial activity against *E. coli*

.

4. CONCLUSION

ZnO and $Zn_{0.5}Mg_{0.5}O$ nanoparticles were prepared by simple and cost-effective co-precipitation method. The formation of hexagonal phase ZnO nanoparticles were confirmed from XRD analysis. There was a decrease in crystallite structure upon Mg doping which ultimately led to increase in surface area of ZnO nanoparticles. The average crystallite size of ZnO and $Zn_{0.5}Mg_{0.5}O$ was 71 nm and 36 nm respectively. The functional groups present in ZnO and $Zn_{0.5}Mg_{0.5}O$ was confirmed from FTIR analysis. There was a formation of nearly spherical shaped morphology of ZnO nanoparticles. An agglomeration of particles observed upon doping Mg in ZnO nanoparticle. ZnO and $Zn_{0.5}Mg_{0.5}O$ was effective in inhibiting *E. coli* bacteria.

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RESEARCH ARTICLE

ISOLATION AND SCREENING OF KERATINSE PRODUCING BACTERIA FROM CHICKEN FARM BED

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ABSTRACT

Currently, there are about 195 nations in the world, with a population of 7.8 billion. This much higher the population directly influences higher food demand. Due to this interlink massive amount of food waste has been collected from the meat industries; especially poultry plays a major role by discharging feathers into dumps. There are about 8.5 billion tons of feathers that are wasted every year which lead to high global environmental risk and cause different human disease. This high risk is due to typical structure of feather. They have a matrix protein called keratin that makes degradation more tedious under normal condition; they are insoluble in nature with high rigidity due to the disulfide bridges. By extraction of keratin from these waste feathers are used for multipurpose such as cosmetics, biomedical, textile use, bio-fertilizers etc. In addition, as per trend by utilizing nature's gift the microorganisms we can breakdown these matrix proteins easily. By this environmental friendly technique our works gets easier and are also cost effective. By consumption of keratin as substrate some microorganisms produce an extracellular enzyme called keratinase which has the ability to cleave the protein. The produced keratinase is a wealth out of waste which has wide biochemical properties and also used for waste water treatments. On whole the feather considers as waste is completely turned to verity of useful products ubiquitous like biodiesel, biofertilizer, biodegradable plastics, biofilms, skin, hair and other biomedical treatments.

Keywords: Poultry waste, chicken feather bioconversion, keratinase, keratin, microbial enzymes, *Bacillus subtilis* strain KBT.

1. INTRODUCTION

The world is facing the consequences of environmental crisis due to enormous pollutions mainly due to land fill dumping with non-degradable wastes. The products like feathers, hair, horn, skin and nail are trashes that are huge in volume containing over 90% of protein which are tedious to degrade. Predominantly due to poor management of chicken feather which is an organic waste that accumulated in bulk quantities as a by-product in poultry industry has become one of the major pollutants due to its refractory nature. In general, each bird has up to 125 gram of feather that represent 5-7% of the total weight of mature chickens [1]. Meanwhile, more than 400 million chickens being processed every week worldwide [2]. Hence, the accumulation of feather waste reaches five million tons [3]. Most feather waste is land filled or burned that cause global environmental issue such as pollution of both air and underground water resources and protein discharge [4, 1].

Chicken feathers contain more than 85% of crude protein, 70% of amino acids, high-value elements, vitamins and growth factors [5]. Researchers have shown great interest in applying these materials to various products such as feed [6], fertilizer [7] and biofilm [8] etc., chicken feathers have high mechanical stability and are not easily hydrolyzed by common proteolytic enzymes. The refractory nature is due the presence of a protein called keratin which is present in it.

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Keratin is an insoluble protein and is resistant to degradation by common peptidases such as trypsin, pepsin, and papain this resistance is due to the constituent amino acid composition and configuration that provide structural rigidity [9, 10]. The mechanical stability of keratin and its resistance to biochemical degradation depend on the tightly packed protein chains in α-helix (α-keratin) and β-sheet (β-keratin) structures. In addition, these structures are cross-linking by disulfide bridges in cysteine's residues also has hydrogen bonding, and hydrophobic interaction [11, 9, 12]. Chicken feathers are degraded mainly by physical methods (pressurized hydrolysis, and puffing) and chemical methods (acid and alkali) [13, 14, 15]. However, these methods have limitations such as high energy consumption during the production process and substantial of damage to the products [16]. In recent years, biotechnological methods have been used to degrade keratin. Microbial processes are not only environmentally friendly [17] but also maintain the original structure and activity of the products [18]. Currently, studies on biodegradation are focused on the screening and identification of microorganisms that can degrade feathers [19, 20, 21].

Hence, this current study was undertaken for screening, isolation and identification of keratinase producing bacteria from poultry dumped soil for feather degradation and production of value added products using chicken feather waste.

2. MATERIALS AND METHODS

2.1. Feather meal powder preparation

Chicken feathers were collected from chicken stall and processing plant and cleansed with tap water. The main purpose is to clean the feathers from stains, oil and grease before processing it. The feathers were then washed with teepol or soap water and dried under sunlight. The dried feathers are then blended and kept carefully in an autoclave cover and sealed.

2.2. Extraction of keratin crude

2.2.1. Dissolving of Chicken Feathers

Two liters of 0.5 M sodium sulfide solution is prepared in a 2 L conical flask. Fifty grams of blended chicken feathers were weighed and added to the sodium sulfide solution. The pH was maintained about 10-13 and incubated in the shaker at 30 °C for 6 hours. The solution is then filtered and centrifuged at 10,000 rpm for 5 min. The liquid supernatant was carefully collected then filtered using filter paper to make it particle free.

2.2.2. Preparation of Ammonium Sulfate Solution

Seven hundred grams of ammonium sulfate was dissolved in 1 L deionized water. The solution was stirred in cold condition until all the ammonium sulfate particles were dissolved. The solution was then filtered to make it particle free.

2.2.3. Protein Precipitation

The feather filtrate solution collected earlier was placed in a beaker and stirred. Ammonium sulfate solution was added slowly in drop wise. The ratio of feather filtrate solution and ammonium sulfate solution added was 1:1. The solution was then centrifuged at 10,000 rpm for 5 min and the solids particles were carefully collected. The liquid supernatant was collected separately.

2.2.4. Protein Purification

Collected solid particles were added into 100 mL deionized water and stirred (washing). The solution was centrifuged at 10,000 rpm for 5 min and the solids were gathered carefully. The collected solid particles were then dissolved in 100 mL of 2 M sodium hydroxide solution. The solution was then centrifuged again at 10,000 rpm for 5 min and all the liquids were collected carefully and stored while the solids were discarded. The precipitating, washing and dissolving steps were repeated 3 times.

2.2.5. Biuret test

One percentage copper sulphate solution and 1 % potassium hydroxide solution was prepared. Five ml of the sample was mixed with 5ml of potassium hydroxide solution in 1:1 ratio. Two to three drops of copper sulphate solution was added then colour changes in the solution occur from blue colour to violet confirms the presence of protein. The precipitating, washing and dissolving steps are repeated 3 times.

2.2.6. Isolation of keratinase producing bacteria

The soil sample was collected from feather waste dumping site of NGGO colony at Coimbatore in sterilized sampling bags. The samples were brought to the laboratory and processed for analysis on a subsequent day. One grams of soil sample suspended into the 10 ml sterile saline contained in a test tube. The saline sample was serially diluted to 10^{-1} to 10^{-9} fold after the tests were labeled based on dilution number. These suspensions were reinoculated in keratin agar media by spread plate technique. 100 µL of serially diluted samples from 10^{-5}, 10^{-6} and 10^{-8} dilution were taken to make spread plate using keratin agar medium. Inoculated keratin agar plates were labeled as 10^{-5}, 10^{-6} and 10^{-8} respectively then incubated for growth at 37 °C for 48 hours. After incubation colonies which were gowned on the keratin agar plates were sub cultured and screened for keratinolytic activity.

2.2.7. Screening of keratin producing bacteria

The selected isolates were screened for keratinolytic activity, this was done by inoculating the colonies on the screening medium (Keratin solution: 10 ml/L, NaCl: 0.5 g/L, KH_2PO_4: 0.7 g/L, K_2HPO_4: 1.4 g/L, $MgSO_4.7H_2O$: 0.1 g/L, Agar: 15 g/L, pH: 7.5). Screening medium was prepared; isolates were plated and incubated at 37 °C for a week. After incubation clear zone around the colonies was confirmed by addition of 10 % of TCA (Trichloro acetic acid) over the agar plate. Clear zone forming colonies were isolated and pure culture was maintained for further studies.

2.3. Morphological identification of keratinolytic bacteria

2.3.1. Gram staining

Bacterial smears of 16-18 h old were made on clean grease free slides, heat fixed and stained as follows. The slide was flooded with crystal violet for a minute, drained and rinsed with water; followed by grams iodine for one minute, drained and rinsed with water. Decolorized with acetone alcohol for 30 seconds and later counterstained with safranin for one minute and observed under an oil immersion microscope. Violet or purple cells were identified as Gram positive and pink ones as Gram negative.

2.4. Molecular identification of keratinolytic bacteria

2.4.1. Genomic DNA Isolation

Culture isolate of 1.5 mL transferred to microfuge tube and centrifuged at 13000rpm for 2 min. supernatant was discarded and the pellet was suspended with 400µl of STE buffer and vortexd well followed by 50 µL of 10 %SDS was added and mixed gently by inversion and incubated in water bath for 55 ºC for 15min. Further 50 µL of tris saturated phenol: chloroform: isoamyl alcohol was added and vortexd well then centrifuged at 14000 rpm for 10min at 4 ºC. Aqueous phase was carefully transferred to another 1.5 mL centrifuge tube with this 2 volume of ice cold 100 % ethanol was added mixed well and incubated at -20 ºc for 30 min and then centrifuged at 12000 rpm for 15 min. Supernatant was discorded and the dry pellet was dissolved in 50 µL of TE buffer. Isolated genomic DNA was stored at -20 ºC.

2.4.2. PCR studies

Isolated DNA was subjected into 16S rRNA gene amplification using universal bacterial primer such as 8(F) 5′-GGTTACCTTGTTACGACH-3′ and UI492R(R) 5′- AGAGTTTGATCCTGGCTCAG -3′. Amplification reaction was carried out by preparing 20µl reaction mixture consisting of : 10 µL of ready master mix (containing the DNTPs, Taq DNA polymerase, MgCl2 and the reaction buffer); 1 µL template genomic DNA, 1 µL each of forward and reverse primers and 7 µL of nuclease free water. Entire reaction was carried out in Bio- Rad thermal cycler and the reaction parameters comprised of one cycle of initial denaturation at 94 °C for 5 min followed by 30 cycles of denaturation at 94 °C for 1 min, annealing at 52°C for 1min and extension at 72 °C for 1.5 min and the final extension was at 72 °C for 10 min. PCR was performed in a thermocycler (Eppendorf, Germany); a Doc Print transilluminator (Vilber Lourmat, France) was used for visualization and documentation. Amplified product was subjected to electrophoresis on a 1.5 % agarose gel and was found to be 1.5 kb in size.

2.4.3. Enzyme production

Bacterial culture of 2.5 mL was inoculated in keratinase enzyme production medium (Media

preparation (g/l): Keratin solution: 10 mL, NH_4Cl: 1 g, NaCl: 1 g, K_2HPO_4: 0.6 g, KH_2PO_4: 0.8 g, $MgCl_2.6H_2O$: 0.48 g, Yeast extract: 0.2 g, pH: 7.5) and incubated at 37 ºC for a week it was kept in the shaker. After a week of incubation culture was centrifuged at 5000 rpm for 10 minutes. Finally supernatant of crude enzyme was collected and stores in cold room.

2.5. Enzyme purification method

2.5.1. Ammonium sulphate precipitation

The crude keratinase enzyme supernatant was partially purified by 70 % saturation with solid ammonium sulfate (47.2g / 100mL). Then the mixture was allowed to stand for 30 min to 1 h. The precipitate was removed by centrifugation at 12000 rpm for 20 min at 4 °C and the pellet obtained was dissolved in 10mM Tris Hcl buffer with pH 7.5. The precipitated enzyme with 70 % saturated ammonium sulfate was assayed for protein and keratinase concentration as before. Protein concentration (mg/mL) of the enzyme was measured as absorbance at 280 nm using UV spectrophotometer. The precipitated enzyme was dissolved in 10 mM Tris-HCL buffer pH 7.5.

2.5.2. Partial purification: Dialyzing the protein sample

The enzyme sample was dialyzed in the membrane by tying one end with a thread and the samples were filled in through the other end. The tubing was checked for leakage. The dialysis bag containing sample was placed in the beaker containing 800 mL of 1mM Tris-HCL buffer pH 7.5 by frequently changing the buffer overnight at 4 °C on a magnetic stirrer. It was then kept on magnetic stirrer in cold room for overnight. It was set in such a way that the dialysis bag slowly floats around the top of the solution. The dialysis buffer was replaced with fresh buffer every 2 hours. After dialysis, the volume was measured and the samples were analyzed for the keratinase and protein concentration as before. Protein and keratinase activity was determined in all these fractions. The proteins were collected in a centrifuge tube and stored at 4 ºC.

3. RESULTS

3.1. Feather meal production

The finely powered feather meal was stored in a sterile autoclave cover and stored for further analysis (Fig 1)

Fig 1. Feather meal from collected chicken feather waste

3.2. Keratin production

The crude keratin was produced from feather meal. Further protein conformation was done by performing Biurets test change in color from blue to violet confirms the presence of keratin protein. Further extracted protein was quantified as 0.65 mg/mL.

3.3. Isolation and Screening of Keratinase producing bacteria

The keratinase producing bacteria were isolated by clear zone formation around the colony which confirms that isolate has keratinolytic activity (Fig 2).

Fig. 2. Screening of keratinolytic bacteria

3.4. Morphological identification of isolate

The Grams staining seems to identify that the isolate is a Gram positive, rod shaped, motile bacteria.

3.5. Molecular Identification of Keratinase producing bacteria

Genomic DNA of the keratinase producing isolate was isolated. The quality and quantity of DNA samples were determined by electrophoresing the extracted DNA on 1% agarose gel (Fig. 3). A single band of high molecular weight were obtained on agarose gel electrophoresis showing that genomic DNA was intact and free from any mechanical or chemical shearing and therefore it was pure enough for subsequent PCR amplification. Isolated genomic DNA was amplified by 16S rRNA Universal primer in PCR and the amplified product was has 1500 bp (Fig 4).

The PCR products after their cleaning-up were sequenced by Sangar sequencing the obtained sequences were subjected to Bioedit. Total of 475bp partial 16S rRNA sequence was retrieved in FASTA format and subjected for BLAST search in GenBank. BLAST result showed that the query sequence was similar to *Bacillus subtilis* with 99.65% similarity and E value 0.0. Hence the isolated bacteria were identified as *Bacillus subtilis* strain KBT.

Fig. 3. Isolated Genomic DNA on agarose gel

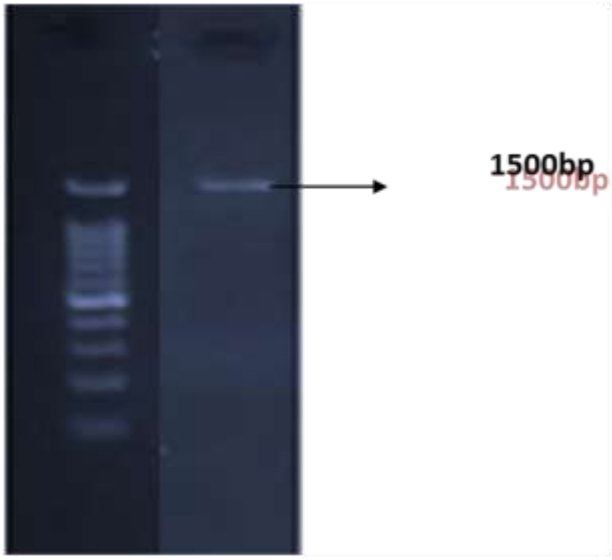

Fig. 4. PCR amplified product

3.6. Feather degradation

Comparing with water and uninoculated control culture treated feathers shown more degradation and also increased percentage of degradation of feather were noted in increased time periods of 7, 14 and 24days of incubation due to the increased production of keratinase enzyme by *Bacillus subtilis* strain KBT (Fig 5). The duration of incubation and feather filtrate weight percentage was given in table 1.

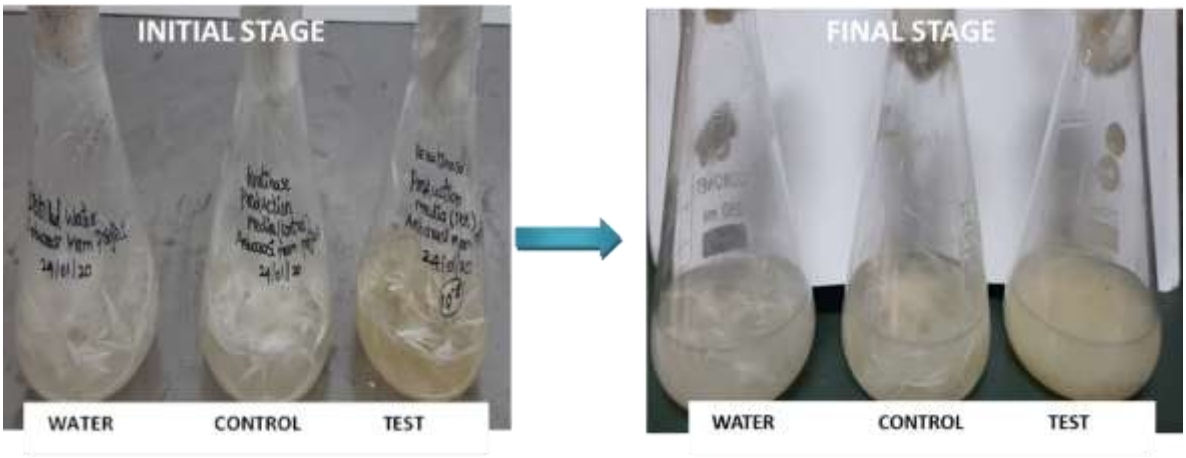

Fig. 5. Chicken feather degradation after inoculation with *Bacillus subtilis* strain KBT

Table 1. Percentage of feather degradation after incubation by the activity of keratinase enzyme

Sample	Initial conc	Final conc	% of Degradation	Keratinase enzyme activity	Keratinase specific activity
Control-1(water)	1.00g	0.99g	1%	-	-
Control-2(medium)	1.00g	0.99g	1%	-	-
7-days	1.00g	0.94g	6%	1.58	0.094
14-days	1.00g	0.86g	14%	2.43	0.144
21-days	1.00g	0.70g	30%	3.60	0.159

3.7. Production and partial purification of keratinase enzyme

Keratinase enzyme was extracted from 48 hrs culture for keratin production medium. The concentration of protein and specific activity of the extracted crude enzyme found to be 2.1mg/ml and 0.13mg/ml, respectively. The After partial purification with 70 % of ammonium sulphate the protein concentration and specific activity noted as 1.9 mg/mL and 0.25 9 mg/mL, respectively. After dialysis Concentration of protein and specific activity quantified as 0.93 mg/mL and 0.27 9 mg/mL, respectively.

3.8. Characterization of extracted keratin solution FTIR

In order to analysis the data, The FT-IR spectrum has been divided into four regions (Fig 6).
Region I (4000 to 3200 cm-1) concerned with water and carboxylic group in chicken feather. In this region the focus is on the revelation of the nature of hydrogen bonding and the carboxylic acids.
Region II (3200 to 1400 cm-1) the bands for functional groups are observed. The functional groups are hydrogen stretching, stretching vibrations lipid acyl group, asymmetric stretching in lipids and proteins were confirmed and the β pleated structures conformation has been obtained.
Region III (1400 – 900 cm-1) has significant importance in the context of biological minerals and

their combinations. The spectra of chicken feather indicates the presence of glucose, deformation of carbohydrates and the characteristics of phosphate ion, carbon ion and also of some functional groups concerned with protein – the Keratin.

Region IV (800 – 600 cm-1) related to cis – double bond (=CH), N-H wagging, CO_2 absorption and SO42- ions and NH_2.

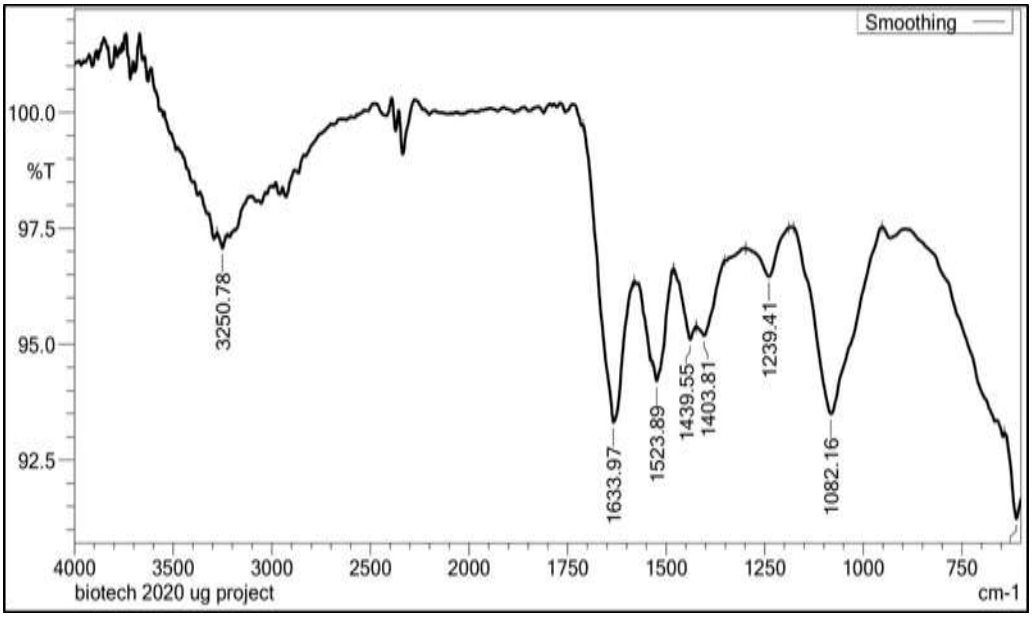

Fig. 6. FT-IR spectroscopy results of extracted keratin

4. CONCLUSION

In this preliminary study, we have isolated and identified chicken feather degrading bacteria *Bacillus subtilis* strain KBT from the soil of a chicken farm bed and screened for their degrading capability of chicken feathers. Result of this study indicates that *Bacillus subtilis* strain KBT is a potential keratinolytic organism and can be used for the biodegradation of keratin in feather industries and can be employed in the production of keratinase. Addition to this, we can turn feather waste into some value added products.

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