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RESEARCH ARTICLE

Evaluation of preliminary Phytochmical, Antidandruff and Antioxidant activity of medicinally and economically important plant, *Wrightia Tinctoria* (Apocynaceae)

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ABSTRACT

The plant samples (leaf and bark) of *Wrightia tinctoria* were collected from the nearby areas of Coimbatore, Tamil Nadu. Preliminary phytochemical analysis, *in-vitro* antioxidant activity and antidandruff activity studies were done using ethanolic and acetone extracts from the leaf and bark of *Wrightia tinctoria.* Phytochemical analysis revealed the presence of Alkaloids, Phenols, Glycosides, Steroids, Terpenoids and Tannins. Antioxidant activity evaluated by the Phosphomoybdenum assay and Metal chelating assay showed that the bark samples exhibited the most antioxidant activity compared to leaf samples in both assays. The antifungal activity of leaf and bark (acetone and ethanol) extracts of *W.tinctoria* on the growth of dandruff causing fungi *Malassezia fufur* done by disc diffusion method, revealed that the extracts of *Wrightia tinctoria* are actively involved in controlling the dandruff causing fungi.

Keywords: *Wrightia tinctoria,* Antifungal activity, Antioxidant activity, *Malassezia furfur*

Introduction

Medicinal plants, rich in bioactive compounds like alkaloids and flavonoids, play a crucial role in traditional and modern medicine. They offer therapeutic benefits for physical, biological, and mental health, and are increasingly popular for their potential to develop new medications. Medicinal plants with antifungal properties provide a natural and effective alternative to synthetic antifungal drugs, reducing health risks and the likelihood of resistance[1],[2],[3].

Phytochemicals are bioactive substances found in plants, offering health benefits beyond basic nutrition. These compounds, such as tannins, alkaloids, terpenoids, and flavonoids, protect plants and enhance their color, aroma, and flavor. Over 4,000 phytochemicals have been identified, each with unique properties. Plants are also rich sources of natural antioxidants, including vitamins C and E, polyphenols, and carotenoids. These antioxidants neutralize free radicals, reducing oxidative stress and the risk of chronic diseases like cancer, cardiovascular, and neurodegenerative disorders. They play a crucial role in the body's defense against free radical-induced damage.

Dandruff

Dandruff, characterized by the shedding of small white flakes from the scalp, affects about 5% of the population, with higher prevalence in males and those aged 20-30[4]. It is commonly caused by the lipophilic fungus *Malassezia*, which thrives on sebum [5],[6],[7]. The condition may be influenced by genetic, environmental factors, and worsens in winter [8],[9]. Treatments include antifungal agents like amphotericin B, clotrimazole, ketoconazole, and other compounds such as salicylic acid and zinc pyrithione.

Wrightia tinctoria

Wrightia tinctoria, a small evergreen tree from the Apocynaceae family, is native to tropical Africa and Asia and known in Tamil as "Paalai" (Vedhanaryanan *et al*., 2013). This plant is rich in secondary metabolites and is used in traditional medicine for various ailments, including skin diseases and liver issues. It exhibits diverse pharmacological properties, such as anti-anticancer activities [10], [11]. Its leaves, seeds,

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and fruits are consumed as vegetables and used in hair oil formulations for their antidandruff properties, with the leaves also producing a blue dye and the bark and seeds treating psoriasis [6].

Materials and Methods

Collection of Plant Material

Wrightia tinctoria leaves and bark were collected in and around Coimbatore, Tamil Nadu, India. The leaves and the bark of *W. tinctoria* were collected from natural and undisturbed areas.

Preparation of Plant Extracts

Twenty-five grams of powdered bark and leaves of *Wrightia tinctoria* were successively extracted with 300 mL of ethanol and acetone for 48 hours using a Soxhlet apparatus. The extracts were then stored in an airtight container at 4°C in a refrigerator until further examination.

Preliminary phytochemical screening of leaf and bark (acetone and ethanol) extracts of *Wrightia tinctoria*

The extracts were subjected to preliminary phytochemical tests to determine the groups of secondary metabolites present in the plant materials [12]

Test for Alkaloids

To 1mL of each extract in two separate test tubes, 2-3 drops of Dragendroff's and Meyer's reagents were separately added. An orange red precipitate/turbidity with Dragendroff's reagent or a white precipitate with Meyer's reagent would indicate the presence of alkaloids.

Test for Flavonoids

To 4 mL of each of the extracts, a piece of magnesium ribbon was added, followed by concentrated HCl dropwise. A colour ranging from crimson to magenta indicates the presence of flavonoids.

Test for Glycosides

Keller Kiliani test: To the 2 mL of extracts, 1 mL of glacial acetic acid with ferric chloride and concentrated sulphuric acid were added. The appearance of a blue colour indicates the presence of glycosides.

Test for Saponins

One mL of extract was added to a test tube and 5 mL of distilled water was added and vigorously shaken. A persistent froth that lasted for at least 15 minutes indicated the presence of saponin.

Test for Tannins

Two mL of the extracts were diluted with distilled water in separate test tubes and 2-3 drops of a 5% ferric chloride ($Fecl_3$) solution were added. A green-black or blue-black colouration indicated the presence of tannin.

Test for Terpenoids

Five mL of extracts were mixed with 2 mL of chloroform and concentrated H_2SO_4 to form a layer. A reddish-brown colouration of the interface showed the presence of terpenoids.

Test for Steroids

Two ml of the extracts were taken in separate test tubes and evaporated to dryness. The residues were dissolved in acetic anhydride, followed by the addition of chloroform. Concentrated sulphuric acid was added to the side of the test tube. The formation of a brown ring at the interphase of the two liquids and the appearance of a violet colour in the supernatant layer indicated the presence of steroids.

Test for phenols

Five mL of the concentrated extracts were taken and 2 mL of a neutral ferric chloride solution was added. The appearance of violet colour indicates the presence of phenols.

Antioxidant Activity

Phosphomolybdenum Assay

The antioxidant activity of the bark and leaf extracts (ethanol and acetone) was assessed using the green phosphomolybdenum complex method[13]. Samples (40 µL) or ascorbic acid (standard) in 1 mM DMSO, or distilled water (blank), were mixed with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The mixture was incubated at 95°C for 90 minutes, then cooled to room temperature. Absorbance was measured at 695 nm, and results were expressed as mg AAE/g extract.

Metal Chelating Activity

The ferrous ion chelating activity of bark and leaf extracts (acetone and ethanol) was measured using the method[14]. Extract samples (100 µL) were mixed with 50 µL of 2 mM FeCl2 and 200 µL of 5 mM ferrozine. After standing at room temperature for 10 minutes, absorbance was measured at 562 nm, with EDTA as the standard. Results were expressed as mg EDTA equivalents/g extract.

Anti-dandruff activity by antifungal method

Sample Collection

Dandruff samples were collected by scraping visible flakes from the scalp using a sterile scalpel. The samples were observed under a compound microscope, treated with 10% KOH, and stained with methylene blue.

Preparation of Saboraud dextrose Agar

The collected dandruff was inoculated onto sterile Sabouraud dextrose agar (SDA) using the spread plate method. SDA was prepared with peptone, dextrose, and agar. The plates were incubated at 32°C to 37°C for 3-5 days. Characteristic white growths around the flakes indicated the presence of organisms causing dandruff.

Growth and Identification

The organism was identified using cultural, microscopic, and biochemical methods. *Malassezia furfur* was isolated in pure culture on Sabouraud dextrose agar with added chloramphenicol. This medium supports the growth of medically significant fungi, while chloramphenicol inhibits unwanted bacterial flora

Inoculum Preparation

The pure culture was inoculated onto Sabouraud Dextrose agar using buds and incubated at 37°C for two days. Discs containing varying concentrations of ethanolic and acetonic extracts of bark and leaf were prepared to determine the minimum inhibitory concentration (MIC) of each extract. A 2% fluconazole disc served as a control. The antifungal activity of the extracts was assessed by the disc diffusion method. After incubation for 48 hours, the plates were removed, and the zone of inhibition around each disc was measured in millimeters. Each concentration was tested in triplicate.

Fresh Leaf and Bark Sample of *Wrightia tinctoria* collected from in and around Coimbatore locality

Powdered sample of leaf and bark of *Wrightia tinctoria*

Culture and Growth pattern of *Malassezia furfur* on Sabouraud's Dextrose Agar Media and Microscopic observation of *Malassezia furfur*

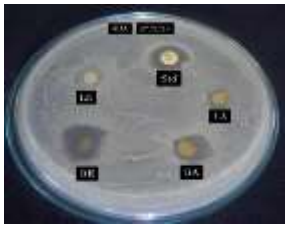
Dandruff

Prepared

isolation of

Microscopic

Antidandruff activity of leaf and bark extracts of ethanolic and acetonic extracts of *Wrightia tinctoria* against *Malassezia furfur* at 5 and 10 mg/mL concentration respectively



Results And Discussion

Table 1. Phytochemical Screening of leaf and bark (acetone & ethanol) extracts of *Wrightia tinctoria*

Sl. No	Phytochemical Constituents	Ethanol Extract			Acetone Extract		Test Name
		Leaf	Bark		Leaf	Bark	
1	Alkaloid	+++	++		-	-	Dragendroff's test
2	Flavonoids	-	-		-	-	Shinoda's test
3	Phenol	-	-		+++	-	Ferric Chloride
4	Glycosides	-	-		-	+	Keller Kiliani test
5	Steroids	-	+		-	-	Liebermann-Burchard's test
6	Terpenoids	+	-		++	-	Salkowski test
7	Saponin	-	-			-	Foam test
8	Tannin	++	-		+++	-	Acetic anhydride

+; moderately present, ++; highly present, -; **absence**

Phytochemical evaluation of *Wrightia tinctoria* extracts (ethanol and acetone) revealed a range of secondary metabolites. Alkaloids were prominent in both the leaf and bark ethanol extracts, showing strong reactions in Dragendroff's test. Flavonoids were absent in all extracts, while phenols were abundant in the acetone leaf extract. Glycosides were detected solely in the acetone bark extract, and steroids were found only in the ethanol bark extract. Terpenoids were present in both ethanol and acetone extracts, and tannins were notably abundant, especially in the acetone leaf extract. Saponins were not detected in any of the extracts.

These findings align with previous studies on *Wrightia tinctoria*. [2] reported the presence of alkaloids, flavonoids, phenols, saponins, steroids, and tannins, although our study did not detect flavonoids or saponins. [15] identified alkaloids and flavones in methanolic extracts, supporting the presence of alkaloids observed in our study. [16] noted various secondary metabolites contributing to the plant's pharmacological activity, which is consistent with our findings of diverse bioactive compounds. Similarly, [17] also identified several secondary metabolites, reinforcing the medicinal potential of *Wrightia tinctoria*.

***In vitro* Antioxidant Activity**

Phosphomolybdenum Assay

The phosphomolybdenum assay revealed significant differences in antioxidant activity among extracts. Ethanol extracts of leaves (249.58 ± SD) showed higher activity than acetone extracts (234.27 ± SD), while acetone extracts of bark (321.9 ± SD) were more effective than ethanol extracts (103.4 ± SD), with all differences being statistically significant ($p < 0.05$). These findings align with previous research by [18] which also demonstrated strong antioxidant activity in *Wrightia tinctoria*.

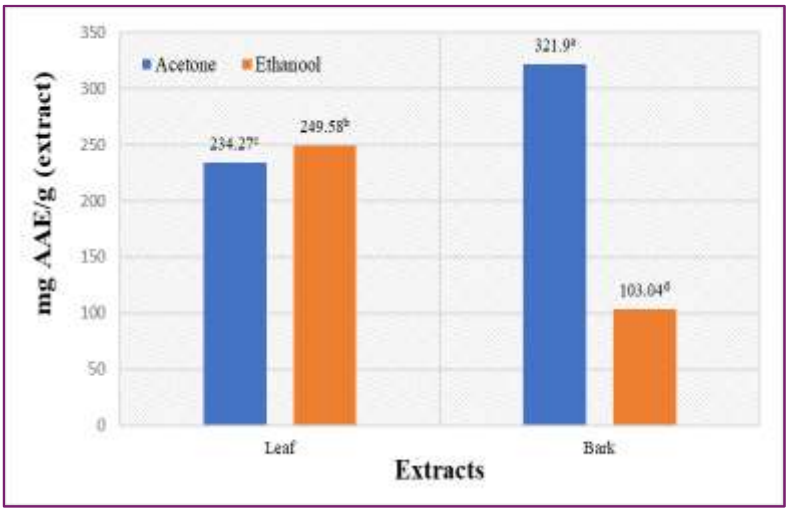


Figure 1: Assay Phosphomolybdenum assay in the leaf and bark (acetonic and ethanolic) extract of *Wrightia tinctoria*. Statistically significant at p < 0.05, where a > b > c. AAE – Ascorbic Acid Equivalents Values are mean of triplicate determination (n=3) ± standard deviation.

Metal Chelating Activity

The metal chelating assay revealed that ethanol extracts from both leaf (63.86 ± SD) and bark (141.54 ± SD) were more effective than acetone extracts, with statistically significant differences ($p < 0.05$). The positive control, BHT (210), exhibited the highest activity. These results are consistent with Dhanabal et al. (2009), who reported high antioxidant activity in *Wrightia tinctoria* leaves with an IC50 of 14.12 ± 0.71 µg/mL, and [17] , who confirmed antioxidant potential using DPPH. [19] also noted significant antioxidant activity (IC50 of 53.64 µg/mL) in TiO2 nanoparticles from *Wrightia tinctoria*. These findings highlight the plant's diverse antioxidant properties and the impact of extraction methods.

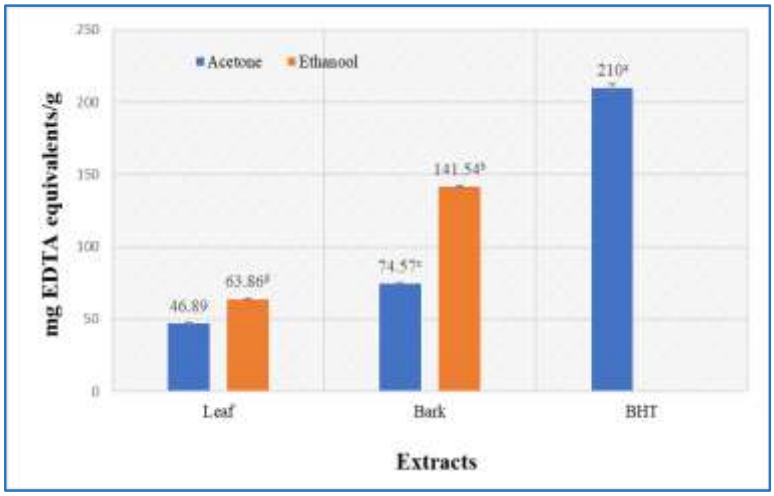


Figure 2: Metal chelating Activity in the leaf and bark (acetonic and ethanolic) extract of *Wrightia tinctoria.* Statistically significant at p < 0.05 where a > b > c. Values are mean of triplicate determination (n=3) ± standard deviation.

Table 2: Mean radius of zone of inhibition of leaf and bark ethanol and acetone extracts of *Wrightia tinctoria* against *Malassezia furfur.*

Zone of inhibition (mm)		
Plant Extract	**5 mg/ml**	**10 mg/ml**
Leaf Acetone	1.3 ±0.11	5 ± 0.11
Leaf Ethanol	0.5±1.5	7 ± 0.17
Bark Acetone	1.5±0.34	10.1 ± 0.38
Bark Ethanol	0.9± 1.0	10.7 ± 0.14
Standard (Flucanazole)	6.0±0.36	11 ± 0.51

The antidandruff activity of *Wrightia tinctoria* extracts varied by solvent and concentration. Ethanol extracts of bark showed the highest inhibition (10.7 ± 0.14 mm) at 10 mg/mL, followed by acetone extracts of bark (10.1 ± 0.38 mm). Leaf extracts, both ethanol and acetone, showed lower inhibition (7 ± 0.17 mm and 5 ± 0.11 mm, respectively). Fluconazole, the standard antifungal, showed consistent inhibition. These results highlight the potential of *Wrightia tinctoria*, especially the bark ethanol extract, for further therapeutic exploration.

Conclusion

The study found that the leaves and bark extracts (acetonic and ethanolic) of *Wrightia tinctoria* contain various phytochemicals with potential to control dandruff-causing fungi and exhibit antioxidant activity.

Acknowledgement

Conflict of Interest

Author has no conflict of interest.

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RESEARCH ARTICLE

The Medicinal potential of weeds: A systematic review of their Phytochemistry

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Abstract

Weeds, often dismissed as unwanted vegetation, have historically been integral to traditional medicine due to their diverse bioactive compounds. This systematic review explores the phytochemical composition and therapeutic potential of medicinal weeds, emphasizing their antimicrobial, anti-inflammatory, and antioxidant properties. Using a comprehensive analysis of peer-reviewed literature, this study identifies key phytochemicals such as alkaloids, flavonoids, terpenoids, and tannins as contributors to their medicinal properties. Findings highlight the untapped potential of weeds in drug discovery, advocating for further exploration and integration into modern therapeutics.

Keywords: weeds, medicinal plants, phytochemistry, bioactive compounds, traditional medicine, drug discovery

1. Introduction

Weeds, defined as plants growing in undesirable locations, have often been overlooked or eradicated in agricultural and urban settings. Despite their reputation as invasive species, many weeds possess significant medicinal value, rooted in traditional medicine practices across the globe (1). Historically, indigenous communities have utilized weeds to treat ailments ranging from infections to inflammatory conditions.Research reveals that weeds are rich in secondary metabolites, including phenolic compounds, alkaloids, flavonoids, terpenoids, and tannins, which contribute to their biological activities (2). However, only a fraction of the world's plant biodiversity has been thoroughly investigated for its therapeutic potential, underscoring the need for systematic research(3). Weeds, often considered as unwanted plants competing with crops for resources, have emerged as significant sources of medicinal compounds, offering a rich tapestry of ethnomedicinal and phytochemical potential. Recent research highlights the diverse medicinal applications of weeds, underscoring their importance in traditional medicine systems across various cultures(4).In regions like the Far-Western Terai of Nepal, weeds are not only abundant but also integral to local ethnomedicinal practices. A study documented 108 weed species used to treat ailments such as fever and heart palpitations, emphasizing the need for conservation and sustainable use of these plants due to their high medicinal value (5). Similarly, Acalypha indica, commonly found in tropical regions, is utilized for its therapeutic properties, including anti-ulcer and anti-bacterial effects, showcasing the plant's potential beyond its classification as a weed(6). The global interest in herbal medicines is rising, driven by their accessibility, cost-effectiveness, and minimal side effects. Weeds, with their diverse phytochemicals such as alkaloids and flavonoids, are increasingly recognized as valuable sources of natural medicine (7). In China, Hypericum species are used for their antidepressant and antimicrobial properties, reflecting the rich traditional knowledge surrounding these plants (8). In South Africa, invasive alien plants and weeds are integrated into traditional medicine, offering potential pharmaceutical properties despite their ecological impact(9). This dual role of weeds as both ecological challenges and medicinal resources underscores the need for innovative approaches to harness their potential(10).In summary, weeds are a valuable yet underexplored source of medicinal compounds, with significant ethnomedicinal applications across different cultures. Their phytochemical diversity offers promising avenues for drug discovery and development, necessitating further research and sustainable management practices. While often viewed as troublesome in agricultural contexts, weeds may harbor a trove of phytochemicals with beneficial medicinal properties(10).

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For centuries, traditional medicine systems around the world have recognized the therapeutic value of plant-derived compounds, and this recognition has sparked a renewed enthusiasm among the scientific community to explore the phytochemical profiles of weed species and their potential applications in modern healthcare. its importance in medicine has become increasingly evident, as a significant portion of contemporary pharmaceutical drugs trace their origins back to natural plant sources (11). phytochemical diversity among weeds could yield novel compounds with unique pharmacological activities, thus positioning them as a promising resource for drug discovery efforts(12). The use of medicinal plants has been a cornerstone of traditional healthcare systems for thousands of years, with various cultures around the world relying on plant-based remedies to treat a wide range of ailments (13)This review aims to consolidate current knowledge on the phytochemistry of medicinal weeds, assess their therapeutic applications, and identify gaps for future research.

1. Methodology

Data Sources: Peer-reviewed articles published between 2000 and 2023 were retrieved from databases including PubMed, Scopus, and Google Scholar.

Inclusion Criteria: Studies focusing on the phytochemical analysis, biological activities, or traditional uses of weeds were included.

Exclusion Criteria: Non-peer-reviewed articles, studies on non-medicinal plants, and papers lacking phytochemical data were excluded.

Search Terms: Keywords such as "weeds," "phytochemistry," "medicinal plants," and "bioactive compounds" were used.

Data Extraction: Information on phytochemicals, biological activities, and therapeutic applications was systematically recorded

2. REVIEW OF LITERATURE

2.1 Medicinal weeds demonstrate broad-spectrum biological activities

Weeds, like other medicinal plants, have been found to possess a wide range of antimicrobial, anti-inflammatory, and antioxidant properties. These properties can be attributed to the diverse phytochemicals present in weeds, such as phenolic compounds, alkaloids, terpenoids, and flavonoids. These bioactive compounds have been shown to modulate inflammatory responses and may contribute to the health-promoting effects of medicinal plants(10)

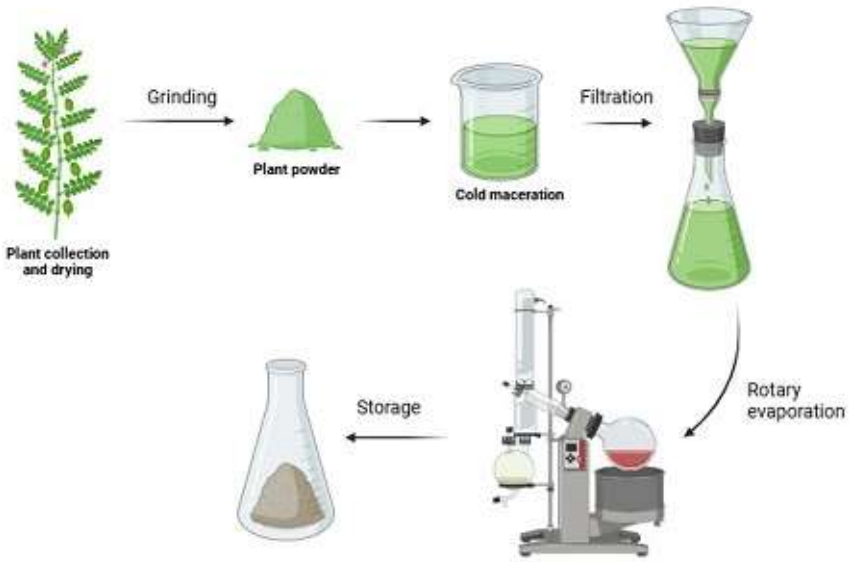

Figure 1. workflow of phytochemical extraction: Starting with plant collection and drying, followed by grinding into powder, cold maceration, filtration, and rotary evaporation, resulting in the storage of concentrated plant extracts for further analysis.

Numerous studies have documented the phytochemical richness of various weed species, highlighting their roles in traditional medicine and modern pharmacology. Weeds, often dismissed as mere nuisances in agriculture, have emerged as significant sources of medicinal compounds with diverse therapeutic potentials. The therapeutic applications of weeds are further

supported by the historical reliance on flowering plants, including various weeds, as foundational sources of medicine(14). This aligns with findings that weeds are beneficial for soil health and serve as untapped reservoirs of medicinal properties due to their phytochemical content(15). For instance, researchers have identified that certain wild weed species possess high concentrations of bioactive compounds, such as sinapic acid and gallic acid, which contribute to their potent antioxidant characteristics and cytoprotective effects(16) Weeds, often dismissed as mere nuisances in agriculture, have emerged as significant sources of medicinal compounds with diverse therapeutic potentials. Numerous studies have documented the phytochemical richness of various weed species, highlighting their roles in traditional medicine and modern citation.json"} (33)The potential of these plants is not limited to traditional uses; modern studies have begun to explore their biochemical properties, revealing significant antioxidant and antimicrobial activities(13). Moreover, the ethnomedicinal practices surrounding weeds have been documented extensively. Nasab and Esmailpour conducted an ethno-medicinal survey that identified several weeds traditionally used to treat gastrointestinal disorders and metabolic issues, underscoring their importance in local healthcare systems (21). This is particularly relevant in developing countries, where access to conventional medicine may be limited. The therapeutic potential of weeds is further illustrated by the findings of Anh et al., who investigated the antioxidant and cytotoxic properties of Andropogon virginicus, revealing its promise in treating various health conditions(22). In addition to their antioxidant properties, many weeds exhibit antibacterial and anti-inflammatory effects. For example, the study by Moh highlighted the allelopathic activities of Croton oblongifolius, suggesting its potential as a natural herbicide while also serving medicinal purposes (23). Similarly, research on Alternanthera sessilis has shown its efficacy in treating conditions like dyspepsia and skin diseases, reinforcing the notion that weeds can be valuable therapeutic agents (24). The diversity of medicinal properties found in weeds is further illustrated by the extensive research on species like Achyranthes aspera and Leucas aspera, which have been shown to possess anticancer and anti-inflammatory properties, pharmacology(17). For instance, Ghosh et al. demonstrated that certain wild weeds possess high levels of bioactive compounds, such as sinapic acid and gallic acid, which contribute to their antioxidant properties and cytoprotective effects (18). This aligns with findings by Shreshtha et al., who noted that weeds are beneficial for soil health and serve as untapped reservoirs of medicinal properties due to their phytochemical content (19). The therapeutic applications of weeds are further supported by Kakar et al., who emphasized the historical reliance on flowering plants, including various weeds, as foundational sources of medicine (20). This is echoed in the review by Jayasundera et al., which discusses the medicinal value of specific weed species from the Asteraceae family, highlighting their use in treating various ailments (17).

Such findings emphasize the need for continued exploration of these plants, as they may offer novel compounds for drug development(25). Recent studies have highlighted the potential of weeds as sources of phytochemicals with medicinal properties. For instance, a comparative study on five wild weeds from West Bengal, India, including Euphorbia hirta and Tridax procumbens, revealed significant antioxidant and antibacterial properties. The study found that aqueous extracts were rich in polyphenols and flavonoids, while ethanolic extracts contained higher levels of alkaloids and cardiac glycosides. Euphorbia hirta, in particular, showed promising antioxidant activity with minimal cytotoxicity (5,26). In South Africa, invasive alien plants and weeds have been integrated into traditional medicine systems. These plants, despite their ecological impact, are rich in secondary metabolites with potential pharmaceutical applications. A review identified 89 plant species used in traditional medicine, with a significant number having documented phytochemical and biological data (9). Parthenium hysterophorus, a notorious weed, has been studied for its medicinal applications despite its harmful nature. It possesses anti-inflammatory, antioxidant, and antimicrobial properties, among others. However, further research is needed to fully understand its clinical and toxicological effects (27). Cyperus rotundus, another widespread weed, has been recognized for its diverse pharmacological activities, including anti-inflammatory and antioxidant effects. Similarly, Chenopodium murale has shown

potential in treating various health conditions due to its rich phytochemical profile (28). Weeds like Chromolaena odorata and Cyperus rotundus have been used in the green synthesis of silver nanoparticles. These nanoparticles have potential medical applications, demonstrating the innovative use of weeds in modern medicine(29) .

Table 1. Recent Research on Medicinal Weeds

Weed Species	Bioactive Compounds	Plant Part Used	Ethnomedicinal Uses	Biological Activity	Extraction Method	Reference
Datura stramonium	Alkaloids	Leaves, seeds	Analgesic, antispasmodic	Antimicrobial, analgesic	Methanolic extraction	(30)
Adina cardifolia	Flavonoids, phenolics	Leaves	Anti-inflammatory	Antioxidant, anti-inflammatory	Aqueous extraction	(11)
Parthenium hysterophorus	Terpenoids, sesquiterpene lactones	Leaves, flowers	Anti-inflammatory, wound healing	Anti-inflammatory, antimicrobial	Ethanol extraction	(10)
Chenopodium album	Saponins, phenolics	Whole plant	Digestive aid, anti-parasitic	Antioxidant, antifungal	Hydroethanolic extraction	(31)
Ageratum conyzoides	Coumarins, alkaloids	Leaves	Antiseptic, wound healing	Antimicrobial, antioxidant	Chloroform extraction	(32)
Eclipta prostrata	Wedelolactone, flavonoids	Whole plant	Hair growth promoter, hepatoprotective	Antioxidant, anti-inflammatory	Aqueous extraction	(33)
Amaranthus spinosus	Phenolic acids, tannins	Leaves, roots	Anti-diabetic, anti-inflammatory	Antioxidant, antimicrobial	Ethanol extraction	(34)
Solanum nigrum	Glycoalkaloids, saponins	Berries, leaves	Anti-ulcer, hepatoprotective	Antioxidant, anti-inflammatory	Methanolic extraction	(35)
Calotropis procera	Cardiac glycosides, flavonoids	Leaves, latex	Anti-arthritic, anti-diarrheal	Anti-inflammatory, antimicrobial	Ethanolic extraction	(36)
Tridax procumbens	Flavonoids, tannins	Leaves, flowers	Anti-diabetic, wound healing	Antimicrobial, antioxidant	Aqueous extraction	(37)

Ipomoea carnea	Alkaloids, phenolics	Leaves, stems	Anti-inflammatory, anti-cancer	Anti-inflammatory, anticancer	Ethyl acetate extraction	(38)

3. Discussion

The therapeutic potential of weeds lies in their phytochemical diversity, which rivals that of cultivated medicinal plants. For example, *Adina cardifolia* and *Datura stramonium* have demonstrated significant antioxidant and antimicrobial properties, attributed to their high flavonoid and alkaloid content (11). Medicinal weeds demonstrate broad-spectrum biological activities, including diverse Phytochemical Constituents: Wild medicinal plants contain a variety of phytochemicals, including phenolics, flavonoids, alkaloids, saponins, and tannins, which contribute to their therapeutic potential (39). Antioxidant Activity: Many wild plants, such as Filipendula ulmaria and Tinospora cordifolia, exhibit significant antioxidant properties due to high levels of phenolics and flavonoids. These compounds help neutralize free radicals, potentially reducing oxidative stress and related diseases (40). Seasonal Variation: The phytochemical content and resulting bioactivity of wild plants can vary with seasons. For instance, the polyphenolic composition of Mediterranean herbs like Thymus longicaulis changes with seasonal stress, affecting their antioxidant and anti-inflammatory properties (41). Comparative Potency: Studies suggest that wild species often have higher phytochemical content and bioactivity compared to their cultivated counterparts. For example, wild Alepidea amatymbica showed higher levels of phenols and flavonoids than cultivated ones, indicating greater antioxidant activity (42). Invasive weeds, often seen as ecological threats, have been found to possess significant ethnobotanical and medicinal uses. These plants are integrated into traditional medicine systems across various regions, offering potential therapeutic benefits.

Future research should focus on:

- Advanced techniques for phytochemical isolation and identification.
- Clinical trials to validate the efficacy and safety of weed-derived compounds.
- Sustainable harvesting practices to mitigate environmental impact

4. Conclusion

Weeds represent an underutilized resource with immense potential for drug discovery and development. Their rich phytochemical profiles and diverse biological activities highlight the need for systematic exploration. By integrating weeds into the broader landscape of medicinal plant research, we can uncover new therapeutic agents and promote sustainable healthcare solutions. In conclusion, the medicinal potential of weeds is vast and multifaceted, encompassing a range of therapeutic applications supported by both traditional knowledge and modern scientific research. The phytochemical diversity found in these plants not only contributes to their efficacy as medicinal agents but also highlights the importance of preserving these species for future medicinal use. In conclusion, weeds offer a valuable source of medicinal compounds with diverse therapeutic applications. However, further research is necessary to fully understand their potential and to address the challenges associated with their use.

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Conflict of Interest

Author has no conflict of interest.

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RESEARCH ARTICLE

Antimicrobial potential of the medicinal plant species of *Acacia Caesia*

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ABSTRACT

This study was designed to evaluate the antimicrobial activity of alcoholic root extracts of *Acacia caesia.* The plant extracts showed strong antibacterial and antifungal activity against both Gram positive and Gram negative microorganisms. The results of the study revealed that the used three alcoholic extracts showed varied degree of antimicrobial activity against the tested human pathogens. However the methanolic root extract exhibited higher inhibition zone (20.33 mm) against the bacterium, *Bacillus subtilis*, whereas the ethyl acetate and methanol extracts showed high degree of inhibition zone against the fungi, *Mucor rouxii* and *Rhizopus* sp. (28.53 and 20.63 mm respectively). Hence, the methanolic root extract was found to be most effective against tested pathogens. These plant extracts which proved to be potentially effective can be used as natural alternative to the various health problems.

Keywords: *Acacia caesia*, Alcoholic extracts, human pathogens, Disc diffusion method

1. INTRODUCTION

The need for less toxic, more potent and non-anti-infective antibiotics, as well as the evolving resistance of microorganisms are some of the medicinal areas that have posed a challenge to therapeutics since 1990s. A corresponding situation exists in the agricultural sectors. These factors have been combined effect of injecting a sense of urgency into the search for new bioactive compounds (1). In agriculture, the crop loss due to plant pathogens has become major concern. Increased usage of different chemicals based products to control these pathogens has resulted in problems like residual effect of chemicals in agri-based products, increased resistance for chemicals in target pathogens and environmental pollution. The plants have been an essential part of human society since the start of civilization.

Medicinal plants represent a rich source of antimicrobial agents. Plants are used medicinally in different countries and are a source of many potent and powerful drugs (2). Plants contain a variety of bioactive compounds that provide them antimicrobial properties, which can be used to develop novel antibiotics(3). Among the large number of plants, the important medicinal species, *Acacia caesia.* The leaves of the study species, *Acacia caesia* are used as vegetable. This part is used in the treatment of asthma and skin diseases (4). The powdered bark and pod are used as substitute for soap and their decoction is used as lice killer (5,6). Further, the bark extract is used as protective coat in boats and fishing nets against corrosive (4). Woody branches are used as tooth-brushes by tribal folk. The soft beaten bark of the plant has cleaning properties and protects the skin against microorganisms. The flowers are reported to be used by sandal women to treat menstrual disorders (7,8,9,10). The bark of the plant is used as shampoo for cleaning the hair, and shoots are used in the treatment of scabies (11). With these multiple values, this species is exploited severely by the local public in western districts of Tamil Nadu. As this plant species occurs principally on hill slopes, it has an important role in the protection of the integrity of the slopes by checking soil erosion. Hence, in the present study an attempt has been made to find out the antimicrobial properties of the root of the species and hence to assess its therapeutic potency.

2. MATERIALS AND METHODS

2.1. Plant Material

Fresh root parts were collected from the population of *Acacia caesia* present in the Maruthamalai Hills of oimbatore District and

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washed under running tap water, air dried and then homogenized to fine powder and stored in air tight bottles.

1.1. Preparation of Extracts

100g air-dried root powder was subjected to 250ml of methanol in soxhlet extraction for 8 hours (50-85°C). The extracts were concentrated to dryness in a flask evaporator under reduced pressure and controlled temperature (50-60°C) to yield crude residue. The crude residue was stored in the refrigerator. To obtain the other chemical extracts, the similar methods as used to obtain methanol extract was adopted by using the solvents *viz*., petroleum ether and ethyl acetate.

1.2. Media Used

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

1.3. Microorganisms

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

1.4. Antimicrobial Assay

The alcoholic extracts were tested for their effect against pathogenic bacteria and fungi by disc diffusion method (12). The organisms bacteria and fungi tested were inoculated into nutrient agar and PDA media respectively. After an incubation period of 24 hrs at a temperature of 35^0C, three or four colonies isolated from these media were inoculated into 4ml of nutrient broth and incubated for 2 hrs at 35^0 C. The cultures were adjusted with sterile saline solution to obtain turbidity. Petri dishes containing Muller- Hinton agar and PDA medium were streaked with these microbial suspensions of bacteria and fungi respectively. Disks of 6mm diameter were impregnated with different extracts *viz.,* petroleum ether, methanol and ethyl acetate. Tetracycline is used as positive control. After equilibrium at 4^0 C, the plates were incubated overnight at 37^0 C and the diameter of any resulting zones of inhibition was measured. Each experiment was repeated at least three times.

2. RESULTS AND DISCUSSION

The results obtained in the present study relieved that the tested medicinal plant extract possess potential antimicrobial activity against ten bacteria and fungi and the findings are summarized. The roots of *Acacia caesia* collected from Maruthamalai hills were dried and powdered. Powdered roots were extracted successively using polar solvents *viz*., petroleum ether, ethyl acetate and methanol. The inhibition effect of alcoholic root extracts of this species is given in Table 1. The study reports that the methanol extract showed highest inhibitory activity against the growth of the bacteria, *Bacillus subtilis* (20.33 mm diameter inhibitory zone) and *B. thuringiensis* (15.87mm diameter inhibitory zone). The other extracts also exhibited the moderate activities against the growth of Bacterial culture used. Petroleum ether extract also showed higher inhibitory zone against the bacterium, *B. thuringiensis* by producing 12.67 mm diameter inhibitory zone and ethyl acetate extract showed maximum zone of inhibition against the bacterium, *Serratia* sp. 12.16 mm diameter inhibitory zone. The antifungal activity of the various alcoholic extracts of root part of the species, *A. caesia* against ten fungal species tested showed the following results: The inhibition effect of alcoholic root extracts of this species is given in Table 2. The study exhibited that the ethyl acetate extract showed highest inhibitory activity against the growth of the fungus, *Mucor rouxii* by producing 28.53 mm diameter inhibitory zone. Petroleum ether and methanol extracts showed higher inhibitory zone against the fungi, *Alternaria*

alternata (20.77 mm diameter inhibitory zone) and *Mucor rouxii* and *Rhizopus* sp. (20.63 mm diameter inhibitory zone) respectively.

The overall study on antimicrobial activity reports that the plant species containing adequate active compounds to reduce or check the growth of microbial colonies. The beneficial medicinal effects of these plant materials typically results from the combinations of secondary products present in the plant. These compounds are mostly secondary metabolites such as alkaloids, steroids, tannins, phenol compounds *etc.* which are synthesized and deposited in specific parts of the study species (13). The heterogeneity of these secondary compounds in wild species is reported to be wide (14). Based on this concept, it is explained that the study species due to the heterogeneity of secondary compounds owing to their wildness could be with higher antimicrobial activity. The higher antimicrobial activity of alcoholic extracts of the present study species may further indicates that the antimicrobial principles/chemical constituents which are either polar or nonpolar can be effectively extracted only through the organic solvent medium (15,16,17,18,19). The positive outcomes observed in the antimicrobial tests coupled with the presence of bioactive compounds in these extracts establish a promising foundation for pharmaceutical exploration (20). It can therefore be suggested that crude extracts contain potential antimicrobial compounds and the obtained results may also be useful for evaluating substances of interest.

3. CONCLUSION

The plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. The result showed the potential antimicrobial effects against tested bacterial and fungal microorganisms. Further investigation need to isolate and purify the active compounds to formulate new drugs.

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Table 1. Antibacterial activity of certain alcoholic root extracts of the species, Acacia caesia.

Plant extract	Diameter of zone inhibition (mm)									
	Gram positive bacteria				Gram negative bacteria					
	Bacillus subtilis	***B. thuringiensis***	***Micrococcus* sp.**	***Lactobacillus* sp.**	***Klebsiella pneumoniae***	***Escherichia coli***	***Pseudomonas stutzeri***	***P. aeruginosa***	***Serratia* sp.**	***Moraxetta* sp.**
Standard *	27.23 ± 0.49	30.67 ± 0.61	23.23 ± 0.49	25.03 ± 0.65	9.77 ± 0.93	25.06 ± 0.70	12.13 ± 0.42	26.63 ± 0.60	30.57 ± 0.67	27.16 ±0.37
Petroleum ether	-	8.63 ± 0.65	9.07 ± 0.40	-	-	6.93 ± 0.40	-	-	12.16 ± 0.37	8.66 ± 0.61
Ethyl acetate	7.87 ± 0.90	12.67 ± 0.61	10.03 ± 0.55	8.97 ± 0.35	8.03 ± 0.45	8.97 ±0.55	8.07 ±0.50	9.06 ± 0.21	11.03 ± 0.65	10.03 ± 0.45
Methanol	20.33 ±0.76	15.87 ± 0.85	10.16 ± 0.38	7.77 ±0.75	-	10.03 ± 0.35	-	10.03 ± 0.35	11.03 ± 0.25	9.83 ± 0.80

*** Tetracycline**

Table 2. Antifungal activity of certain alcoholic root extracts of the species, *Acacia caesia*.

Plant extract	Diameter of zone inhibition (mm)									
	Aspergillus niger	***A. flavus***	***A. baumanii***	***Fusarium oxysporum***	***F. solani***	***Mucor rouxii***	***Alternaria alternata***	***Candida albicans***	***Cladosporium* sp.**	***Rhizopus* sp.**
Standard *	27.03 ± 0.21	30.63 ± 0.60	26.77 ± 0.65	30.67 ±0.61	25.63 ± 0.57	28.67 ±0.65	27.77 ±0.71	9.67 ± 0.65	12.77 ± 0.71	33.67 ± 0.61
Petroleum ether	7.73 ± 0.54	12.73 ± 0.75	-	-	-	10.77 ±0.75	20.77 ±0.75	-	-	10.73 ±0.67
Ethyl acetate	14.77 ±0.58	10.73 ± 0.70	9.77 ± 0.71	10.73 ±0.70	8.73 ± 0.67	28.53 ±0.55	10.73 ±0.70	7.63 ± 0.60	-	13.63 ±0.57
Methanol	19.63 ± 0.49	13.63 ± 0.65	15.67 ± 0.61	15.67 ± 0.65	12.13 ± 0.32	20.63 ±0.80	14.73 ±0.67	-	15.73 ±0.67	20.63 ± 0.60

*** Tetracyclin**

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RESEARCH ARTICLE

Corrosion inhibition of mild carbon steel in well water medium L-TRYPTOPHAN - Zn^{2+} System

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ABSTRACT

The environmentally friendly inhibitor system, L-Tryptophan-Zn^{2+}, was investigated using the weight loss method. A synergistic effect was observed between L-Tryptophan and Zn^{2+} system. The formulation comprising 250 ppm of L-Serine and 50 ppm of Zn^{2+} demonstrated an impressive inhibition efficiency of 91%. UV-Visible spectroscopy revealed the formation of a protective film on the metal surface. FTIR analysis suggested that the Zn^{2+}-L-Tryptophan complex formed at the anodic sites of the metal surface, which inhibited the anodic reaction, while $Zn(OH)_2$ formed at the cathodic sites, controlling the cathodic reaction. A corrosion inhibition mechanism was proposed based on the results from weight loss studies and surface analysis techniques. Synergism parameters were calculated and found to be greater than 1, confirming the synergistic effect between L-Tryptophan and Zn^{2+}. The surface morphology of the compound was examined using SEM and EDAX.

1. INTRODUCTION

Corrosion is a natural process that converts a refined metal into a more chemically stable oxide. It is the gradual deterioration of materials (usually a metal) by chemical or electrochemical reaction with their environment. In the most common use of the word, this means electrochemical oxidation of metal in reaction with an oxidant such as oxygen, hydrogen or hydroxide. Rusting, the formation of iron oxides, is a well-known example of electrochemical corrosion.(1)

Corrosion inhibitors are chemicals that, when added in small amounts to a hostile environment, reduce the rate of attack on a material such as a metal. The corrosion inhibitor slows down the rate at which a metal in that environment corrode.(1) Uniform corrosion, pitting, crevice corrosion, filiform corrosion, galvanic corrosion, environmental cracking, and fretting corrosion.(1) Corrosion prevention refers to the implementation of strategies and techniques aimed at reducing or eliminating the deterioration of materials caused by chemical reactions with the environment. There are effective corrosion prevention methods that can extend the life of metal equipment by up to 250%. Not all corrosion prevention methods are equal.(2) An inhibitor is a chemical compound that effectively reduces the corrosion rate of metal when added in small concentration or otherwise mildly aggressive medium or environment. Inhibitors are classified as anodic, cathodic or mixed inhibitors. Inhibitors reduces corrosion by interfering with one or more of the corrosion reactions and affecting the corrosion process as a whole. As most corrosion inhibitors have a certain amount of toxicity, care must be taken to ensure that their application confirms to prevaile environmental and health regulations. Economic consideration also governs their selection.(3)

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Amino acids contain amino group and carboxyl group. They contain electron rich nitrogen atom and oxygen atom. These electrons can be released to the metal surface and thus corrosion of metals can be prevented. Corrosion of many metals have prevented by amino acids in acidic, basic, and neutral medium. Usually, weight loss method and electrochemical studies have been employed to evaluate the corrosion inhibition efficiency of amino acids. Adsorption of amino acids on metal surface obey Frumkin, Langmuir, Temkin and Freundlich adsorption isotherms. The protective film formed on metal surface in presence of amino acids have been analyzed by FTIR spectra, SEM, EDAX and XRD pattern.

Amino acids have amino group (-NH2) and carboxyl group (-COOH). Lone pair of electrons are available on nitrogen atom and oxygen atom. Hence coordination of amino acids with metal atoms can take place through both these atoms. When there is flow of electrons from these electron rich centres, to electron deficient centres of the metal, corrosion rate will be reduced. Hence amino acids are widely used as, corrosion inhibitors [1-26].

The principal application areas for inhibitors are,

i. Industrial water systems - for cooling, processing, boiler and condenser systems.
ii. Natural water for human use in 6 to 8.5 pH range.
iii. In aqueous solutions of acids used for metal cleaning
iv. In non- aqueous media and in the mineral oil industry during production of crude and its subsequent refining and processing.
v. For the protection of metallic components e.g., machinery tools, electronic hardware etc., [2]

2. MATERIALS AND METHODS

2.1. Weight loss method

The weight losses are found by keeping identical specimens for a constant time and temperature in the solution under study. Inhibition efficiency (%) is calculated by using the formula.

$$IE(\%)=100\ [1 - W2/W1]\ \%$$

Where W1 is the weight loss in the absence of inhibitor

W2 is the weight loss in the presence of inhibitor.[10]

2.2. FTIR Spectra

The carbon steel specimens immersed in various test solutions for one day were taken out and dried. The film formed on the metal surface was carefully removed and thoroughly mixed with KBr, so as to make it uniform throughout. The FTIR spectra were recorded in a Perkin- Elmer 1600 spectrophotometer. [10]

2.3. Ultraviolet and visible spectroscopy

While interaction with infrared light causes molecules to undergo vibrational transitions, the shorter wavelength, higher energy radiation in the UV (200-400nm) and visible (400-700nm) range of the electromagnetic spectrum causes many organic molecules to undergo electronic transitions. What this means is that when the energy from UV or visible light is absorbed by a molecule, one of its electrons jumps from a lower energy to a higher energy molecular orbital.[6]

2.4. Surface Morphology methods

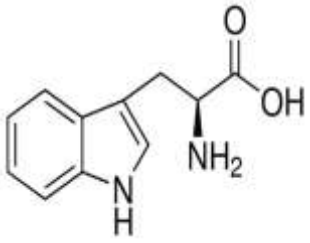
Interfacial tension measurements, double layer capacity measurements, radio tracer technique, X- ray photoelectron spectroscopy, soft X-ray spectro microscopy, auger electron spectroscopy, electron microscopy, magnetic susceptibility, XRD, SEM, ESCA, AFM methods have also been useful to study the mechanism of some inhibitor.[6]

2.5. Characteristics of inhibitor

L-Tryptophan Acid

Molecular weight = 204.23 g/mol
Molecular Formula = $C_{11}H_{12}N_2O_2$

Structure of L-Tryptophan Acid



Glutamic acid is an essential amino acid. L-Tryptophan Acid is an antioxidant. It may help to protect the body from damage caused by ionizing radiation. It also prevents liver damage from acetaminophen poisoning. L-Tryptophan is the L-enantiomer of glutamic acid. It has a role as a nutraceutical, a micronutrient, an antidote to paracetamol poisoning, a human metabolite and a mouse metabolite. It is used in the biosynthesis of

proteins. In plants and microorganisms, tryptophan acid biosynthesis belongs to the aspartate family, along with threonine and lysine. The main backbone of glutamic acid is derived from aspartic acid, while the sulphur may come from cysteine or hydrogen sulfide.

2.6. Determination of corrosion rate

The weighed specimens in triplicate were suspended by means of glass or plastic hooks in 100 ml beakers containing 100 ml of various test solutions. After 7 days of immersion, the specimens were taken out, dried and washed. From the change in weight of the specimens, corrosion rates were calculated using the following relationship.

Corrosion rate = Loss in weight (mg)Surface area of the specimen dm2 × Period of immersion (days)

The corrosion rate is expressed in mdd units [mdd = mgm/(dm2)(day)]

Corrosion inhibition efficiency (I.E) was then calculated using the equation given below.

$$IE=100\ (1-W2W1)\%$$

Where W1 = Corrosion rate in the absence of inhibitor

W2 = Corrosion rate in the presence of inhibitor

2.7. Synergism Parameters (S_I)

The synergism parameters (SI) were calculated using the relation as stated below[]

$$S_i = 1+ \theta_{1+2}/1-,\theta_{1+2}$$

Where $\theta_{1+2} = (\theta_1 + \theta_2) - (\theta_1 - \theta_2)$

$\theta 1+2'$ = Combined inhibition efficiency of substance 1 and substance 2

If the resultant value of SI is greater than 1, then the result confirms the synergistic effect between the inhibitor and the additives.

3. MATERIALS AND METHODS

3.1. Preparation of mild carbon steel specimen

The mild carbon steel specimen used for the experiment is in the composition following composition.

Table 1 : Composition of mild carbon steel

Elements	C	Mn	P	Si	S	Cr	Ni	Mo	Fe
Composition (%)	0.017	0.196	0.009	0.007	0.014	0.043	0.013	0.015	99.686

Mild carbon steel specimen of the above composition was analysed by using vacuum emission spectrometer DV-4 (supplied by BAIRD Corporation of India) and of the dimensions 1.0 cm × 4.0 cm × 0.2 cm were polished to mirror finish using emery sheets, washed with distilled water, dried and were used for the weight loss and surface examination studies.

3.2. Chemicals used

L-Tryptophan
Zinc sulphate
Ferrous sulphate

3.3. Well Water

For the present study, well water of The American College, Madurai was used. Corrosion behaviour of mild carbon steel in this water was evaluated.

Table 2: Water Parameters

S.No	Parameters	Test Method	Unit	Range
1	PH	IS 3025 part 11- 1983	-	7.93
2	Total Hardness as $CaCO_3$	IS 3025 Part 21- 2009	mg/L	620
3	Total Alkalinity	IS 3025 Part 23- 1986	mg/L	540

4	Chloride as Cl	IS 3025 part 32- 1988	mg/L	600
5	Sulphate as SO_4	IS 3025 part 24- 1986	mg/L	99
6	Nitrate as NO_3	APHA. 23rd Edition 2017- 4500 NO_3 B	mg/L	15
7	Fluoride as F	APHA. 23rd Edition 2017 - 4500 - F- D	mg/L	Less than 0.2

3.4. Preparation of stock solution

1g of L - Tryptophan Acid was dissolved in well water and made up to 100ml in a standard measuring flask. 1ml of this solution was diluted to 100ml to get 100ppm of L - Tryptophan Acid.

Zinc sulphate solution

Exactly 1.1g of Zinc sulphate was dissolved in well water and made up to 250ml in a standard measuring flask.

The details regarding the preparation of various environments used for weight loss method in the present study is given in the Table 1,2, 3.

Table 3: L- Tryptophan Acid and $ZnSO_4$

S.No	L- Tryptophan Acid (ppm)	$ZnSO_4$ Solution (ppm)	Total volume made up to 100 ml with well water
1	-	-	100
2	50	0	100
3	100	0	100
4	150	0	100
5	200	0	100
6	250	0	100

Table 4: L- Tryptophan Acid and $ZnSO_4$ (10 ppm)

S.No	L- Tryptophan Acid (ppm)	$ZnSO_4$ solution (ppm)	Total volume made up to 100 ml with well water
1	-	-	100
2	50	10	100
3	100	10	100
4	150	10	100
5	200	10	100
6	250	10	100

Table 5: L - Tryptophan Acid and $ZnSO_4$ (50 ppm)

S.No	L- Tryptophan Acid (ppm)	$ZnSO_4$ solution (ppm)	Total volume made up to 100 ml with well water
1	-	-	100
2	50	50	100
3	100	50	100
4	150	50	100
5	200	50	100
6	250	50	100

The environment chosen for the surface examination studies his is given in the Table 6. Among the several inhibitor combinations, the one which offered the highest inhibition efficiency was chosen as the environment.

Table 6: Preparation of environment for surface examination studies

S.No	Inhibitor	Environment Chosen
1	**L-** Tryptophan Acid	Well Water + L- Tryptophan Acid (250ppm) + Zn^{2+}(50 ppm)

4. RESULT AND DISCUSSION

4.1. Analysis of results of the weight loss method

Inhibition efficiency (IE%) of L-trytophan -Zn^{2+} systems in controlling corrosion of carbon steel immersed in well water in the presence and absence of inhibitor system (Immersion period = 7 days) are given in the table 6-8. It is observed that L-Tryptophan alone has poor inhibition efficiency. In the presence of various concentrations of Zn^{2+}(10 and 50 ppm) the IE of L-tryptophan increases. A synergistic effect exists between L - tryptophan and Zn^{2+}.[16]

Table 6: Corrosion rates (CR) of mild steel immersed in well water in the presence and absence of inhibitor system at various concentrations and the inhibition efficiencies (IE) obtained by weight loss method.

Inhibitor system : L-Tryptophan Acid Zn^{2+}(0 ppm)
Immersion period. : 7 days
pH : 5.5 - 7.0

L- Tryptophan Acid (ppm)	Zn^{2+} ppm	CR (mdd)	IE (%)
0	0	35.06	-
50	0	19.0	13.9
100	0	17.4	22.0

150	0	15.2	31.1
200	0	14.0	36
250	0	12.6	42.9

Table 7: Corrosion rates (CR) of mild steel immersed in well water in the presence and absence of inhibitor system at various concentrations and the inhibition efficiencies (IE) obtained by weight loss method.

Inhibitor system : L- Tryptophan Acid Zn^{2+} (10 ppm)
Immersion period : 7 days
pH : 5.5 - 7.0

L- Tryptophan Acid (ppm)	Zn^{2+}ppm	CR(mdd)	IE(%)
0	10	35.06	-
50	10	10.11	61
100	10	4.5	82
150	10	4.3	83
200	10	3.63	86
250	10	2.38	90

Table 8: Corrosion rates (CR) of mild steel immersed in well water in the presence and absence of inhibitor system at various concentrations and the inhibition efficiencies (IE) obtained by weight loss method.

Inhibitor system : L-Tryptophan Acid Zn^{2+}(50 ppm)
Immersion period : 7 days
pH : 5.5 - 7.0

L-Tryptophan Acid. (ppm)	Zn^{2+}ppm	CR (mdd)	IE (%)
0	0	35.06	-
50	50	20.07	51
100	50	18.18	60
150	50	11.68	74
200	50	5.19	88
250	50	3.89	91

4.2. Synergism parameter (S_i)

Synergism parameter (SI) has been used to know the synergistic effect existing between two inhibitors [9 - 14]. Synergism parameter (SI) can be calculated using the following relationship.

$$SI = 1-\theta_{1+2} / 1- \theta^{1}_{1+2}$$

Where

Θ = surface coverage

θ_{1+2} = $(\theta 1+\theta 2) - (\theta 1\theta 2)$

θ_1 = surface coverage by L-Tryptophan

θ_2 = surface coverage by Zn^{2+}

$\theta`_{1+2}$ = surface coverage by both L-tryptophan and Zn^{2+}

and where $\theta = IE/100\%$

The synergism parameters of L-Tryptophan -Zn^{2+}system were given in Table 7 and Table 8 corresponding to various concentration of Zn^{2+}ion. For different concentrations of inhibitors, SI approaches 1 when no interaction between the inhibitor, compounds exist. When SI >1, it points to synergistic effects. In the case of SI < 1, it is an indication that the synergistic effect is not significant. From table 9, it is observed that value of synergism parameters (SI) calculated from surface coverage were found to be one and above. This indicates that the synergistic effect exists between L-tryptophan and Zn^{2+}. Thus the enhancement of the inhibition efficiency caused by the addition of Zn^{2+} ions to L-Tryptophan is due to the synergistic effect. [15]

Table 9. Inhibition efficiencies and synergism parameters for various concentrations of L-Tryptophan Acid and Zn^{2+} (10 ppm) system.

L-Tryptophan Acid (ppm)	Inhibition efficiency IE(%)	Surface coverage (	Zn^{2+} (Ppm)	IE %	Surface coverage	Combined IE% I_{1+2}	Combined surface coverage	Synergism parameters (S_i)
50	13.9	0.13	10	10	0.10	61	0.61	1.22
100	22.0	0.22	10	10	0.10	56	0.56	1.54
150	31.12	0.31	10	10	0.10	63	0.63	1.59
200	36.	0.36	10	10	0.10	69	0.69	1.74
250	42.9	0.42	10	10	0.10	90	0.90	1.88

Table 10: Inhibition efficiencies and synergism parameters for various concentrations of L-Glutamic acid-Zn2+ (50 ppm) system.

L-Tryptophan Acid (ppm)	Inhibition Efficiency IE%	Surface coverage (θ_1)	Zn^{2+} (ppm)	IE %	Surface coverage (θ_2)	Combined IE% I_{1+2}	Combined surface coverage	Synergism parameters (S_i)
50	13.9	0.13	50	15	0.15	51	0.51	1.46
100	22.0	0.22	50	15	0.15	60	0.60	1.57
150	31.1	0.31	50	15	0.15	74	0.74	2.07
200	36	0.36	50	15	0.15	88	0.88	4.08
250	42.9	0.42	50	15	0.15	91	0.91	4.77

4.3. UV- Visible Absorption Spectroscopy

UV-visible absorption spectrum of an aqueous solution containing tryptophan and Fe^{2+}(freshly) prepared $FeSO_4$. $7H_2O$ is shown in Fig. 1

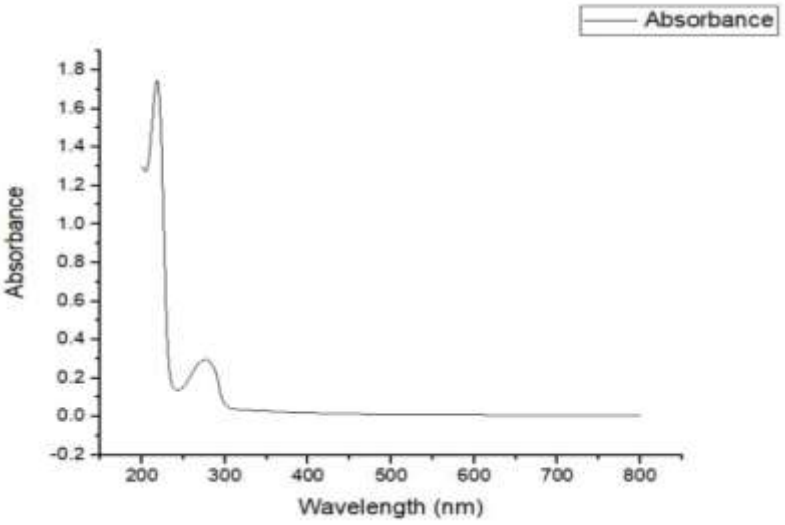


Fig .1. A peak appears at 280 nm. This peak is due to the formation of Fe^{2+} L- Tryptophan complex formed in solution.

4.4. Analysis of FTIR spectra

The FTIR spectrum of the film formed on the metal surface after immersion in the well water, 200 ppm of L-tryptophan and 50 ppm $Zn^{2+}$is shown in Figure 2. The -C=O stretching frequency of carboxyl group appears at 288.79 cm-1. The -CN stretching frequency appears at 1666.88 cm-1. The -NH stretching frequency appears at 3466.74 cm-1. [16] The C-S stretching frequency is at 806.98 cm-1. This observation suggests that L-tryptophan has coordinated with Fe^{2+} through the oxygen atom of the carboxyl group and nitrogen atom of the amine group resulting in the formation of Fe^{2+}-L-tryptophan acid complex on the anodic sites of the metal surface. The peak at 521.8 cm-1 corresponds to Zn-O stretching. The peak at 3404.95cm-1 is due to -OH stretching. This confirms that $Zn(OH)_2$is formed on the cathodic sites of metal surface . Thus the FTIR spectral study leads to the conclusion that the protective film consist of Fe^{2+}-L-tryptophan complex and $Zn(OH)_2$

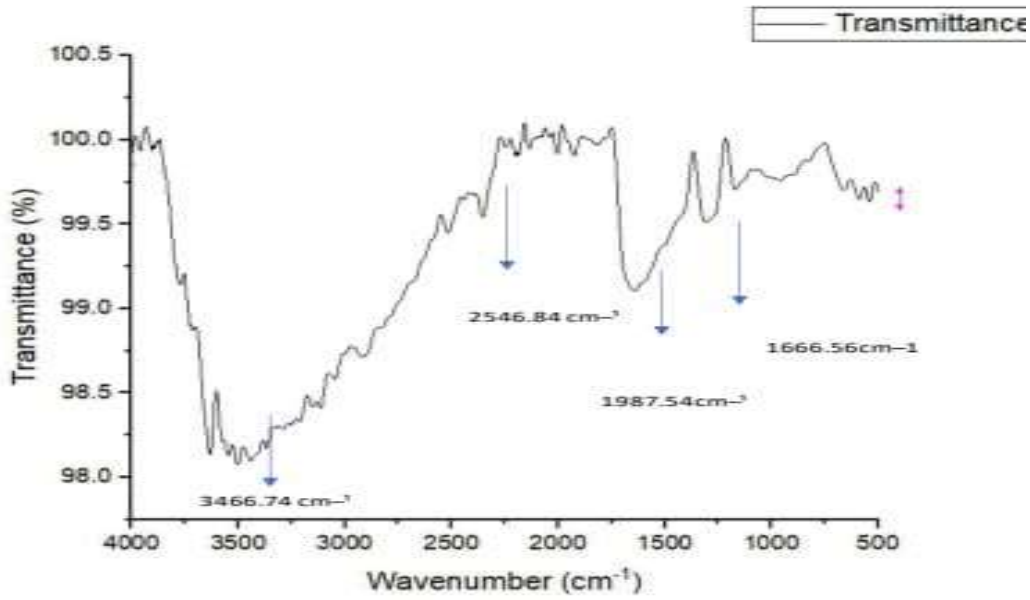


Fig.2 FT_IR Spectrum of L-tryptophan and 50 ppm Zn^{2+}

4.5. SEM Analysis of Metal Surface

SEM provides a pictorial representation of the surface. To understand the 00nature of the surface film in the absence and presence of inhibitors and the extent of corrosion of carbon steel, the SEM micrographs of the surface are examined. The SEM images of different magnification of carbon steel specimen immersed in well water for 7 days in the absence and presence of inhibitor system are shown in Figure 3 (a, b, c, d, e, f) respectively. The SEM micrographs of polished carbon steel surface (control) in Figure 3 (a,b) shows the smooth surface of the metal. This shows the absence of any corrosion products (or) inhibitor complex formed on the metal surface. The SEM micrographs of carbon steel surface immersed in well water (Figure 3 (c, d)) show the roughness of the metal surface which indicates the highly corroded area of carbon steel in well water. However in Figure 3 (e, f) indicate that in the presence of inhibitor (250 ppm L-L- tryptophan and 50 ppm Zn^{2+}) the rate of corrosion is suppressed, as can be seen from the decrease of corroded areas. The metal surface almost free from corrosion is due to the formation of insoluble complex on the surface of the metal. In the presence of L-Tryptophan and Zn2+, the surface is covered by a thin layer inhibitors which effectively controls the dissolution of carbon steel.

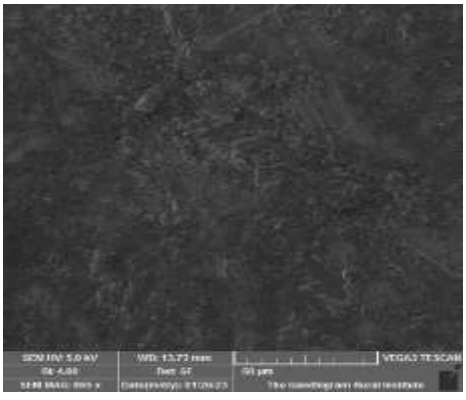


Fig (a).

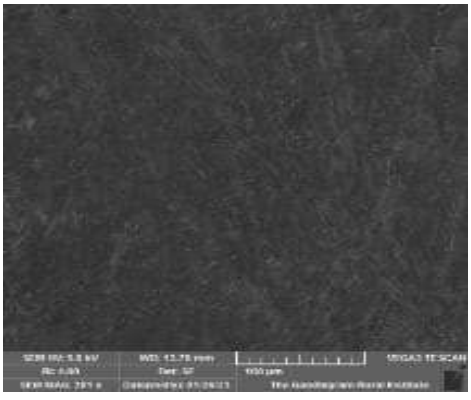


Fig (b)

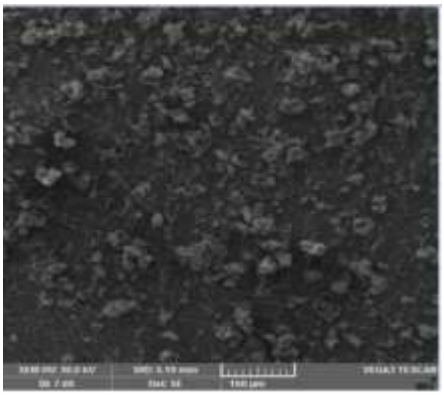


Fig (c)

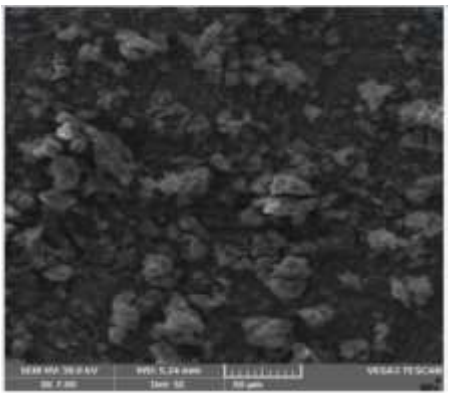


Fig (d)

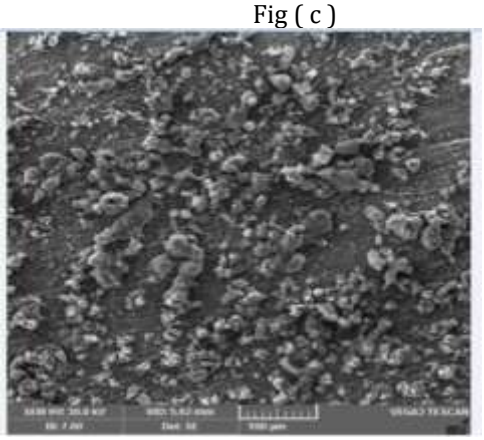


Fig (e).

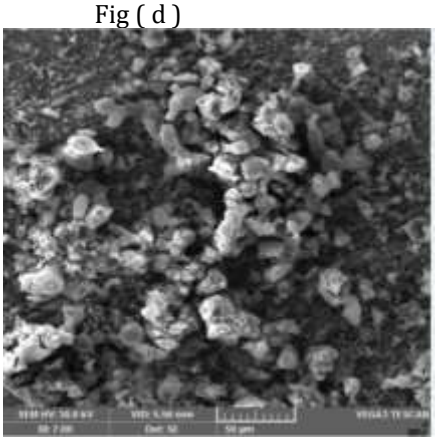


Fig (f)

Figure 3. SEM Analysis of
(a) Polished mild Carbon Steel; Reference Sample (100m)
(b) Polished mild Carbon Steel; Reference Sample (50m)
(c) Mild Carbon Steel immersed in well water; blank (100m)
(d) Mild Carbon Steel immersed in well water; blank (50m)
(e) Mild Carbon Steel immersed in well water +250 ppm L-Tryptophan +50 ppm Zn^{2+} (100m)

4.6. Energy dispersive analysis of X-rays (EDAX)

The EDAX survey spectra were used to determine the elements present on the metal surface before and after exposure to the inhibitor solution. The objective of this section was to confirm the results obtained from chemical and electrochemical measurements that a protective surface film of inhibitor is formed on the metal surface. To achieve this, EDAX examinations of the metal surface were performed in the presence of inhibitors system. They show the characteristic peaks of some of the elements constituting the mild carbon steel sample. The EDAX spectrum of carbon steel immersed in well water containing 250 ppm of L-Tryptophan and 50 ppm of Zn^{2+} is shown in Figure 4. It shows the characteristic lines for the existence of N, and Zn. In addition, the intense C and O signals. The appearance of the N, and Zn signal and this enhancement in C and O signal is due to the presence of inhibitor. These data show that metal surface is covered the N, O, C and Zn atoms. This layer is undoubtedly due to the inhibitor system. Figure 4 shows that the Fe peaks observed in the presence of inhibitor. The suppression of the Fe peaks occurs because of the overlying inhibitor film. This observation indicates the existence of an adsorbed layer of inhibitor that protects steel against corrosion. These results suggest that N, O, and C of L-Tryptophan has coordinated with Fe^{2+}, resulting in the formation of Fe^{2+}-L-Tryptophan acid complex on the anodic sites of metal surface and presence of Zn atoms are precipitated as Zn (OH)2 on the cathodic sites of metal surface.

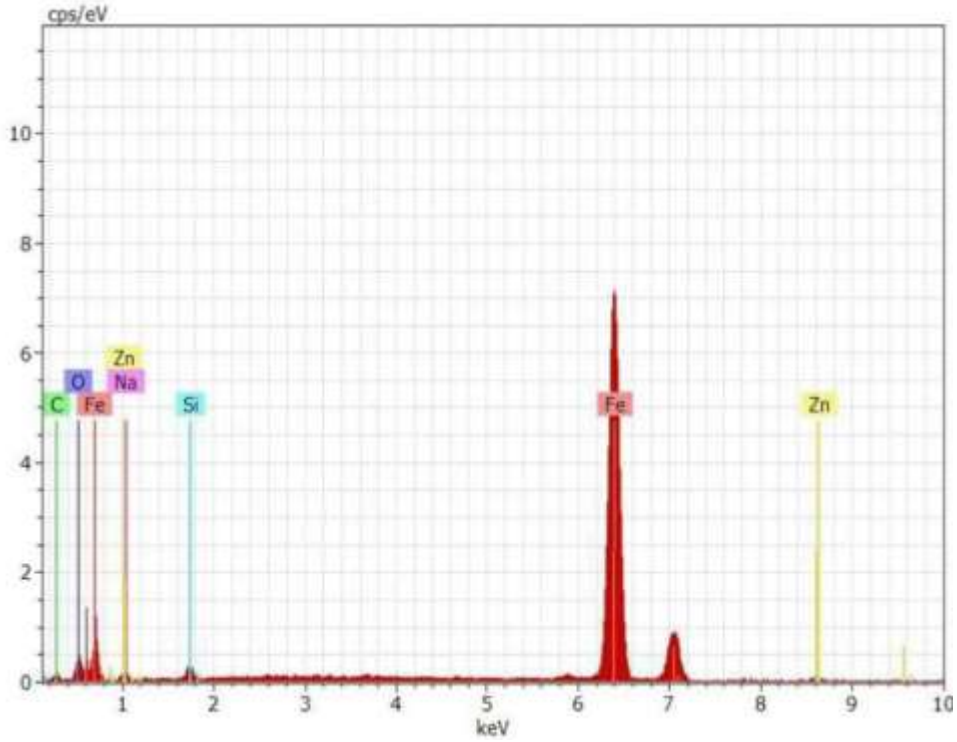


Figure 4. EDAX spectrum of carbon steel immersed in well water containing 250 ppm of L-Tryptophan and 50 ppm of Zn^{2+}

4.6. Mechanism of corrosion inhibition

The results of the weight-loss study show that the formulation consisting of 250 ppm L-Tryptophan and 50 ppm of Zn^{2+} has 91 % IE in controlling corrosion of carbon steel in well water. A synergistic effect exists between Zn^{2+} and L-trytophan FTIR spectra reveals that the protective film consists of Fe-L-tryptophan complex and $Zn(OH)_2$. In order to explain these facts the following mechanism of corrosion inhibition is proposed When the solution containing well water, 50 ppm Zn^{2+} and 250 ppm of L-trytophan acid is prepared, there is formation of Zn^{2+}-L-tryptophan complex in solution. When mild carbon steel is immersed in this solution, the Zn^{2+}L-tryptophan complex diffuses from the bulk of the solution towards metal surface. Zn^{2+}-L- tryptophan complex diffuses from the bulk solution to the surface of the metal and is converted into a Fe^{2+}-L-tryptophan complex, which is more stable than Zn^{2+}-L-tryptophan . On the metal surface Zn^{2+}-L-tryptophan complex is converted in to Fe^{2+}-L-tryptophan acid on the anodic sites. $Zn^{2+}$is released.

Zn^{2+}-L-tryptophan+ Fe^{2+} /Fe^{2+}-L-tryptophan+ Zn^{2+}

The released Zn2+ combines with OH^- to form $Zn(OH)_2$ on the cathodic sites.

$$Zn^{2+} + 2OH^- \longrightarrow Zn(OH)_2$$

Thus the protective film consists of Fe2+-L-tryptophan complex and $Zn(OH)_2$.[16]

5. CONCLUSION

A Synergistic effect exists between L-tryptophan and Zn^{2+}in controlling corrosion of carbon steel immersed in well water. The formation consisting of 250 ppm of L- Tryptophan and 50 ppm of Zn^{2+} provided 91% IE. The formation of immersion period on the above inhibitor systems has been studied. Polarization study reveals that this formation functions as a mixed type of inhibitor system. The protective film on carbon steel is studied by UV and IR spectrum studies. The morphology of the metal is studied by SEM, EDAX images.

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RESEARCH ARTICLE

Evaluation of Newly Formulated Biostimulant on Improving Productivity of Chilli (*Capsicum Annuum*)

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ABSTRACT

Agriculture provides continuous supply of vegetables and fruits. Chilli is one among the vegetables which is consumed by all people from all parts of the world. Production of chilli meets the demand and supply gradually due to lack of higher yield. In this scenario, the biostimulant quench the thrust and make up the productivity higher to supply huge quanitiy. Biostimulants are natural or synthetic substances that can be applied to seeds, plants, and soil. These substances cause changes in vital and structural processes in order to influence plant growth through improved tolerance to abiotic stresses and increase seed and/or grain yield and quality.

The present study was designed to evaluate a newly formulated biostimulant on increasing the productivity of chilli. This biostimulant contains a mineral extract of natural compounds based powder formulation as a broad spectrum plant protector. The formulation has growth regulators and plant nutrients with NPK the primary and S, Ca, Mg secondary macro nutrients. Trace amounts of iron and copper are also included. Among the four dosages tested (1g, 2g, 3g and 4g in one litre of water), proved that increased the yield and biometric values. While the concentrations of biostimluant increased will influence the gradual yield improvement of chilli in both pot and field experiments. Since the cost of biostimulant plays major role in farmer's economy point of view, the optimum dose 2g / Litre of water could be considered for recommendation for availing the higher yield of chilli.

Keywords: biostimulant, chilli, productivity, biometric values

1. INTRODUCTION

Agriculture is the backbone of human civilization, encompassing the cultivation of crops, livestock rearing, and the management of natural resources to produce food, fibers, and raw materials. The Chilli plant, scientifically known as *Capsicum annuum*, is a member of the nightshade family Solanaceae. Chilli plants thrive in warm climates with well-drained soil and plenty of sunlight [11]. They are relatively easy to grow, making them popular among other crops. Besides their culinary use, Chilli peppers have been associated with various health benefits due to their high content of vitamins, minerals, and capsaicin, the compound responsible for their heat [8]. The yearly productivity of Chilli in India varies due to several factors. Understanding soil types, composition, and their interactions with crops is crucial for sustainable and productive agriculture [9]. Issues like erosion, nutrient depletion, and soil degradation highlight the importance of preserving and improving soil quality for long-term agriculture success [1]. Some of the factors are directly influencing the balancing and maintaining the sustainability of soil.

A plant Bio-stimulant is any substance or microorganism, in the form in which it is supplied to the user, applied to plants, seeds or the root environment with the intention to stimulate natural processes of plants benefiting nutrient use efficiency and/or tolerance to abiotic stress [2] regardless of its nutrient content, or any combination of such substances and/or microorganisms intended for this use" The bio-stimulants are those products which reduces a plants requirement of nutrients and fertilizers [1]. Many definitions of bio stimulants have been reported [16]. By the application of Bio-stimulants the plant uses the low available nutrients efficiently, so as that there is no requirement of fertilizer application. These Bio- stimulants serve a great purpose and ensure the agricultural sustainability in those areas which possess agricultural lands with less availability of nutrients [15]. These products when applied at low

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concentrations are pretty much beneficial to the plant but, when applied in high concentration there will be noticeable fatality responses shown by the plants [7].

The present study was designed to evaluate a newly formulated biostimulant on increasing the productivity of chilli. This biostimulant contains a mineral extract of natural compounds based powder formulation as a broad spectrum plant protector. The formulation has growth regulators and plant nutrients with NPK the primary and S, Ca, Mg secondary macro nutrients. Trace amounts of iron and copper are also included. There are four dosages planned to test (1g, 2g, 3g and 4g in one liter of water), at pot and experiment level. In order to consider for recommendation for availing the higher yield of chilli.

2. MATERIALS AND METHODS

2.1 *Field and Pot experiment*

A commercial grade of biostimulant was procured from vin lab mysore Karnataka .A newly formulated biostimulant on increasing the productivity of chilli. This biostimulant contains a mineral extract of natural compounds based powder formulation as a broad spectrum plant protector. The formulation has growth regulators and plant nutrients with NPK the primary and S, Ca, Mg secondary macro nutrients. Trace amounts of iron and copper are also included.

We selected 20 cent plot of chilly field for conducting experiment area located in Thirumangalam taluk , T kunnathur village Madurai district, Tamil nadu. We divided that plot in to five plots. A single plot carried 2 cents (10 plants). We named that plot into plot A, plot B, plot C, plot D, plot E. Then a pot mixture was prepared by the composition of Garden soil: Red soil: Sand in the ratio of 2:1:1. Vermicompost and humus were added were added to this pot mixture. The above soil mixture was prepared and removed the small stones and sieved well. After adjusting the soil pH, the mixture was filled in the grow bags as per treatment scheduled in the experiment. There are 7 numbers of treatments each replicated five times. Each pot was planted with two number of chilli seedlings and experiment was started. The following treatments were undertaken to carry out the experiments

Various concentration of Bio stimulant were taken for this study and they were evaluated at 1g/l, 2g/l, 3g/l, 4g/l of water as four different dosages in chilly field for its efficacy on yield, disease and pest control

2.2 Observation of Parameters

The biometric and biochemical data were taken and the mean values of replicated plots are given in the tables and figures. At end of the experiments, the height of the plant, leaf area, number of leaves per plant, number of flowers per plant, fruit weight per plant, single fruit weight, shoot fresh and dry weight, root fresh and dry weight, yield per plot, Chlorophyll estimation and Protein estimation Lowry et al (1979).

3. RESULTS AND DISCUSSION

3.1 Observation of Plant Biometric Parameters of Pot Experiment

At the end of the experiment the plant biometric parameters were measured and recorded such as height (Stem and Root), fresh weight (Stem and root), dry weight (Stem and Root), number of leaves per plant, Leaf area, number of buds per plant, number of flowers per plant, Number of fruits per plant, and fruit Length. The results showed the plant biometric parameters (vegetative) of pot experiment; the plants which were maintained in the control soil they had 26cm stem height and 5cm of root height. Then the plant root and stem portion cut down the both are weighted for fresh weight the fresh weight of the root portion is 3.2g and the stem 15g, then it was kept incubated in hot air oven to take the dry weight, the dry weight of stem portion is 5.6g and the weight root portion is 0.50g (Table 1) and the average of the leaves per plot is 45 and the leaf size is (6×3). Also the same trend of observation was given by [14). Similar results were noticed in the present study and our findings were substantiated with their publication [5].

The plants which are all maintained in 1g bio stimulant treated with liquid, the height of the stem 33cm and height of the root 09cm. then the root and stem portion will be weighted for fresh weighted the fresh weight of stem is 21g and the fresh weight of root portion is 5.4g then it will be dries in incubator after that the dry weight will be get the dry weight of stem 6.1g and the dry weight of root is 0.68g (Table 1). The total number of leaves per plants in the treatment is 48, and the leaf size (7x 3.4). The plants which are all maintained in 2g bio stimulant, the height of the stem 38cm and height of the root 11cm. then the root and stem portion will be weighted for

fresh weighted the fresh weight of stem is 25g and the fresh weight of root portion is 5.8g then it will be dries in incubator after that the dry weight will be get the dry weight of stem 7.4g and the dry weight of root is 1.24g [11]. The total number of leaves per plants in the treatment is 38, and the leaf size (7x 3.4). The plants which are all maintained in the 3g bio stimulant they have, the height of the stem 42cm and height of the root 14cm (Table 1). then the root and stem portion will be weighted for fresh weighted the fresh weight of stem is 31g and the fresh weight of root portion is 6g then it will be dries in incubator after that the dry weight will be get the dry weight of stem 7.9g and the dry weight of root is 1.37g. The total number of leaves per plants in the treatment is 18, and the leaf size (8x3.5).

We observed the growth parameter of the plants in chilly field. The parameters like plantlets per bunch, height of the plant, number of leaves, width of the leaf, fresh weight, dry weight, weight of seed (100) & weight of the total seed.

First application of bio stimulant was given on thirty days old chilly plants. Totally three rounds of application was implemented keeping 15 days interval. The plants were uprooted after two months from third spraying of bio stimulant and respective biometric parameters were noted and tabulated. They can act on plant productivity as a direct response of plants or soils to the bio stimulant application or an indirect response of the biostimulant on the soil and plant microbiome with subsequent effects on plant productivity [17] proposed the following definition of a bio stimulant as a formulated product of biological origin that improves plant productivity because of the novel or emergent properties of the complex of constituents; and not as a sole consequences of the presence of known essential plant nutrients, plant growth regulators, or plant protective compounds. Several researches have been developed in order to evaluate the use of bio stimulants in improving plant growth subjected to abiotic stresses [13].

In small concentrations, these substances are efficient, enhancing nutrition efficiency, abiotic stress tolerance, and/or crop quality traits, regardless of its nutrients content [10]. These substances when applied exogenously have similar actions to the groups of known plant hormones, whose main ones are auxins, gibberellins, and cytokinins [8]. In the plants which are all maintained in 1g bio stimulant, they are produce a 2 buds and 6 flowers, and also have 2 fruits and the fruit have a 2.1cm length and 1.4g weight.(Table 2). In the plants which are all maintained in 2g bio stimulant, they are produce a 5 buds and 9 flowers, and also have 9 fruits and the fruit have a 2.5cm length and 2.7g weight.(Table 2). In the plants which are all maintained in 3g bio stimulant, they are produce a 7 buds and 10 flowers, and also have 10 fruits and the fruit have a 3.8cm length and 3.3g weight.(Table 2). And the plants which are all maintained in 4g bio stimulant, they are produce a 7 buds and 10 flowers, and also have 14 fruits and the fruit have a 4.1cm length and 3.5g weight. Similar trend was observed and substantiated the present findings by [18].

3.2 Observation of Plant Biometric Parameters of Field Experiment

Results showed the plant biometric parameters (vegetative) of field experimented, in the table the plants which are all maintained in the control soil they have a 11cm stem height and 7cm of root height. Then the plant root and stem portion cut down the both are weighted for fresh weight the fresh weight of the stem portion is 10.7g and the root 1.9g, then it will be incubated to take the dry weight, the dry weight of stem portion is 4.01g and the weight root portion is 0.34g and the average of the leaves per plot is 30.0 and the leaf size is (5×3). (Table 3). Also the same trend of observation was given by [13]. Similar results were noticed in the present study and our findings were substantiated with their publication. The plants which are all maintained in 1g bio stimulant, the height of the stem 15cm and height of the root 9cm. then the root and stem portion will be weighted for fresh weighted the fresh weight of stem is 13.5g and the fresh weight of root portion is 2.3g then it will be dries in incubator after that the dry weight will be get the dry weight of stem 0.54g and the dry weight of root is 0.54g. The total number of leaves per plants in the treatment is 35, and the leaf size (5x3.2). Similar results were observed by [12].

The plant biometric parameters (Reproductive) also measured in the field experiment plants, such as a Number of buds plant, Number of flower per plant, Number of fruits per plant, Fruit Length, Fruit weight (Table 4). In the plants which are all maintained in the control field soil mixture they are produce a 2 buds and 4 flowers, and also have 2 fruits and the fruit have a 1.9cm length and 1.7g weight, total production by this plot 106.4g and per acre 6250kg. In the plants which are 1g bio stimulant they

produced 5 buds and 7 flowers, and also have 3 fruits and the fruit have a 2.0cm length and 2.3 g weight. Total production by this plot 119.5g and per acre 6980kg (Table 4).Same trend of observations also were noticed by [3,6].

In the plants which are all maintained in 3g bio stimulant, they are produced10 buds and 12 flowers, and also have 6 fruits and the fruit have a 3.0cm length and 3.4g weight, total production by this plot 165.0g and per acre 8055kg,(Table 4).In the plants which are all maintained in 4g bio stimulant, they are produce a 13 buds and 15 flowers, and also have 9 fruits and the fruit have a 3.2 cm length and 4.1g weight, total production by this plot 179.5g and per acre 8250kg. (Table 4). They proved their results as like that of these present observations in the chilli crop [8].

3.3 Chlorophyll and Protein Estimation

In the pot experiment plants both chlorophyll and protein contents were estimated, by the result of this estimation the chlorophyll a, b is combined together and get a finally total value of chlorophyll content. In the result of chlorophyll estimation shows the amount of chlorophyll content of each plant which is all maintained and treated bio stimulant (Fig.1). They proved their results as like that of the present observations in the Chilli crop [6]. Chlorophyll content was observed in field experiment (Fig 2) was as like that of trend which received in pot experiment as like that of finding by [17].

In the chlorophyll and protein estimation in pot experimented plants are given values such as the plant which was given with 2g of biostimulant per litre of water provided satisfactory results of chlorophyll content in both pot and field experiments (Fig 3). The optimum dose of biostimulat registered satisfactory results on chlorophyll and protein content of Chilli plant, this result was substantiated by [5].

Table 1: Effect of biostimulant on biometric parameters (vegetative) of chilli plants under pot experiment

Treatment	Height (cm)		Fresh Weight (g)		Dry Weight (g)		No. of leaves per plant	Leaf area
	Shoot	Root	Shoot	Root	Shoot	Root		
Control	26	05	15	3.2	5.6	0.50	12	6x3
1g bio stimulants/l	33	09	21	5.4	6.1	0.68	14	7x3.4
2g bio stimulants/l	38	11	25	5.8	7.4	1.24	15	7x4.1
3g bio stimulants/l	42	14	31	6.3	7.9	1.37	18	8x3.5
4g bio stimulants/l	48	18	35	6.9	8.5	1.48	23	8x4.8

Values are mean of three replicates

Table 2: Influence of biostimulant on Chilli biometric parameters (reproductive) under pot experiment

Treatment	No. of buds per plant	No. of flowers per plant	No. of fruits	Fruit length (cm)	Fruit weight (g)
Control	02	06	2	2.1	1.4
1g bio stimulants/l	04	07	5	2.5	2.2
2g bio stimulants/l	05	09	9	3.5	2.7
3g bio stimulants/l	07	10	10	3.8	3.2
4g bio stimulants/l	07	18	14	4.1	3.5

Values are mean of three replicates

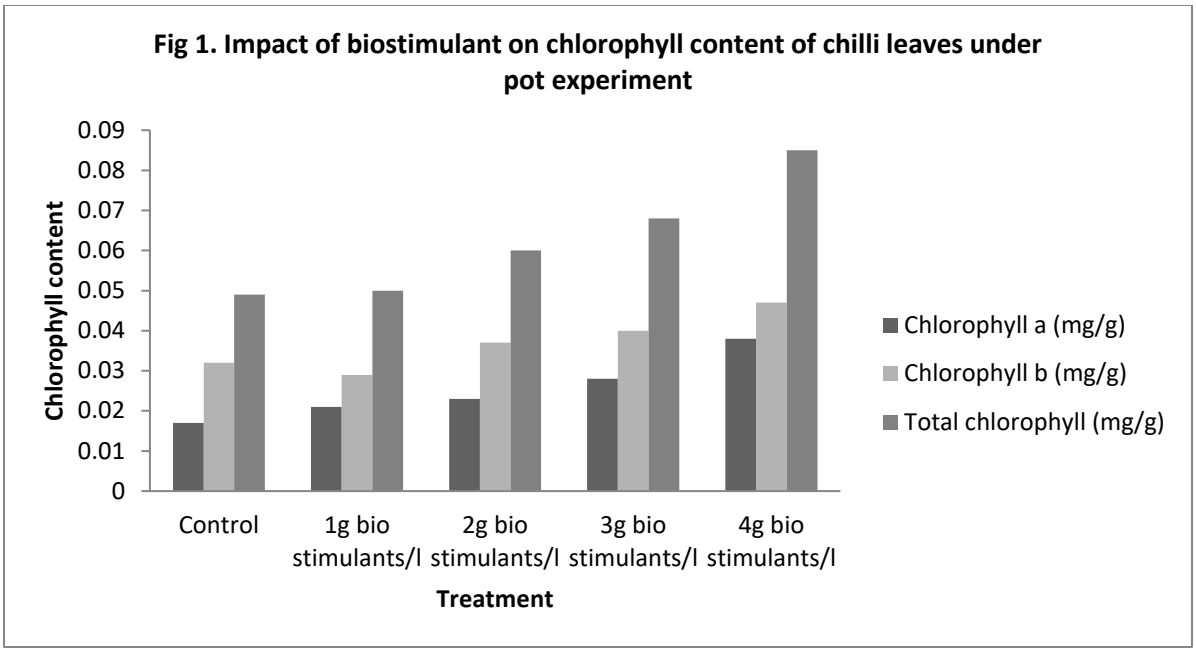


Table 3: Effect of bio stimulants on biometric parameters (vegetative) of Chilli under field experiment

Treatment	Height (cm)		Fresh Weight (g)		Dry Weight (g)		No. of leaves per plant	Leaf area (L×B) cm
	Stem	Root	Stem	Root	Shoot	Root		
Control	11	7	10.7	1.9	4.01	0.34	30.0	5×3
1g bio stimulants/l	15	9	13.5	2.3	5.09	0.54	35.5	5×3.2
2g bio stimulants/l	19	12	17.5	2.8	5.06	0.88	45.5	5.6×3.4
3g bio stimulants/l	25	14	20.7	2.0	7.72	0.91	50.5	7×3.5
4g bio stimulants/l	28	17	21.5	3.2	6.62	1.86	54.5	8.7×5.1

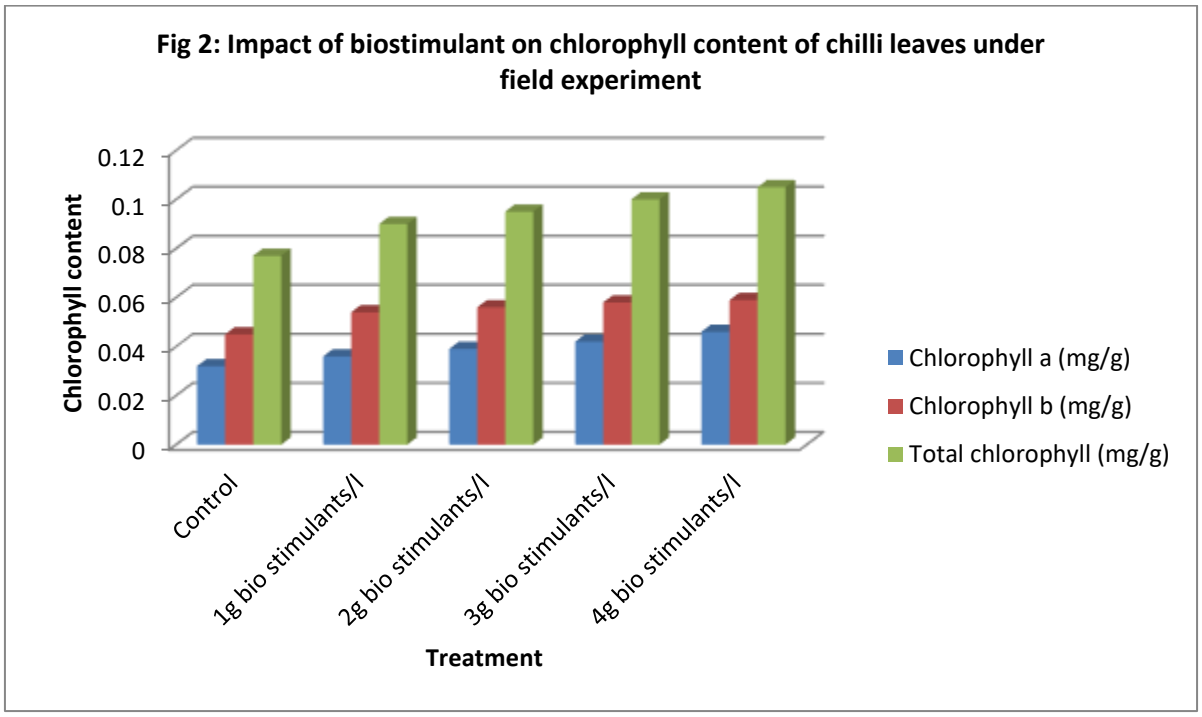
Values are mean of three replicates

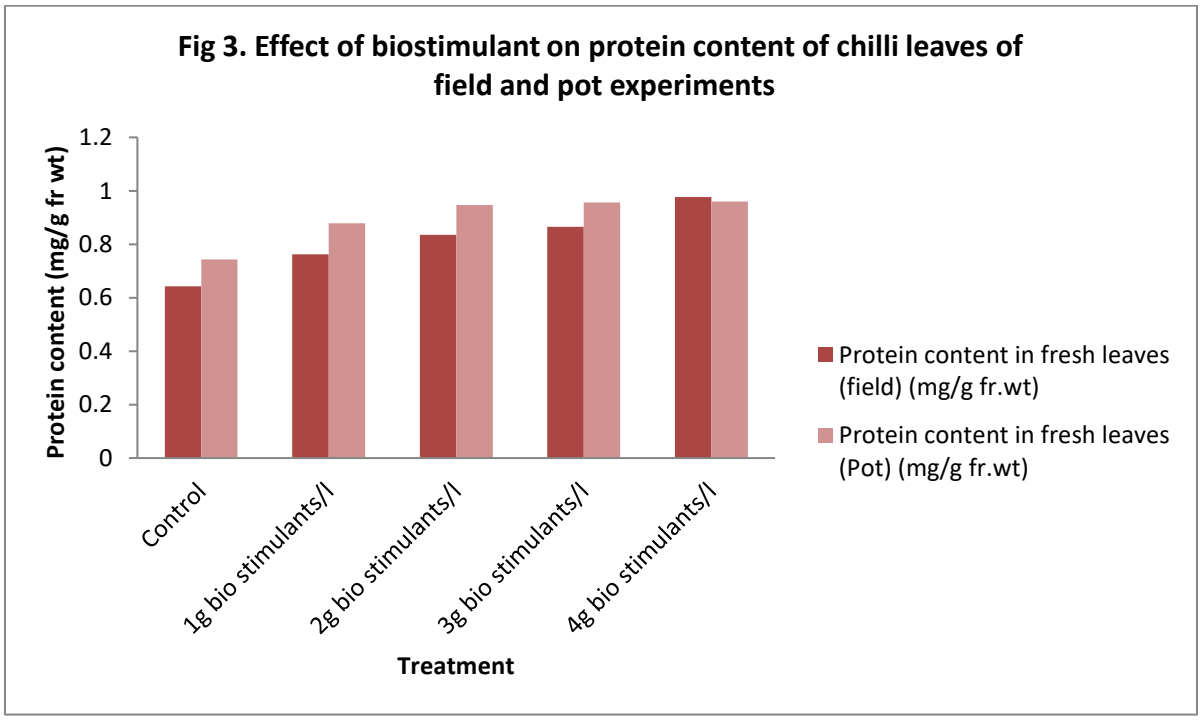
Table 4: Effect of bio stimulants on biometric parameters (reproductive) of Chilli under field experiment

Treatment	No. of buds per plant	No. of flowers per plant	No. of fruits Per plant	Fruit length (cm)	Single fruit weight (g)	Yield per plot (g)	Yield per acre (Kg)
Control	2	4	2	1.9	1.7	106.2	6250
1g bio stimulants/l	5	7	3	2.0	2.3	119.5	6980
2g bio stimulants/l	8	10	5	2.8	2.9	135.5	7850

3g bio stimulants/l	10	12	6	3.0	3.4	165.0	8055
4g bio stimulants/l	13	15	9	3.2	4.1	179.5	8250

Values are mean of three replicates





4. CONCLUSION

The present study concluded that a newly formulated biostimulant proved its influence on increasing the productivity of chilli. Since it contains the required mineral content, it fulfills the maximum supplement of crop energy and increasing the biometric values.

Among the four dosages tested (1g, 2g, 3g and 4g in one litre of water), proved that increased the yield and biometric values. While the concentrations of biostimluant increased will influence the gradual yield improvement of chilli in both pot and field experiments. Since the cost of biostimulants plays major role in farmer's economy point of view, the optimum dose 2g / Litre of water could be considered for recommendation for availing the higher yield of chilli.

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Efficacy of biogenic film from bioactive waste of Agaricus Bisporous

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ABSTRACT:

This study investigates the development of chitosan/polyvinyl alcohol (PVA) films incorporated with mushroom extract and ascorbic acid (Vitamin C) for enhanced mechanical, antioxidant, and antimicrobial properties. Chitosan, a biodegradable biopolymer, was blended with PVA to form a stable, flexible film matrix, while mushroom extract, rich in bioactive compounds, and ascorbic acid were added to improve the films' functionality. The films were characterized using various techniques, including X-ray diffraction (XRD), Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and thermogravimetric analysis (TGA), to assess their structural, chemical, and thermal properties. XRD analysis revealed the crystalline and amorphous nature of the films, with changes observed upon the incorporation of mushroom extract and ascorbic acid. FTIR spectra confirmed the successful incorporation of the bioactive compounds, with characteristic peaks indicating the interaction between chitosan, PVA, mushroom extract, and ascorbic acid. SEM images showed the surface morphology and homogeneity of the films, revealing a smooth surface and uniform distribution of the additives. TGA analysis provided insight into the thermal stability, demonstrating that the films exhibited improved thermal resistance with the addition of mushroom extract and ascorbic acid. Building on these results, the chitosan/PVA films by varying mushroom extract and ascorbic acid concentrations.

KEYWORDS: Bioactive film, Crosslinking, Thermal stability, Film functionality

INTRODUCTION:

The development of biodegradable polymer films has gained significant attention due to the growing need for sustainable alternatives to conventional materials. A key area of research involves creating polymeric composite films that integrate both natural and synthetic polymers. These composite films are particularly valuable in various industries, offering enhanced mechanical properties, such as increased strength and reduced weight, along with improved functionality. Additionally, they exhibit effective antibacterial activity, which makes them ideal for applications aimed at preserving product integrity and extending shelf life. Among the various polymers utilized for this purpose, Polyvinyl Alcohol (PVA) is a standout material. PVA is a colorless, water-soluble, non-toxic, and biocompatible polymer known for its excellent film-forming properties, high optical transparency, and remarkable thermal stability. As a semi-crystalline material, PVA is easy to process and is biodegradable, making it a prime candidate for environmentally friendly alternatives in a wide range of applications, such as medical devices, agricultural films, and biodegradable coatings.[1]

Chitosan, another key biopolymer, is derived from chitin through deacetylation, which is primarily sourced from the shells of crustaceans such as shrimp, crabs, and lobsters. Chitosan is a polysaccharide that possesses inherent antimicrobial properties, which make it effective in preventing microbial growth. These properties are particularly beneficial in sectors like food preservation, wound care, and drug delivery. The degree of deacetylation and the molecular weight of chitosan influence its characteristics and suitability for various uses. In biomedical and agricultural applications, chitosan-based films are advantageous because they provide antimicrobial activity while also contributing to the mechanical strength and flexibility of the film. However, chitosan alone has limitations, such as water solubility and mechanical weakness. To overcome these drawbacks, it is often combined with other polymers, such as PVA, to create a more durable and functional composite material.[2]

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The main objectives of this research were to explore how the incorporation of ascorbic acid into chitosan/PVA films influences their structural integrity Specifically, the study aimed to:

- Synthesize PVA/chitosan films integrated with ascorbic acid using the solution casting method.
- Investigate the chemical composition of the films by analyzing the functional groups using FTIR spectroscopy.
- Analyze the surface morphology and structural uniformity of the films using SEM.
- The Decomposition of material were done by TGA analysis.

METHODOLOGY:

2.1 MATERIALS:

The materials employed in this study include fungal chitosan, polyvinyl alcohol (PVA), and ascorbic acid, each selected for their specific properties and compatibility in the preparation of mushroom chitosan-based PVA films.

2.1.1 Polyvinyl Alcohol (PVA)

PVA is a synthetic polymer derived from the polymerization of vinyl acetate, which is hydrolyzed to form PVA. The degree of hydrolysis influences its properties, making it a versatile material with a wide range of applications. PVA is water-soluble, non-toxic, and has excellent film-forming, adhesive, and emulsifying properties. It is also resistant to oils, solvents, and grease, making it suitable for preparing hydrophilic membranes with good mechanical properties. Additionally, PVA exhibits transparency, softness, and flexibility, with a high strength-to-weight ratio, which enhances its application in various industries, including as a binding agent in composite films.

2.1.2 Chitin and Chitosan

Chitin is a naturally occurring polysaccharide found abundantly in the exoskeletons of arthropods and in fungal cell walls. Chitosan, derived from chitin through deacetylation, is soluble in acidic solutions and possesses antimicrobial properties. It is biocompatible, biodegradable, and non-toxic, making it ideal for food preservation and medical applications. Chitosan-based films offer low oxygen permeability, which is critical for preserving food, and have moderate water vapor barrier properties. Blending chitosan with other hydrocolloids, such as PVA, improves the mechanical strength and barrier properties of the resulting films.

2.1.3 Ascorbic Acid

Ascorbic acid, commonly known as Vitamin C, is a water-soluble antioxidant that plays an essential role in neutralizing free radicals, which can damage cells. It aids in collagen synthesis, promoting wound healing, and has been shown to have protective effects against oxidative stress. Derived from citrus fruits and various vegetables, ascorbic acid also contributes to food preservation by improving the stability and shelf life of food products. In this study, ascorbic acid is incorporated into the composite films to enhance their antioxidant and antimicrobial properties.[3]

2.2 METHODS:

2.2.1 Preparation of Chitosan:

The materials required for the preparation of mushroom chitosan include mushroom biomass, such as the fruiting bodies or mycelium of edible varieties like *Pleurotus ostreatus* or *Agaricus bisporus*. Sodium hydroxide (NaOH) is used for the deproteinization step, while ascorbic acid or hydrochloric acid (HCl) serves for demineralization to remove inorganic salts. Ethanol and acetone are needed for washing and drying the extracted chitin, and deionized water is used throughout the process for rinsing and purification. Finally, calcium hydroxide ($Ca(OH)_2$) is used in the deacetylation step to convert chitin into chitosan. These materials are essential for efficiently extracting and processing chitosan from mushroom sources.

STEP 1: Collection and Drying: Dry mushrooms (e.g., *Pleurotus ostreatus*, *Agaricus bisporus*) to remove moisture at 50–60°C.

STEP 2: Demineralization: Soak dried mushrooms in 0.1 M HCl or ascorbic acid for 2–3 hours to remove minerals (calcium, magnesium). Rinse with deionized water.

STEP 3: Deproteinization: Treat the demineralized biomass with 5-10% NaOH at 80–90°C for 1–2 hours to remove proteins. Rinse with water.

STEP 4: Chitin Extraction: After demineralization and deproteinization, wash the biomass with ethanol or acetone to purify chitin.

STEP 5: Deacetylation (Chitosan Formation): Immerse chitin in 40–50% $Ca(OH)_2$ at 90°C for 2–4 hours to remove acetyl groups, forming chitosan. Rinse with water.

STEP 6: Drying and Powdering: Dry the chitosan (air, vacuum, or oven at <50°C) and grind into a fine powder.

Chitosan purity can be adjusted based on the deacetylation process.[4]

2.2.2 PVA/ Chitosan/AA Film Preparation:

Chitosan-based PVA films were prepared using a chemical casting method, ideal for producing thin polymer films. To begin, a 1% PVA solution was made by dissolving PVA in deionized water at 90°C with stirring until clear. A 1% chitosan solution was prepared by dissolving chitosan in ascorbic acid at room temperature with constant stirring. The PVA and chitosan solutions were mixed, with ascorbic acid acting as a crosslinker, and stirred for 1 hour to complete the crosslinking reaction.

The resulting viscous solution was cast onto a Petri dish and air-dried, with film thickness controlled by solution volume. After drying, the films were peeled off and preconditioned at a constant temperature for testing. The film's formation involved hydrogen bonding and ionic cross-linking between chitosan's amino groups and PVA's hydroxyl groups.[5]

2.2.3 ASCORBIC ACID:

To prepare ascorbic acid from strawberries using the maceration method, the materials required include fresh, ripe strawberries (preferably organic), distilled water, a blender, a clean glass jar or beaker, and a fine mesh strainer or cheesecloth for filtering. A knife and cutting board are also needed to hull the strawberries, removing the leaves and stems. The process involves blending the strawberries to create a puree, which helps release the ascorbic acid into the solvent (distilled water) during maceration. The mixture is then strained to extract the vitamin C-rich liquid.

STEP 1: Prepare Strawberries: Wash, hull, and remove the stems from the strawberries.

STEP 2: Blend: Puree the strawberries in a blender to increase surface area.

STEP 3: Macerate: Place the pureed strawberries in a glass jar, add enough distilled water to cover them, seal the jar, and let it sit in a cool, dark place for 6–24 hours.

STEP 4: Strain: After maceration, strain the mixture using a fine mesh strainer or cheesecloth to separate the liquid extract.

STEP 5: Store: Store the liquid in an airtight container in the refrigerator to preserve the ascorbic acid (vitamin C).

This method extracts the vitamin C, which is sensitive to heat, light, and air, so proper storage is essential.[6]

2.3 CHARACTERIZATION:

2.3.1 Fourier transform infrared (FTIR) analysis:

Fourier Transform Infrared Spectroscopy (FTIR) identifies chemical bonds and functional groups in a material by measuring its infrared absorption spectrum, where molecules absorb specific wavelengths corresponding to their bond vibrations.

2.3.2 Scanning Electron Microscopy (SEM)

Scanning Electron Microscopy (SEM) uses a focused electron beam to capture high-resolution images of material surfaces, structure, and composition at the nanometer scale.

2.3.3 X-ray Diffraction (XRD)

X-ray Diffraction (XRD) is a technique used to analyze the structure, phase composition, and properties of crystalline materials by measuring X-ray diffraction patterns. It is widely used in materials science, chemistry, and geology**.**

2.3.3Thermogravimetric Analysis (TGA)

Thermogravimetric Analysis (TGA) measures mass changes in a sample with temperature or time, under a controlled atmosphere. It is used to study thermal stability, decomposition, moisture content, and material composition**.[9]**

RESULT AND DISCUSSON:

The characterization of mushroom chitosan PVA film was successfully analysed and the result is given below.

3.1 FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR) ANALYSIS:

FTIR spectroscopy was used to determine the functional groups and also to confirm the successful formation of the PVA film from mushroom chitosan. The observed peaks and their interpretation as follows :

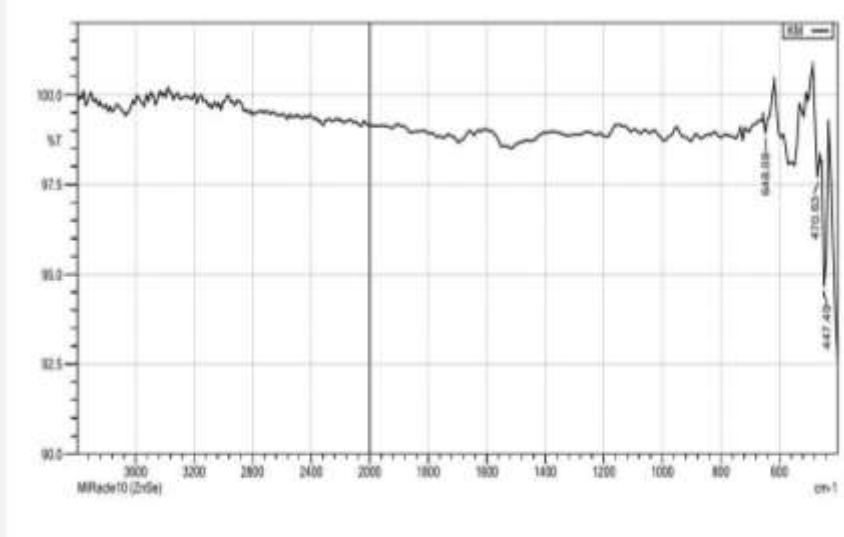


Figure 3.1 FTIR analysis

From the figure 3.1, the peak 648.08 cm^{-1} indicates the C-H bending indicating the presence of aromatic. The peak 470.63 cm^{-1} indicates the metal-halide stretching (M-X), such as C-Cl or C-Br stretching, particularly organometallic compounds or halogenated compounds. The peak 447.49 cm^{-1} indicates M-O stretching (metal-oxygen) commonly seen in inorganic compounds containing metal oxygen bond. These peaks suggest the presence of halogenated organic compounds (like chlorinated compounds) or organometallic compounds, potentially involving M-Cl bonds, or C-H bending in aromatic systems. The FTIR result confirms that the PVA or chitosan chains have aromatic components like contamination or aromatic cross-linkers used in film formation. PVA backbone which also contains alkene-like structures or even any aromatic impurities.

4.2 SCANNING ELECTRON MICROSCOPY (SEM) WITH EDAX:

Scanning Electron Microscopy (SEM) provides high resolution surface image, which also gives information about size, morphology, surface features.

4.2.1 SEM Analysis of Mushroom Chitosan PVA Film:

The chitosan has the network like structure which has a rough surface with micro or nano sized pores, due to its dense network they cause some wrinkling or non-uniformity with PVA. The PVA film is generally smooth and homogeneous at the microlevel which provides an improving mechanical properties. The mushroom derived chitosan has a irregular surface structure influence in textured surface. The cross linking agent ascorbic acid lead to rougher or more rigid surface structure.

4.2.2 EDAX Analysis of Mushroom Chitosan PVA Film:

(i) Composition: The EDAX spectrum confirms the presence of carbon (C) and oxygen (O) as primary element along with nitrogen (N) and sodium (Na) from chitosan.

(ii) Elemental Ratios: The atomic ratios of the elements are given in different peaks

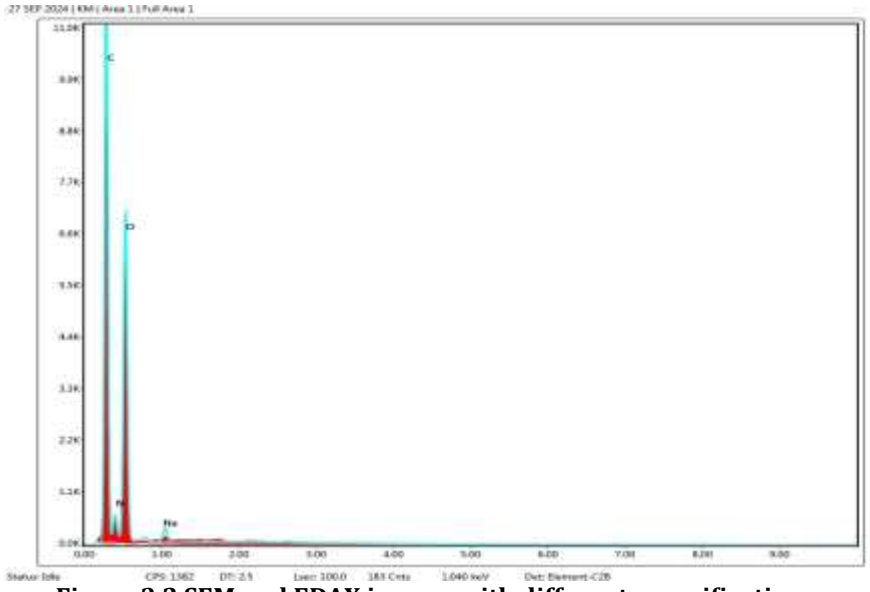
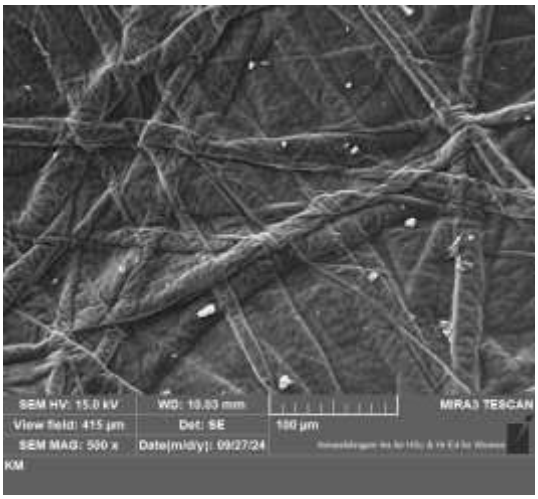
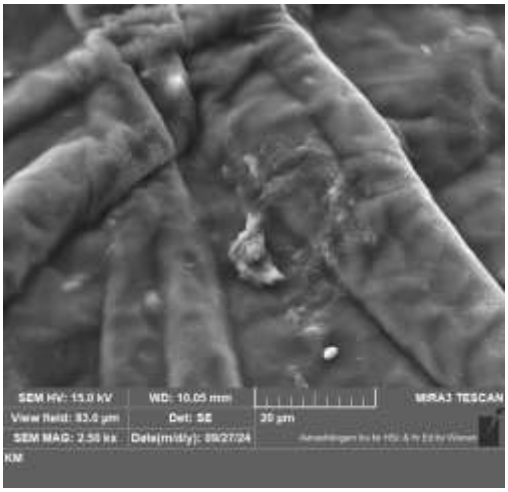
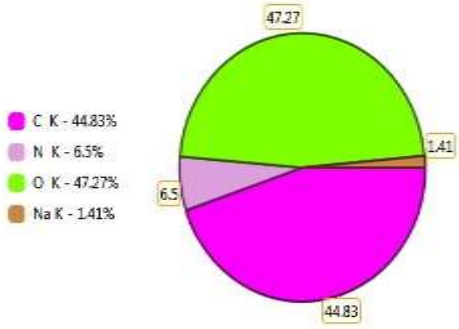


Figure 3.2 SEM and EDAX images with different magnifications





Element	Weight%	Atomic%	Error%
C K	44.83	51.75	5.31
N K	6.50	6.43	16.73
O K	47.27	40.27	9.31
Na K	1.41	0.85	11.18



3.3 X- RAY DIFFRACTION Analysis:

The result for X-Ray Diffraction (XRD) analysis of mushroom chitosan PVA film focuses on the peak identification, crystalline size calculation (Scherrer Equation), Lattice Parameter Calculation, Strain and Defects and crystallinity estimate the degree of crystallinity

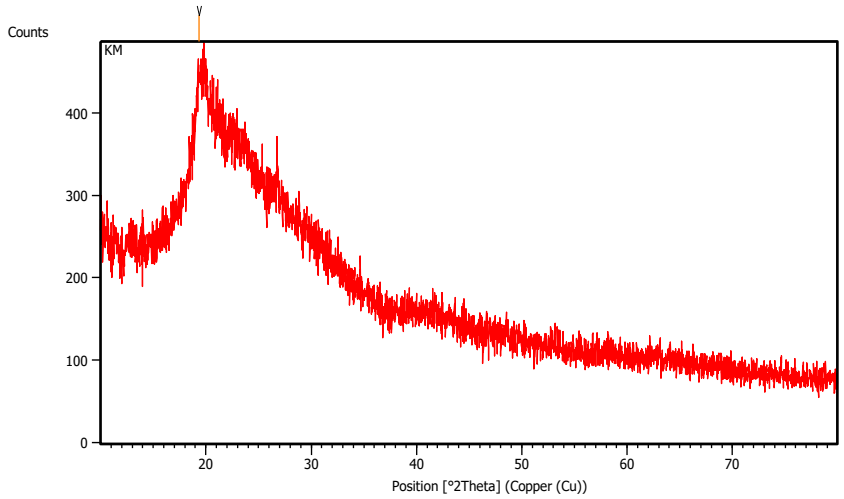


Figure 3.3 XRD Main Graphics, Analyze View

Table 3.1 Calculation of Crystallite Size, Micro strain, Lattice parameters

2θ	FWHM	d-spacing [Å]	D	Delta	Micro strain	Lattice parameter	Relative intensity
19.3275	1.1424	4.58877	70.4	0.00949	0	7.95	100

(i) Peak Position (2θ):
The characterization peak was observed at: 19.3275
(ii) Crystallite Size:
The crystallite size in chitosan is of the range 70.4 nm
(iii) Structure: The peak 19.3275 indicates the semicrystalline structure due to hydrogen bonds between -OH groups of PVA backbone. These peaks were observed in XRD pattern of AA-crosslinked CS/PVA but the intensity of these peaks was decreased because of the reduction in crystallinity because of the reduction of the H-bonding between polymer chain during crosslinking reaction.

4.4 Thermogravimetric (TGA) Analysis:
A TGA curve plots the mass loss of a sample as a function of temperature or time under controlled heating conditions and derive important information about the material's thermal stability, composition, and decomposition behaviour.

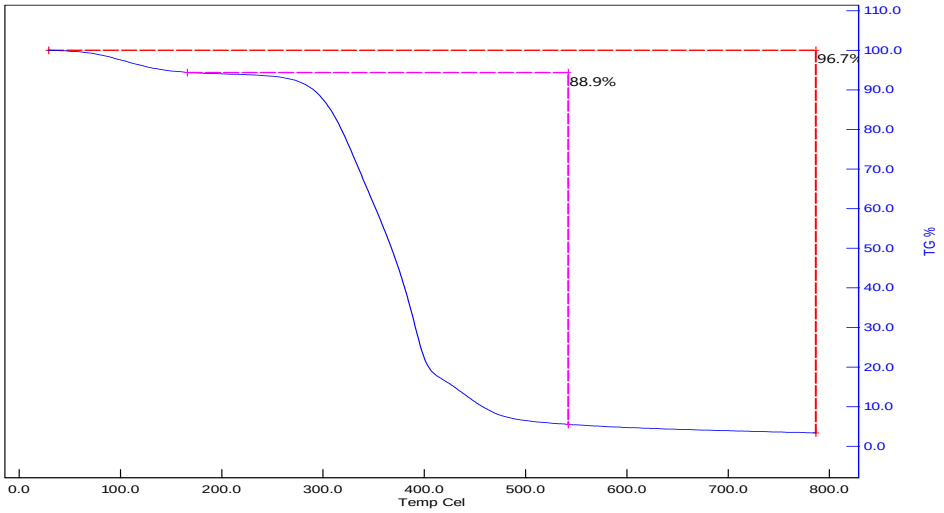


Figure 3.4 : TGA Graph

The material remains stable till 300° C and starts to decomposes above that temperature and TGA percentage was at 96.7% at the initial stage and upon increase in temperature the percentage was lowered to 88.9%.

CONCLUSION:

The PVA/chitosan composite films crosslinked with ascorbic acid demonstrated enhanced mechanical, antimicrobial, and antioxidant properties, making them ideal for sustainable applications. FTIR analysis confirmed successful crosslinking, revealing functional groups indicative of ascorbic acid incorporation. SEM images showed that chitosan provided a rough surface texture, while PVA remained smooth; the addition of ascorbic acid resulted in a more rigid surface, improving mechanical strength. XRD analysis indicated a semi-crystalline structure with a crystallite size of 70.4 nm. The crosslinking process reduced crystallinity by disrupting hydrogen bonding between polymer chains, contributing to greater flexibility. TGA results showed good thermal stability, with minimal mass loss up to 300°C, indicating that the composite films are suitable for use at higher temperatures. These findings suggest that the PVA/chitosan films with ascorbic acid are promising for biodegradable packaging, food preservation, and biomedical applications, offering both mechanical strength and antimicrobial properties. The study underscores the potential of ascorbic acid as an effective crosslinker to improve the functionality and sustainability of composite films, providing an eco-friendly alternative to conventional materials.

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RESEARCH ARTICLE

Comparative study on spectrophotometric analysis and antibacterial activity of homely prepared turmeric powder and commercially available turmeric powder

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Abstract

The study was conducted t o determine and compare the amounts of curcumin that are present in three different brands of turmeric powder as well as homemade powder (*Curcuma longa, Curcuma aromatica, Curcuma amada, Curcuma caesia* and *Curcuma angustifolia*) using spectrophotometric analysis and antibacterial activity also done with four different bacterial species. The results showed that the absorbance of the acetone extracts of the three turmeric powder samples was read at 420 nm in spectrophotometer. The absorbance of pure curcumin was noted in 1.12 at 420 nm, brand A showed 1.76 brand B showed 1.32 and brand C showed 1.66. The concentration of the curcumin was found to be different for each sample. Antibacterial results showed that brand A,B,C were sensitive to *Klebsiella, Proteus* and *Bacillus* species , but in the case of *E.coli* species A,B are sensitive and C was not sensitive.

Key words: Antibacterial, Spectrophotometer, Curcuma, turmeric power

Introduction

Spices occupy a vital component of agriculture products that are used as spice or condiment and for medical reasons. They are well recognised to contain considerable amounts of bioactive substances and natural antioxidants. (1). The *Zingerberaceae* family includes the tropical perennial monocotyledonous herbaceous plant known as turmeric (*Curcuma longa*) (2). Although it comes originally from South and South-East Asia, the majority of it is cultivated in Bangladesh, China, Thailand, Malaysia, Cambodia, Philippines, Indonesia and Nigeria, which are all tropical regions (3). The rhizome, often known as the root, has a bitter and bright orange color that is covered in a rough brown skin. In traditional medicine, turmeric's rhizomes have been used to treat inflammations, signs of cancer, diabetes, stomachaches, high cholesterol as a natural remedy (4). Ikpeama *et al.* looked at the nutritional profile of turmeric and it was said to contain 67.38% carbohydrates, 2.85% ash, 6.85% fat, 9.40% crude protein, and 8.92% moisture(5) . Additionally, according to Gopinathan *et al.,* the granulated rhizome contains 70–76% curcumin (6). The biological effects of turmeric are due to curcumin, a yellow-colored active compound that is a powerful antioxidant (7). Vital components including vitamin C, betacarotene, polyphenol, fatty acids, and essential oil are also included in curcumin (5). Curcumin has been shown to have promise as a preventative and therapeutic agent for a number of malignancies, including those of the gastrointestinal tract, breast, lungs, head and neck, nervous system, and sarcoma (8, 9, 10,11). *Curcuma longa* comprises 2-9% curcuminoids, which are made up of the demethoxycurcumin, diarylheptanoids curcumin as well as bisdemethoxycurcumin (12, 13). The present research was focused on the comparative study of homely prepared turmeric with commercially available turmeric powder.

Materials and Method

Collection of samples

Curcuma longa, Curcuma aromatica, Curcuma casiea, Curcuma amada, Curcuma angustifolia were taken from Peermade, Idukki district it was then cleaned, rinsed with deionized water, scraped off, sliced, and dried for a week in the sun shade. For the current investigation, dried rhizomes were broken down into tiny pieces and powdered. Turmeric powder of the commercial brands A, B, and C was obtained from Konni.

Sample extraction

In 100ml of solvents such as acetone, butanol, and petroleum ether, 50g of the powder of home

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made dried rhizomes *of C.longa, C.aromatica, C.amada, C.caesia* and *C.angustifolia* and brand A, B and C were taken for 3 days with periodic shaking. A filter was used to separate the solvent from the whole extract and the remaining solvent was then allowed to evaporate before being concentrated. The extracts were kept chilled in capped conical flasks until use. Extracts were then utilized to quantitatively analysis of curcuminoids using a spectrophotometer and to check the antibacterial activity.

Spectrophotometric analysis of curcumin

The five *Curcuma* samples's absorbance were measured on a spectrophotometer at 420 nm in comparison to the blank solvents, and the amount of curcumin obtained using this approach was calculated and represented as a percentage.

UV-Visible spectra of curcuminoids

By refluxing the substance in acetone, curcumin was extracted quantitatively from the samples and measured spectrophotometrically using a spectrophotometer in the 200–700nm wavelength. At around 425 nm, curcumin displays a prominent, wide maximum absorption. As a result, the spectrophotometric estimation of curcumin concentration for all turmeric samples was in the range of around 425nm.

Antibacterial activity

The disc diffusion technique was used to evaluate the anti-microbial activity of the extracts. For the antibacterial test, nutritional broth was inoculated with overnight cultures of *E. coli, Proteus, Bacillus sp.,* and *Klebsiella sp.* Each of the petri plates contained around 20ml of nutritional agar, which was added and let to set. After making wells in the agar, the bacterial cultures were swabbed onto the plates. Each well received a single drop of extract from five different species in a specific solvent. The petri dishes were incubated for 48 hours at 37^0C. All five of the samples underwent the test. The diameter of the clear inhibitory zone around the well was measured to assess its antibacterial activity. Additionally, pictures were taken to document the effects of curcuminoid extracts on cultures.

Result

According to reports, the amount of curcumin in each kind of turmeric powder varies. In the current research, the spectrophometric analysis and antibacterial activities of commercially available turmeric powders are contrasted with those of naturally occurring turmeric powder that is made at home.

Three different kinds of turmeric powder were purchased from Konni for the comparative study, and the powders were then extracted in acetone, butanol, and petroleum ether. Using a spectrophotometer, 1ml of the sample was taken to measure absorbance at 420 nm in order to determine the quantity of coloured substance (curcumin) in the sample.Antibacterial activities were also investigated by placing a drop of extract into the wells made on the agar plates swabbed by strains of bacteria including *Bacillus sp, E.coli, Proteus sp* and *Klebsiella.*

Spectrophotometric analysis

The absorbance of the acetone extracts of the three turmeric powder samples was read at 420nm in spectrophotometer. The concentration of the curcumin was found to be different for each sample (Table.1).

Table 1.Spectrophotometric analysis

SI No.	Turmeric powder	OD at 420nm
1	A	1.76
2	B	1.32
3	C	1.67

Antibacterial activity

The antibacterial activity of selected brands of turmeric powder with homemade was carried out andtabulated.

Table .2

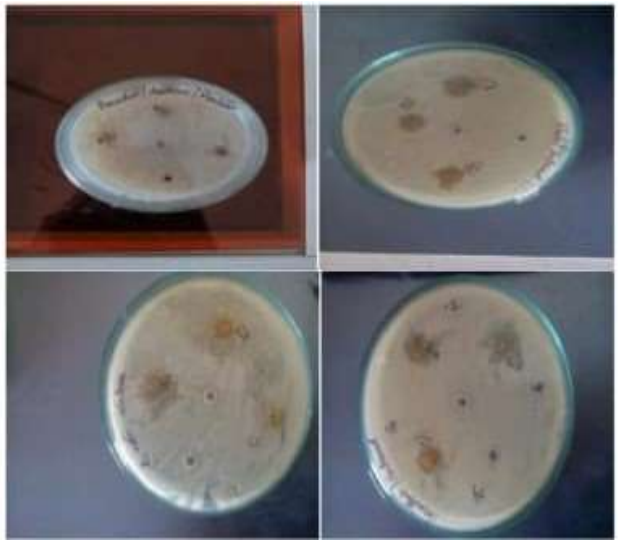
Sl. No.	Name of bacteria	Home made turmeric powder					Brand code of turmeric powder		
		C.longa	***C.aromatica***	***C.amada***	***C. caesia***	***C.angustifolia***	A	B	C
1	*Klebsiella*	+	+	-	-	-	+	+	+
2	*Proteus*	+	-	-	-	-	+	+	+
3	*Bacillus*	+	+	+	+	-	+	+	+
4	*E.coli*	-	-	-	-	-	+	+	-

\+ Sensitive
\- Not Sensitive

The diameter of the zone of inhibition was used to assess the antibacterial activity. Observable results were obtained when the antibacterial potential was tested against several bacterial strains. This experiment employed commercial brands A, B, and C of turmeric powder and the results revealed that *Klebsiella sp.* was sensitive to those brands (Plate.2). In the case of *E,coli* , it was sensitive to Brand A and B, not sensitive to brand C. The homemade powder result showed that *C.longa* was sensitive to *Klebsiella, Proteus* and *Bacillus* whereas E.coli was not sensitive. *C.aromatica* was sensitive to *Klebsiella* and *bacillus* ; not sensitive to *Proteus* and *E.coli. C.amada* and *C.caesia* was sensitive to *Bacillus* only and not sensitive to remaining species. *C.angustifolia* was not sensitive to all species like *Klebsiella, Proteus, Bacillus and E.coli* (Plate.1, Table.2).

Plate.1 Showing antibacterial activity of homemade turmeric powder

Plate.2 Showing antibacterial activity of commercial turmeric powder A,B,C



Discussion

Along with the three brands of turmeric powder obtained from the market, the antibacterial and Spectrophotometric analyses of the five chosen species were also studied. Thus, it is shown that turmeric, a common dietary item, may help to protect humans against some of nature's enemies like germs. In the three various brands of turmeric powder that are commercially available, a comparative study was carried out to determine how much curcumin was present. The results showed that colouring agents were present. Brand A displayed an absorbance of 1.76, Brand B displayed 1.32, and Brand C displayed 1.66 whereas pure curcumin had an absorbance of 1.12 at 420nm. It is evident that the samples include colouring agents since the spectrophotometer detects the coloured component in the sample. By measuring the diameter of the zone of inhibition, antibacterial activity was determined. Observable results were obtained when the antibacterial was tested against several bacterial strains. When tested against specific bacterial strains such *Klebsiella sp, Bacillus sp, E.coli,* and *Proteus sp.,* the extract from each species shown clear antibacterial activity.

Conclusion

The pigment curcumin, which is derived from five distinct *Curcuma* species, including *Curcuma longa, Curcuma aromatica, Curcuma amada, Curcuma caesia* and *Curcuma angustifolia* has been investigated thoroughly using a spectrophotometer. The antibacterial activity was also observed for the five species chosen as well as the three brands of turmeric powder owned from the market. Thus, it clearly showed that turmeric, a natural food component, may provide some kind of defence against our natural adversaries like germs.

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RESEARCH ARTICLE

Invitro Antioxidant Activity of *Embelia basaal* Burm.f. (Myrsinaceae)

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Abstract

This study evaluated the phytochemical screening, secondary metabolite content of different extracts and antioxidant activity of acetone extracts of *Embelia basaal* leaf and bark. Using Soxhlet extraction the different solvents such as petroleum ether, chloroform, acetone and ethanol were used for determining the qualitative phytochemical analysis and it confirmed the presence of primary and secondary metabolites. The quantification of total phenolic, tannin and flavonoid contents were found to be higher in the acetone extract of *E.basaal* bark. Subsequently the acetone extracts were subjected to antioxidant assays (DPPH, ABTS, nitric oxide, ferric reducing, superoxide, lipid peroxidation, and reducing power) and the results revealed that the acetone extract of *E.bassal* bark demonstrated superior free radical scavenging activity compared to the leaf. Thus, the findings highlight *E.basaal* has potential for developing health promoting drugs.

Keywords: *Embelia basaal*, *In-vitro* Antioxidant, Secondary metabolites, Phytochemical, Polar solvents.

1. Introduction

The genus *Embelia* is a member of the Primulaceae family, with over 130 species, (Angiosperm Phylogeny Group IV 2016). *Embelia ribes* Burm. f. is a shrubby creeper with woody climbers that belongs to the Primulaceae family and is found in semi-evergreen and deciduous forests in India. It is widely referred to as "Vidanga," one of the oldest traditional medicinal species in India, and is mostly used in the ayurvedic medical system. in various forms like churna, asava, aristha, lauha and taila.

E. basal is a highly valued material in many formulations and has strong anthelmintic and anti-oxidant effects in Ayurvedic medicine[1]. The bigger elliptical leaves of the plants are used with ginger, as a gargle for sore throats, the dried root bark is used as a toothache cure, and the finely ground berries are used as an ointment to treat pleuritis [2]. Fruits are carminative, astringent, light, arousing, stimulant, anthelmintic, and alternative. It is renowned for its ability to drive out tapeworms. A seed decoction helps with skin conditions, fevers, and chest issues [3].

In traditional medicine, plants are the most precious source of medicines used by many diverse populations by various communities [4]. The World Health Organization (WHO) states that the greatest source of a wide range of medications is medicinal plants [5]. Approximately 80% of people in wealthy nations utilize traditional medicines [6], that contains phytochemical compounds, which are responsible for their potential to cure many diseases.

All the parts of medicinal plants naturally contain phytochemicals that act as defense mechanisms and offer protection against a variety of illnesses. Secondary metabolites are naturally occurring plant chemicals that are the byproducts of primary metabolites, which include phenolic compounds, alkaloids, steroids, flavonoids, terpenoids, glycosides, saponins, tannins, and others [7].

Secondary metabolites are natural plant chemicals. These compounds play a key role in many medications, helping to neutralize a harmful free radicals produced during metabolism [8]. Excess free radicals, caused by abnormal metabolism increase the risk of diseases [9]. Natura antioxidant have gained popularity because of synthetic ones, like BHA and BHT that are linked to toxicity, side effects and high production costs [10]. This study focuses on analyzing the phytochemicals and antioxidant properties of *E. basaal* to improve global health.

2.Materials and Methods

2.1. Collection and extraction of Plant material

The *E. basaal* leaves and bark, were gathered from Manjoor Village in the Nilgiri District and authenticated by the Botanical Survey of India (voucher specimen no: BSI/SR/5/23/2024/Tech –

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234.), the Southern Regional Center in Coimbatore, Tamil Nadu. After being cleaned with tap water, the plant material was shade-dried for 20 days, ground into a fine powder, packed in thimbles, and then extracted successively using a Soxhlet apparatus utilizing solvents such as petroleum ether, chloroform, acetone, and ethanol. The extract was placed in an airtight container for future studies.

2.2 Qualitative phytochemical analysis

Standard protocols were used to evaluate the extracted samples for the initial phytochemical screening in order to identify the presence of primary and secondary metabolites [11].

2.3 Quantitative analysis of Secondary metabolites

2.3.1 Determination of total phenolics

The method described by [12] was used to determine the total phenolic content. In the test tubes, fifty microliter triplicates of the extracts (20 mg/20 mL) were obtained, and distilled water was added to bring the volume up to one millilitre. Next, each tube was filled with 2.5 mL of sodium carbonate solution (20%) and 0.5 mL of Folin-Ciocalteu Phenol Reagent (1:1 with water). The absorbance at 725 nm was measured against the reagent blank shortly after the reaction mixture was vortexed and the test tubes were left in the dark for 40 minutes. Gallic acid equivalents were used to express the results of the triplicate analysis.

2.3.2 Determination of total Tannins

The tannins were quantified using the same extract after the treatment with polyvinyl polypyrrolidine (PVPP) following, [13] procedure. A 100 × 12 mm Eppendorf tube was filled with 100 mg of PVPP, 1 mL of distilled water, and 1 mL of the sample extracts. After vortexing the material, it was frozen for 15 minutes at 4°C. The sample was then centrifuged at 4000 rpm for 10 minutes at room temperature, and the supernatant was gathered. Apart from the tannins, which would have precipitated with the PVPP, this supernatant solely contains simple phenolics. Using the foregoing method, the phenolic content of the supernatant was determined and reported as the amount of non-tannin phenolics.

2.3.3 Determination of total flavonoids

The method by [14] was used to quantify the flavonoid levels of the plant extracts. Two milliliters of distilled water were added to each test tube containing about 100 microliters of the plant extracts. The blank was a test tube filled with 2.5 mL of distilled water. After adding 150 µL of 5% $NaNO_2$ to each test tube, the tubes were incubated for six minutes at room temperature. Following incubation, all test tubes, including the blank, received 150 µL of 10% $AlCl_3$. At room temperature, every test tube was incubated for six minutes. After adding 2 mL of 4% NaOH to each test tube, the volume was increased to 5 mL with distilled water. After thoroughly vortexing the contents in each test tube, they were let to stand at room temperature for fifteen minutes. At 510 nm, the pink hue that resulted from the presence of flavonoids was detected using spectrophotometry. The quercetin equivalent (mg/g) was represented as the standard during the absorbance.

2.4 Antioxidant activity

2.4.1 DPPH radical scavenging activity

According to [11] method, the extract's antioxidant activity was assessed using the stable radical DPPH to measure its capacity to donate hydrogen or scavenge radicals. Different concentrations of sample extracts were obtained, and methanol was used to adjust the volume to 100 µL. approximately 5 mL of a 0.1 mM methanolic solution of DPPH was added Sample and standard aliquots (BHA, BHT, rutin, and quecertin) and shaken vigorously. 100 µL of methanol was added to 5 mL of 0.1 mM methanol solution DPPH as the negative control. At 27°C, the tubes were allowed to stand for 20 minutes. At 517 nm, the sample's absorbance was measured in comparison to the blank (methanol). The IC_{50}, or the concentration of the sample needed to inhibit 50% of the $DPPH^{\cdot}$ concentration, was used to express the samples' radical scavenging ability.

2.4.2 ABTS radical cation scavenging activity

Using the [1] methodology, the ABTS cation radical decolorization assay was used to measure the samples antioxidant activity. 7 mM ABTS aqueous solution and 2.4 mM potassium persulfate were mixed in the dark for 12 -16 hours at room temperature to yield ABTS•+. This solution was diluted in ethanol (about 1:89 v/v) and allowed to equilibrate at 30°C prior to the experiment, to exhibit an absorbance of 0.700 ± 0.02 at 734 nm. The sample extracts stock solution was diluted so that, when 10 µL aliquots were added to the experiment, the blank absorbance was inhibited by 20% to 80%. After the initial mixing of 10 µL of sample or Trolox (final concentration 0–15 µM) in ethanol with 1 mL of diluted ABTS solution, absorbance was measured at 30°C precisely 30 minutes. Each standard dilution was determined in triplicate, and the % inhibition was shown as a function of Trolox concentration after being

compared to the blank (ethanol) at 734 nm. The concentration of trolox with comparable antioxidant activity, given as μM/g sample extracts, is the unit of total antioxidant activity (TAA).

2.4.3 Phosphomolybdenum assay

The green phosphomolybdenum complex production, as per the [15] method, was used to assess the antioxidant activity of the samples. One milliliter of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was added to a test tube containing 40 microliters of sample or ascorbic acid in 1 milliliter of dimethyl sulfoxide (standard) or distilled water (blank). After being wrapped in foil, the test tubes were placed in a water bath set at 95°C for 90 minutes. Following the samples' cooling to room temperature, the mixture's absorbance at 695 nm was measured in comparison to the reagent blank. Total antioxidant capacity data are mean values in milligrams of ascorbic acid equivalents per gram of extract, or mg AAE/g.

2.4.4 Ferric reducing antioxidant power (FRAP) assay

A method outlined by [16] was used to determine the antioxidant capabilities of various sample extracts. 30 μL of test sample or methanol (for the reagent blank) and 90 μL of distilled water were combined with 900 μL of freshly made FRAP reagent that had been incubated at 37°C. In a water bath, the test samples and reagent blank were incubated for 30 minutes at 37°C. The test sample was finally diluted to 1/34 in the reaction mixture. 2.5 mL of 20 mM TPTZ in 40 mM HCl, 2.5 mL of 20 mM FeCl3. 6H2O, and 25 mL of 0.3 M acetate buffer (pH-3.6) were combined to create the FRAP reagent. A spectrophotometer was used to acquire absorbance measurements at 593 nm against the reagent blank immediately as the incubation was completed. The calibration curve was prepared using methanolic solutions with known Fe (II) concentrations, ranging from 100 to 2000 μM (FeSO4.7H2O). The concentration of antioxidant with a ferric-TPTZ reducing capacity equal to 1 mM FeSO4.7H2O was defined as the Equivalent Concentration parameter. The antioxidant concentration that increased absorbance in the FRAP assay to the theoretical absorbance value of a 1 mM concentration of Fe (II) solution was determined to be the equivalent concentration.

2.4.5 Superoxide radical scavenging activity

The experiment was predicated on the ability of different extracts to scavenge the superoxide radicals produced in the riboflavin–light–NBT system, hence inhibiting the synthesis of formazan [17]. 50 mM sodium phosphate buffer (pH-7.6), 20 μg riboflavin, 12 mM EDTA, 0.1 mg NBT, and 40 μL of an aliquot of sample solution or BHA and BHT (standard) were all included in each 3 mL reaction mixture. The reaction was initiated by exposing the sample extract on the reaction mixture for ninety seconds. And the negative control, with identical tubes containing the reaction mixture were stored in the dark, the absorbance was measured immediately after exposure in comparison to the reagent blank (reaction mixture devoid of plant sample) at 590 nm, and the scavenging activity (%) was calculated.

2.4.6 Nitric oxide scavenging activity

This process is based on the approach [18] Sodium nitroprusside in aqueous solution at its natural pH spontaneously produces nitric oxide, which combines with oxygen to form nitrite ions that may be calculated using the Greiss reagent. Nitric oxide scavengers compete with oxygen, which lowers the amount of nitrite ions produced. For the experiment, different concentrations of sample solution of different extracts or BHT and rutin (standard) were combined with sodium nitroprusside (10 mM) in phosphate buffered saline (0.2 M, pH-7.4), and the mixture was incubated for 150 minutes at room temperature. The negative control was the same reaction mixture without the sample. 0.5 mL of Griess reagent (1% sulfanilamide, 2% H3PO4, and 0.1% N-1-naphthyl) ethylene diamine dihydrochloride) was added following the incubation period. At 546 nm, the chromophore's absorbance was measured in comparison to the blank (phosphate buffer) and the scavenging activity (%) was calculated.

2.4.7 Lipid peroxidation assay

Using egg yolk homogenates as lipid-rich media, the amount of lipid peroxide produced was measured using a modified thiobarbituric acid-reactive species (TBARS) assay [19]. malondialdehyde (MDA), a subsequent byproduct of the oxidation of polyunsaturated fatty acids reacts with two molecules of TBA yielding a pinkish red chromogen with a maximum absorbance at 532 nm. Egg homogenate (500 μl of 10%, v/v in phosphate-buffered saline pH 7.4) and 200 μl of sample were added to a test tube and made up to 1.0 ml with distilled water. Then, 50 μl of $FeSO_4$ (0.075 M) and 20 μl of L-ascorbic acid (0.1 M) were added and incubated for 1 h at 37˚C to induce lipid peroxidation. Thereafter, 0.2 ml of EDTA (0.1 M) and 1.5 ml of TBA reagent (3 g TBA, 120 g TCA and 10.4 ml 70% $HClO_4$

in 800 ml of distilled water) were added in each sample and heated for 15 min at 100˚C. After cooling, samples were centrifuged for 10 min at 3000 rpm and absorbance of supernatant was measured at 532 nm and inhibition (%) of lipid peroxidation was calculated.

2.4.8 Reducing power assay

The reducing power of different solvent extracts was determined by the method adopted by [20]. One milliliter of phosphate buffer (pH 6.6) was mixed with 50–250 µg of extract. After adding 5 mL of a 1% potassium ferricyanide solution, the mixture was incubated for 20 minutes at 58°C. Five milliliters of 10% TCA were added following the incubation. For five minutes, the material was centrifuged at 5000 rpm. 0.5 mL of 1% ferric chloride and 5 mL of distilled water were combined with the top layer of the supernatant (5 mL). Using spectrophotometry, the absorbance of the reaction mixture was determined at 700 nm. The following formula was used to determine the percentage increase in reducing power.

2. Results and Discussion

Table 1: Phytochemical screening of various leaf and bark extracts of *E.basaal*

Phytochemicals	Leaf				Bark			
	P.E	C.F	A	E	P.E	C.F	A	E
Carbohydrates	+	+++	++	+++	+	+++	++	+++
Proteins	_	+++	_	++	++	++	++	_
Alkaloids	+++	_	+	_	+++	+++	_	++
Saponins	_	_	_	_	_	_	_	_
Flavonol Glycosides	_	_	++	++	_	_	++	_
Glycosides	+++	+	++	_	++	+	+++	++
Cardiac glycosides	+++	_	++	_	+	+	+++	++
Phytosterols	++	+++	++	+	++	+	+++	++
Flavonoids	++	++	++	+	+	++	++	+
Phenolic compounds	++	++	+++	++	++	++	+++	++
Tannins	+++	+++	+++	++	+++	+++	+++	_
Steroids	+++	_	_	+	+++	_	_	++
Terpenoids	+	+	+++	+	+	+	+++	+

P.E - Petroleum ether, C.F. - Chloroform, A - Acetone, E - Ethanol

(+): Presence of chemical compound, (-): Absence of chemical compound

(+) < (++) < (+++): Based on the intensity of characteristic colour produced

Table 2: Total phenolic, tannin and flavonoid in various leaf and bark extracts of *E.basaal*

Extracts	Plant part	Total Phenolics (mg GAE/g extract)	Total Tannin (mg GAE/g extract)	Flavonoid (mg RE/g extract)
Petroleum ether	Leaf	88.79 ± 2.56	78.02 ± 2.85	34.6 ± 0.58
	Bark	71.42 ± 3.02	57.63 ± 2.84	24.52 ± 0.51
Chloroform	Leaf	44.53 ± 1.68	30.84 ± 1.71	11.76 ± 0.26
	Bark	134.73 ± 0.48	116.69 ± 0.73	47.56 ± 0.81
Acetone	Leaf	952.94 ± 2.11	610.15 ± 2.26	62.7 ± 0.51
	Bark	959.66 ± 0.84	800.31 ± 1.78	98.12 ± 0.19
Ethanol	Leaf	682.35 ± 0.84	497.24 ± 3.31	19.46 ± 0.33
	Bark	120.17 ± 3.85	119.09 ± 4.01	35.53 ± 0.12

GAE – Gallic Acid Equivalents; RE – Rutin Equivalents
Values are mean of triplicate determination (n=3) ± standard deviation.

***Invitro* antioxidant activity**

Table 3: *Invitro* antioxidant activity in various in leaf and bark extracts of *E.basaal*

Antioxidant assay	Solvent	Leaf	Bark	Standard	
DPPH IC_{50} (µg/mL)	Acetone	49.53	54.74	Rutin	5.56
				BHT	4.44
ABTS (µgTE/g extract)	Acetone	139444.4 ± 240.56	137500 ± 208.33	Rutin	144167 ± 416.6
				BHT	145347 ± 636.4
FRAP mM Fe (II)/mg extract)	Acetone	512.09 ± 1.13	225.92 ± 1.95	Rutin	522.83 ± 4.46
				BHT	566.91 ± 3.5
Nitric oxide (% of inhibition)	Acetone	42.3 ± 1.56	50.02 ± 1.61	Rutin	52.7 ± 0.25
				BHT	54.2 ± 0.1
Phosphomolybdenum assay mg AAE/g (extract)	Acetone	550.99 ± 0.65	1274.16 ± 0.79	AAE	
Superoxide radical (% of inhibition)	Acetone	26.47 ± 0.66	40.91 ± 0.21	Rutin	53.2 ± 0.1
				BHT	54.7 ± 0.25
Lipid peroxidation (% of inhibition)	Acetone	52.39 ± 0.13	54.65 ± 0.05	Rutin	73.4 ± 0.87
				BHT	74.2 ± 0.94

TE – Trolox Equivalents; Fe (II) - Ferric Equivalents; AAE- Ascorbic acid equivalent
Values are mean of triplicate determination (n=3) ± standard deviation

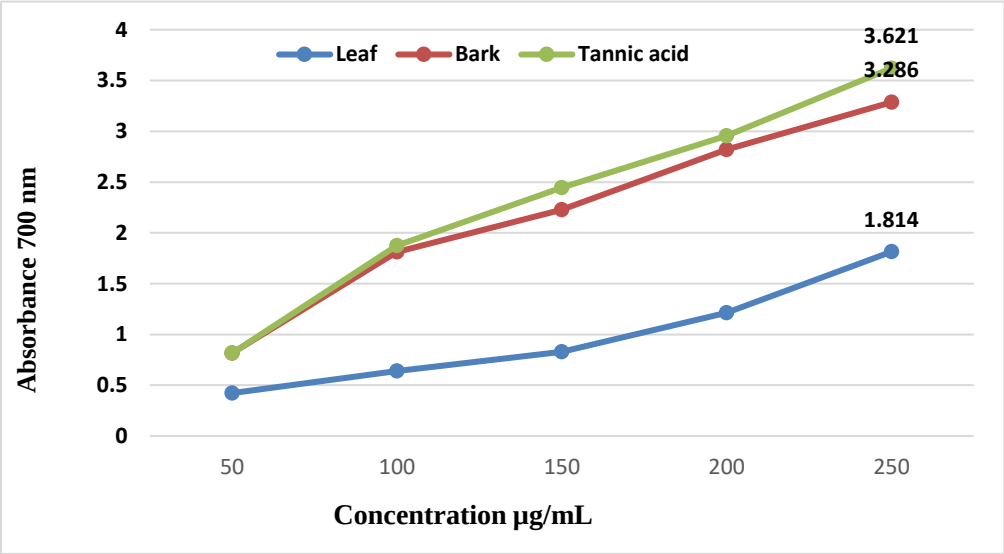

Figure. 1. Reducing power assay of acetone leaf and bark extract of *E.basaal*

3.1 Phytochemical analysis

3.1.1 Qualitative phytochemical screening

In general, the phytochemicals found in plants and plant-derived products are nontoxic and have therapeutic qualities [21] Table 1. shows the findings of the qualitative phytochemical analysis that was performed on the different extracts of *E.basaal* leaf and bark.

In all extracts, carbohydrates were found, but they were more prevalent in ethanol and chloroform. Proteins were found in chloroform and ethanol leaf extracts. Petroleum ether and chloroform bark extracts had high levels of alkaloids, while ethanol bark and petroleum ether leaf extracts had minimal quantities. Acetone and ethanol leaf extracts and acetone bark extracts consisted of flavonol glycosides, whereas petroleum ether and acetone bark extracts contained significant amounts of glycosides and cardiac glycosides. The findings of the phytochemical screening of *E. basaal* leaf and bark extracts are consistent with earlier research conducted by [2] in *E. ribes* leaf extracts, which found important bioactive substances like alkaloids, flavonoids, proteins, carbohydrates, and amino acids in various solvent extracts (acetone, ethanol, methanol, aqueous). In general, flavonoids and phenolic compounds were found, with acetone extracts having the highest concentration of phenolics. Tannins were not present in ethanol bark extracts, although they were present in petroleum ether, chloroform, and acetone extracts of bark and leaf of *E.basaal*. This study also supports the findings of [22] they observed similar compounds in the stem extracts, including alkaloids, carbohydrates, cardiac glycosides, quinones, phenols, and tannins, with saponins and starch being absent. Terpenoids and phytosterols were both highly present in the acetone and chloroform extracts, respectively. All extracts were devoid of saponins.

Overall, a notable phytochemical diversity was shown in the bark and leaves extracts of *E.basal*. However, the bark was the richer part in terms of phytochemical contents, as evidenced by the increased presence of important secondary metabolites such as alkaloids, glycosides, cardiac glycosides, and tannins in bark extracts. The high intensity of the colour showed a high concentration of specific secondary metabolites, denoted by the +++ sign and the lack of a chemical constituents is indicated by the - sign. The phytochemical richness of the plant and its potential for more pharmacological research are highlighted by these findings.

3.2 Quantitative analysis of secondary metabolites

3.2.1 Determination of total phenolic and tannin contents of *E.basaal*

Phenolic substances are comparatively persistent phenoxyl radicals that interfere with biological components' chain oxidation reactions [23]. These play a specific role in scavenging of free radicals [8]. The yields of the phenolic contents of *E. basaal* using various solvents varied. Acetone was the most efficient solvent, with the highest yields from both the leaf (952.94 ± 2.118 mg GAE/g) and the bark (959.66 ± 0.848 mg GAE/g). The results were

compared with previous reports, where [4] analyzed the total phenolic content of *E.bassal* dried ethanolic extract (in terms of gallic acid equivalents) and found that it was higher (5.8 mg GAE/g) than other extracts. However, ethanol yielded more phenols from the leaf (682.35 ± 0.848 mg GAE/g), while petroleum ether and chloroform yielded more phenols from the bark (134.73 ± 0.488 mg GAE/g), (71.42 ± 3.028 mg GAE/g) respectively.

The maximum tannin content was found in acetone (610.15 ± 2.26 mg GAE/g) leaf and (800.31 ± 1.78 mg GAE/g) and bark extracts of *E.basaal*. Followed by ethanol (497.24 ± 3.31 mg GAE/g) in the leaf and (119.09 ± 4.01 mg GAE/g) in the bark extract of *E.basaal*. Both leaf and bark yields lower tannin content in petroleum ether and chloroform extracts of *E.basaal*. These outcomes are consistent with those of Saraf *et al*., (2016), who found that flavonoids and tannins were the main phytochemicals present in fruit extracts from *E. ribes* and that they were soluble in polar solvents such as acetone. Similarly, Ananth and Anand Gideon, (2021) results depicted that tannins are a major secondary metabolite in the stem extracts of *E. ribes* are supported by the notable tannin concentration found in acetone and chloroform extracts.

3.2.2 Quantification of flavonoids

Table 2 displays the analysis and flavonoid content of *E.basaal.* Acetone produced the maximum flavonoid concentration in the leaf (62.7 ± 0.518 mg RE/g) and bark (98.12 ± 0.198 mg RE/g) of *E. basaal* when compared to other solvents. This demonstrates that acetone is the best solvent for extracting flavonoids from *E. basaal.* Acetone extracts high flavonoid content is in line with research by [2], which showed that *E. ribes* leaf extracts containing flavonoids were abundant when polar solvents were used.

3.3 Invitro Antioxidant Activity

3.3.1 DPPH Radical Scavenging activity

Table 3. displayed the extracts of *E.basaal* capacity to scavenge DPPH radicals. The strongest free radical scavenging activity was represented by the lowest IC_{50} values. The assay was contrasted with BHT and standard rutin. Acetone leaf extract has the strongest DPPH radical scavenging activity among the extracts tested, as evidenced by its higher IC_{50} values compared to *E.basaal* bark (54.74 µg/mL). The standard rutin and BHT were found to have radical scavenging activities of 5.56 µg/mL and 4.44 µg/mL, respectively. According to [24], the ethanolic extract of *E.ribes* had a superior DPPH value of 67.48±0.17 µg/mL, which is significantly greater than that of the *E.basaal* research sample.

3.3.2 ABTS radical cation scavenging activity

An oxidation process with potassium persultate produced the ABTS (2, 2-azinobis-3-ethylbenzothiazolino-6-sulfonic acid) radicals. Using ABTS, the total antioxidant capacity of *E.basaal* acetone extracts was calculated as indicated in Table 3. The acetone extract of the leaf has a slightly higher activity (139,444.4 µg TE/g) than the bark extract (137,500 µg TE/g) when compared to the synthetic antioxidant BHT (145,347 µg TE/g) and the natural standard antioxidant rutin (144,167 µg TE/g). In accordance with [25], *E. ribes* methanolic extracts have high ABTS scavenging activity because of their abundance of bioactive compounds, such as embelin, which produced results comparable to those of *E.basaal* and is essential for scavenging free radicals and shielding biological systems from oxidative stress.

3.3.3 Ferric Reducing antioxidant power assay

The ferrous complex, which has a strong blue colour and is detected at 593 nm, is produced when ferrictripridyltridyltriazine is reduced. Comparing the acetone extract from the leaf to the standard antioxidants rutin (522.83 mM Fe (II)/mg extract) and BHT (566.91 mM Fe (II)/mg extract), the FRAP results show that the leaf extract has the highest activity at 512.09 mM Fe (II)/mg extract, while the bark extract has a lower capacity at 225.92 mM Fe (II)/mg. Similarly, it was observed that *E.ribes* had the maximum FRAP activity in a berry methanolic extract, measuring 66.73 ± 0.60 mg Fe(II)/g [24]. It said that the species *Embelia* contains a wealth of phytocompounds that are excellent natural antioxidant sources.

3.3.4 Nitric oxide Scavenging activity

In comparison to the leaf extract (42.3%) bark has a higher percentage of inhibition (50.02%) in contrast to the common antioxidants, BHT and rutin. The substantial nitric oxide scavenging action of embelin-rich extracts of *E. ribes* is highlighted in a study by [26]. This property is attributed to the phenolic hydroxyl groups in the extracts, which efficiently donate electrons to neutralize NO radicals.

3.3.5 Phosphomolybdenum assay

The results demonstrate that the acetone extract of bark of *E.basaal* (1274.16 mg AAE/g) has a significantly greater capacity to reduce free radicals than the leaf extract (550.99 mg AAE/g), indicating superior antioxidant potential over the leaf extract

and being equivalent to the natural antioxidant ascorbic acid. Based on the findings of the earlier study by [27] the presence of polyphenols and flavonoids, which function as electron donors in lowering molybdenum ions to their lower oxidation states, was associated with the significant antioxidant activity that the methanolic extracts of *E. ribes* demonstrated in this assay.

3.3.6 Superoxide radical scavenging activity

Superoxide radicals are a precursor to additional reactive oxygen species, they are known to be harmful to biological components. However, the results, which are displayed in Table 3, depicts that the acetone extract of bark of *E.basaal* shows the highest percentage of inhibition at 40.91%, while the leaf extract shows the lowest percentage of inhibition at 26.47%. BHT (54.7%) and standard rutin (53.2%) showed better radical inhibition. Research shows that the presence of embelin, flavonoids, and phenolic acids in the methanolic and ethanolic extracts of *E. ribes* results in considerable superoxide radical scavenging action [28], emphasized the fact that *E. ribes* extracts scavenge superoxide radicals, which is consistent with their rich phytochemical profile, especially embelin. These bioactive components shield cells from harmful effect by stopping the cascade of oxidative stress brought on by superoxide.

3.3.7 Lipid peroxidation

The findings indicate that, in comparison to normal Rutin (73.4%) and BHT (74.2%), the acetone extract from the bark has an effective percentage of inhibition of 54.65%, while the leaf extract has an inhibition of 52.39%. Similarly, [29] found that *E. ribes* extracts antioxidant qualities considerably lower malondialdehyde (MDA), a biomarker of lipid peroxidation, indicating their potential to lessen damage brought on by oxidative stress.

3.3.8 Reducing power assay

An effective indicator of the plant's antioxidant activity is the reducing power assay. According to the graph, the reducing power of *E.basaal* acetone extracts showed a concentration-dependent activity between 50 and 250 µg/mL of samples and absorbance at 700 nm. The acetone extract of *E. bassal* exhibited a concentration-dependent increase in reducing power. As the absorbance of the leaf extract increased, it peaked at 250 µg/mL with a value of 1.814. The bark acetone extract, with the greatest absorbance of 3.286 at 250 µg/mL, showed superior reducing ability compared to the leaf. Significant reducing power has been demonstrated in studies on *E. ribes*, especially in extracts like methanol, ethanol, and acetone. Both methanolic and ethanolic extracts of *E. ribes* contain high number of phenolic compounds such as embelin, which is known to have electron-donating qualities[30].

4. Conclusion

E. basaal contains many phytochemicals, including alkaloids, flavonoids and tannins. The bark extract has been shown to contain the highest concentration of these compounds. Antioxidant studies reveal that both leaf and bark extracts possess strong free radical scavenging properties. With the bark extract demonstrating superior antioxidant capacity. These finding suggest the *E.basaal* has the potential to produce bioactive substances with significant medicinal value.

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RESEARCH ARTICLE

Under-utilized lesser-known wild edible plants of Tamil Nadu, India

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Abstract

The present study highlights the diversity of wild edible plants used by tribal and local communities in Tamil Nadu's phytogeographical regions, covering both the Western Ghats and Eastern Ghats. The ethnobotanical documentation includes 62 plant species across 36 families and 39 genera which are lesser-known wild edibles. The plants are categorized as 27 fruits, 26 leafy edibles, 3 tuberous edibles and 3 species of whole plants, stem and flower of single species each. These lesser-known wild edibles have significant potential but require further investigation to determine their nutritional value, potential for cultivation, agronomic practices, and propagation methods. Their sustainable conservation is also crucial to ensure their availability for future generations. This study sheds light on the importance of preserving traditional knowledge and biodiversity through scientific exploration.

Introduction

The growing global population, urbanization, and various crises have exacerbated hunger and malnutrition, particularly among vulnerable populations such as women, youth, and indigenous peoples. The text emphasizes the importance of harnessing wild plants in modern agriculture to create novel varieties that can contribute to a more diversified and resilient food supply. The current trends suggest that without significant changes, global malnutrition and undernourishment will persist, making it difficult to meet nutrition targets by 2030. While a limited number of plant species (around 30) provide the bulk of the world's energy needs, rural and tribal communities rely on a much broader diversity of wild edible plants, which are not part of conventional agriculture and are primarily found near local vegetation. The statistic that 582 million people are expected to be chronically undernourished by 2030, with more than half of them residing in African countries, underscores the urgent need for more sustainable and diverse food sources [1]. The fact that 45,000 wild edible plants have been recorded globally, with 3,900 species commonly consumed by remote populations, highlights the potential of these plants to contribute to food security if integrated into modern agricultural systems.

Tamil Nadu state's forests, covering approximately 27,079 km^2, support an impressive diversity of plant life, with 6,723 taxa, making Tamil Nadu the state with the highest number of plant species in India [2]. The region's forest vegetation includes 11 major and 40 minor forest types, creating a habitat for numerous endemic plants with economic and nutritional potential. The state's tribal communities, particularly the 12 major hill tribes residing in forested areas like the Nilgiris, Anamalais, Palnis, Agasthiyamalais, and the Eastern Ghats of Kolli and Shervarayan Hills, heavily rely on wild edible plants for their dietary needs. Ethnobotany of tribal communities of Western Ghats such as Palliyars [3]. and Kanis [4]. This reliance highlights the importance of these plants not only as a source of food but also as a genetic resource for developing high-yielding crop varieties and discovering new phytomolecules. The documentation and inventory of these wild edible plants are essential for identifying new crop species and sustainable dietary resources, especially as the global food system faces growing challenges related to population growth, climate change, and food insecurity. Exploring and utilizing these plant resources could help meet both regional and global food security needs, while preserving the ecological and cultural heritage of the tribal communities in Tamil Nadu. It is an opportunity for agricultural innovation, particularly in developing countries, to explore the cultivation of these underutilized wild plants, which could alleviate hunger and malnutrition, especially in rural and remote communities. The documentation and inventory of wild edible plants

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are essential for identifying new crop species and sustainable dietary resources, especially as the global food system faces growing challenges related to population growth, climate change, and food insecurity. Exploring and utilizing these plant resources could help meet both regional and global food security needs, while preserving the ecological and cultural heritage of the tribal communities in Tamil Nadu. It is an opportunity for agricultural innovation, particularly in developing countries, to explore the cultivation of these underutilized wild plants, which could alleviate hunger and malnutrition, especially in rural and remote communities. The documentation and inventory of wild edible plants are much essential for identifying the new crop plants and dietary resources to meet the global challenges.

Methodology

This study was conducted as part of an ethnobotanical documentation on plant diversity in the Eastern and Western Ghats of Tamil Nadu, led by the senior author. Ethnobotanical information was collected through informal, pre-scheduled interviews with local people and respondents of 12 different tribal communities residing across Tamil Nadu. Information on edible plant uses was documented, including specific plant parts used, modes of consumption, and preparations with supplemental ingredients. The documented areas and the distribution of the selected tribal communities are illustrated in Map 1. The chosen communities represent a diversity of ethnic cultures and traditions. Voucher specimens of the plants were collected, preserved as herbarium samples, and housed at the Sri Ganesan Herbarium (SGH) at The Madura College, Madurai. A total of 968 observations regarding locally used edible plants were recorded across Tamil Nadu. These records were carefully reviewed and cross-referenced with earlier literature and field observations, resulting in a final list of 62 lesser-known edible plant species. Specimens were identified in the field and verified against local Floras, with the current scientific names confirmed using Plants of the World Online [5] and the World Flora Online [6]. For each plant, we provide its botanical name, family, local name, edible plant part(s), and uses.

Observation

The present study highlighted a total of 62 plant species which are contributing 49 genera and 36 families with their edible uses. Based on the useful parts, fruits (27 species) and leaves (26 species) have been rated highly used wild edibles, followed by tuber and whole plant (3 species each), seeds with two species, stem and flowers with single species (Table 1). Out of these edible plants, 5 species belong to Capparidaceae, followed by Begoniaceae, Fabaceae with 4 species each, Amaranthaceae, Apocynaceae, Melastomataceae and Phyllanthaceae with 3 species each, Elaeocarpaceae, Primulaceae, Myrtaceae, Molluginaceae, Malvaceae, Oxalidaceae, Solanaceae and Rosaceae with 2 species and remining 20 families with single species each. Among these, about 20 plants species are eaten as fresh leafy edibles followed by ripened fruits with 9 species. Tubers of *Aponogeton natans* and *Ceropegia megamalayana* (Fig.2d) are eaten as raw and a tuber of *Maerua oblongifolia* is cooked and eaten. Succulent stem of *Ceropegia juncea* is used as fresh edible. Some plants used to prepare parched traditional recipes with adding common spices *viz*, *Arisaema leschanultii* (Fig. 2c), *Celosia polygonoides*, *Hypertelis cerviana*, *Malachra capitata*, etc. Plant species such as *Capparis diversifolia*, *Embelia adnata*, *Medinilla beddomei*, *Medinilla malabarica* and *Ceropegia meamalayana* are reported here as first entity for edible plants.

Discussion

Use of wild edible is coping mechanism in times of food shortage, provides important safety energy source for the rural poor. In Tamil Nadu, out of 37 scheduled tribes listed, about 12 are residing completely in forested areas (Map 1). Some prominent hills tribes are Irular, Kadar, Kanikaran, Kattu Naicken, Kurumban, Malasar, Malayali, Mudhuvan, Palliar, Panian, Toda and Urali. Several studies have already been documented the wild edibles of Tamil Nadu from the forest dwelling people [3, 7-15]. Palliyar tribes alone used more than 150 wild edible plants from the Western Ghats of Tamil Nadu [3].

Several wild edible fruit plants like *Artocarpus lakoocha*, *Aegle marmelos*, *Annona squamosa*, *Carissa carandas*, *Cordia dichotoma*, *Eugenia jambos*, *Limonia acidissima*, *Grewia tenax*, *Manilkara hexandra*, *Morus indica*, *Pithecellobium dulce*, *Schleichera oleosa*, *Spondias pinnata*, *Syzygium cumini*, *Tamarindus indica*, and *Ziziphus mauritiana* are contributing significantly to the nutritional supplements in rural areas [16]. A study documented about 28 important wild edible fruits from Western Ghats [17] and most species of wild edible fruit plants belong to the families of Anacardiaceae, Clusiaceae, Malvaceae, Myrtaceae, Phyllanthaceae, Moraceae, Rutaceae, etc.

Conventionally, tribal and rural people often rely on wild edible fruits for their food, which could provide primary dietary constituents and natural bioactive compounds [18]. Several of wild edibles have rich antioxidant principles which are protecting the human body from cancers and other infective disease [19]. Whereas wild edible plants are contributing more than 7.5% of local people dietary needs with other supplements like healing of medicinal ailments [20]. Most of the wild edible tubers have rich of carbohydrate resources along with number of other phytochemicals [9].

The study highlights that the scheduled tribes and forest communities in Tamil Nadu continue to rely on lesser-known wild edibles as part of their food sources, maintaining a deep understanding of their habitats, collection times, identification methods, preparation techniques, and consumption practices. This traditional knowledge, preserved through verbal communication, serves as a valuable cultural resource. It holds potential significance for future food security and nutrition, offering a sustainable way to support both the communities and broader needs for natural nutrition sources.

Conclusion

Table 1. Under-utilized Lesser-known wild edible plants of Tamil Nadu

SI.No.	Name of the plant	Family	Part used	Local name	Uses
1.	*Antidesma montanum* Blume	Phyllanthaceae	Fruit	Aasaripuli	Ripened fruits edible
2.	*Aponogeton natans* (L.) Engl. & K.Krause	Aponogetonaceae	Tuber	Kotti kizhangu	Raw and cooked tubers edible
3.	*Arisaema leschenaultii* Blume	Araceae	Leaves	Kaattukarunai	Fried leaves edible
4.	*Asystasia gangetica* (L.) T.Anderson	Acanthaceae	Leaves	Manjalthavasi	Cooked leaves edible
5.	*Bambusa bambos* (L.) Voss	Poaceae	Seed (grains)	Moongilarasi	Cooked grains edible
6.	*Begonia crenata* Dryand.	Begoniaceae	Leaves	Kalthamarai	Raw leaves edible
7.	*Begonia cordata* Vell.	Begoniaceae	Leaves	Kallurukki	Fresh raw leaves edible
8.	*Begonia floccifera* Bedd.	Begoniaceae	Leaves	Kalthamarai, Paraipulichai	Fresh raw leaves edible
9.	*Begonia roxburghii* (Miq.) A.DC.	Begoniaceae	Leaves and stem	Narayanasanjevi	Leaves edible as raw, stem coked along with green vegetable
10.	*Boerhavia erecta* L.	Nyctaginaceae	Leaves	Sirumukirattai	Cooked leaves edible
11.	*Bridelia retusa* (L.) A.Juss.	Phyllanthaceae	Fruit	Mulvaengai	Ripened fruits edible
12.	*Bulbophyllum fuscopurpureum* Wight	Orchidaceae	Leaf bulb	Seethai manjal	Fresh bulb edible
13.	*Canavalia gladiolata* J.D.Sauer	Fabaceae	Fruit	Thampatavarai	Cooked fruits edible

14.	*Capparis diversifolia* Wight & Arn..	Capparidaceae	Fruit	Koratti pazham	Ripened fruits edible
15.	*Capparis sepiaria* L.	Capparidaceae	Fruit	Karumsoorai	Ripened fruits edible
16.	*Celosia polygonoides* Retz.	Amaranthaceae	Leaves	Pulisirukeerai	Cooked leaves edible
17.	*Cereus pterogonus* Lem.	Cactaceae	Fruit	Sathurakalli	Ripened fruits edible
18.	*Ceropegia juncea* Roxb.	Apocynaceae	Stem	Vaelipulichai	Raw stem edible
19.	*Ceropegia megamalayana* (Karupp.) Kottaim.	Apocynaceae	Tuber	Paraikizhangu	Tubers edible as raw
20.	*Citrullus lanatus* (Thunb.) Matsum. & Nakai	Curcubitaceae	Fruit	Mithukkai	Unripe fruits made into dry pickles and ripened fruits edible
21.	*Cordia diffusa* K.C.Jacob	Boraginaceae	Fruit	Narivizhi	Ripened fruits edible
22.	*Crateva adansonii* DC.	Capparidaceae	Fruit	Mavalingam	Ripened fruit pulp edible
23.	*Elaeocarpus* serratus L.	Elaeocarpaceae	Fruit	Kattukotlan	Ripened fruits edible
24.	*Elaeocarpus variabilis* Zmarzty	Elaeocarpaceae	Fruit	Kottampazham	Ripened fruits edible
25.	*Embelia adnata* Bedd. ex C.B.Clarke	Primulaceae	Leaves	Pulippanthalai	Raw leaves edible
26.	*Embelia tsjeriam-cottam* (Roem. & Schult.) A.DC.	Primulaceae	Leaves and fruits	Kaattupulichai	Ripened fruits and leaves edible
27.	*Eugenia discifera* Gamble	Myrtaceae	Fruit	Kaattukoyya	Ripened fruits edible
28.	*Eugenia singampattiana* Bedd.	Myrtaceae	Fruit	Singampatti koyya	Ripened fruits edible
29.	*Flueggea leucopyrus* Willd.	Phyllanthaceae	Fruit	Vetpula	Ripened fruits edible
30.	*Garcinia indica* (Thouars) Choisy	Clusiaceae	Fruit	Kokkam puli	Ripened fruits edible
31.	*Hugonia mystax* Lam.	Linaceae	Fruit	Mothirakkanni	Ripened fruits edible
32.	*Hydrocotyle conferta* Wight	Araliaceae	Leaves	Siruvallarai	Cooked leaves edible
33.	*Hypertelis cerviana* (L.) Thulin	Molluginaceae	Plant	Parpadagam	Cooked plants edible
34.	*Ixora chinensis* Lam.	Rubiaceae	Fruit	Thetti pazham	Ripened fruits edible

35.	*Kadsura heteroclita* (Roxb.) Craib	Schisandraceae	Fruit	Thaenmilagu	Ripened fruits edible
36.	*Maerua oblongifolisa* (Forssk.) A.Rich.	Capparidaceae	Tuber	Poochakra kizhangu	Cooked tuber edible
37.	*Malachra capitata* (L.) L.	Malvaceae	Leaves	Punnaku keerai	Cooked leaves edible
38.	*Medinilla beddomei* C.B.Clarke	Melastomataceae	Leaves	Neervalam	Fresh leaves edible
39.	*Medinilla malabarica* Bedd. & C.E.C.Fisch.	Melastomataceae	Leaves	Pooncharai	Fresh leaves edible
40.	*Waltheria indica* L.	Malvaceae	Leaves	Sempodu	Cooked leaves edible
41.	*Morisonia flexuosa* L.	Capparidaceae	Fruit	Milagaipazham	Ripened fruits edible
42.	*Oxalis latifolia* Kunth	Oxalidaceae	Leaves	Malai aarai	Cooked leaves edible
43.	*Oxalis spiralis* Kunth	Oxalidaceae	Leaves	Sevappu aarai	Cooked leaves edible
44.	*Paramollugo nudicaulis* (Lam.) Thulin	Molluginaceae	Plant	Pura keerai	Cooked plants edible
45.	*Phoenix loureiroi* Kunth	Arecaceae	Fruit	Eecham	Ripened fruits edible
46.	*Physalis angulate* L.	Solanaceae	Fruit	Sodakkuthakkali	Ripened fruits edible
47.	*Physalis peruviana* L.	Solanaceae	Fruit	Sodakkuthakkali	Ripened fruits edible
48.	*Portulaca quadrifida* L.	Portulacaceae	Plant	Pasiri keerai	Cooked plants edible
49.	*Potentilla indica* (Andrews) Th.Wolf	Rosaceae	Fruit	Ponvandukannu	Ripened fruits edible
50.	*Psilotrichum patulum* (Willd.) I.M.Turner	Amaranthaceae	Leaves	Antharakeerai	Cooked leaves edible
51.	*Pupalia lappacea* (L.) Juss.	Amaranthaceae	Leaves	Magilikeerai	Cooked leaves edible
52.	*Rivea hypocrateriformis* (Desr.) Choisy	Colvolvulaceae	Leaves	Potthikeerai	Cooked leaves edible
53.	*Rivina humilis* L.	Petiveriaceae	Fruit	Kuruthinelli	Ripened fruits edible
54.	*Rubus fairholmianus* Gardner	Rosaceae	Fruit	Siru-unni	Ripened fruits edible
55.	*Sarcostigma kleinii* Wight & Arn.	Icacinaceae	Fruit	Kodikkalli	Cooked fruits edible
56.	*Senna italica* Mill.	Fabaceae	Seeds	Nilavagai	Cooked leaves edible

57.	*Senna obtusifolia* (L.) H.S.Irwin & Barneby	Fabaceae	Leaves	Oosithagarai	Cooked leaves edible
58.	*Sesbania sesban* (L.) Merr.	Fabaceae	Flower	Chithagarathi	Cooked flowers edible
59.	*Smilax wightii* A.DC.	Smilacaceae	Leaves	Kodiyelanthai	Tender leaves edible
60.	*Sonerila tinnevelliensis* C.E.C.Fisch.	Melastomataceae	Leaves	Kaattupulichai	Leaves used to substitute tamarind
61.	*Stephanotis* volubilis (L.f.) S.Reuss, Liede & Meve	Apocynaceae	Leaves	Perumkurinjan	Cooked leaves edible
62.	*Zaleya decandra* (L.) Burm.f.	Aizoaceae	Leaves	Uppukeerai	Cooked leaves edible

Map 1. Tribal distribution in Tamil Nadu

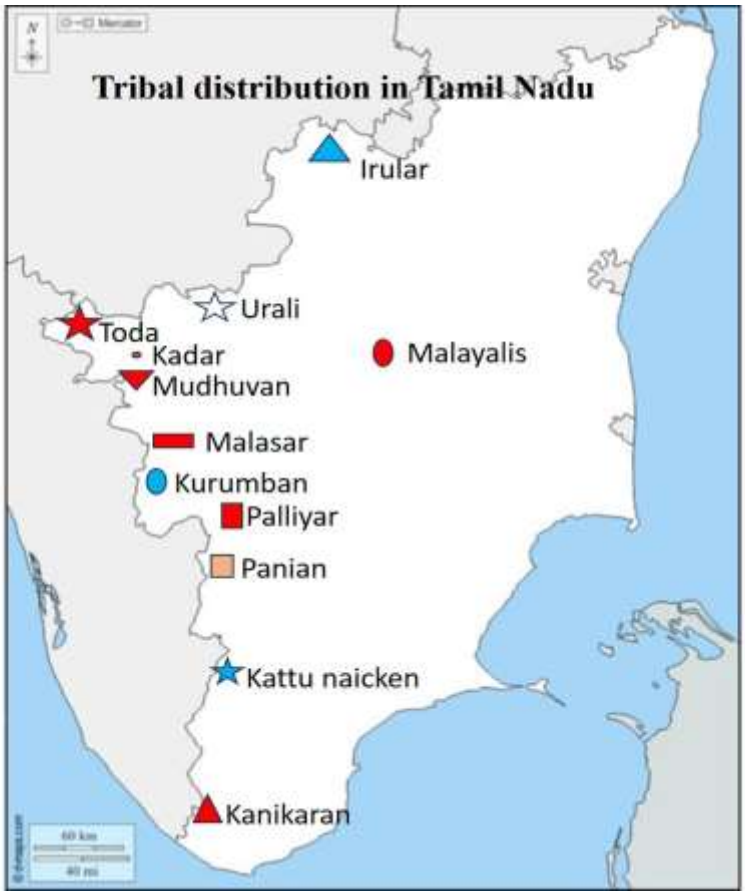

Figure 2. a. *Begonia floccifera*; b. *Begonia malabarica*; c. *Arisaema leschenaultii* (leaves); d. *Ceropegia megamalayana*; e. *Capparis diversifolia*; f. *Embelia adnata*; g. *Medinilla beddome*; h. *Medinilla malabarica*; i. *Sonerila tinnevelliensis*

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RESEARCH ARTICLE

Assessing riparian floristic diversity in the Gayathripuzha river basin of Palakkad district

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Abstract

The riparian flora of the Gayathripuzha river of Palakkad district was studied by a series of survey from July2022 to February 2023.The study revealed that about87 species of species belonging to 31 families were identified. The habit wise analysis of the species suggests that 65% of the plants were herbs, 16% shrubs and 8% climbers and 11% creepers. The present study revealed that Asteraceae,Fabaceae and Malvacea were the dominant families. 74 dicot species and 12 monocot species make up the variety of angiosperm plants. The habit wise analysis of the species suggests that 65% of the plants were herbs, 16% shrubs and 8% climbers and 11% creepers. Asteraceae family dominated the vegetation analysed.

Key Words: Riparian Vegetation.

Introduction

The word 'Riparian' is defined as vegetation ecosystem and habits that are associated with water bodies. The Latin term "Ripa," means "belonging to the banks of a river". This area is frequently referred to as riparian flora since it is a transition zone between water upland environments. It is called as gallery forest or stream side forest because the riparian zone, which extends from the beginning to the end of a river, is greatly influenced by the quantity and flow of water in the river channel[1] (Brinson 1990). The riparian zones supply shelter and food for many aquatic animals and the shade that limit stream temperature change. The roots of riparian trees and shrubs helps to hold stream banks in place and prevents erosion. Riparian vegetation also traps sediment and pollutants helping to keep the water clean by their root system. Different latitudes and altitudes can support very difficult riparian communities. The average width of the riparian area may vary from between 5 - 20cm, depending on the species used and the site.

Riparian landscapes are highly threatened ecosystem as they are inherently rare habits occupying a mere one thousand of earth surface [2](Hynes 1970). Studies on riparian vegetation have been carried out all over the world, identifying floristic diversity and the role of riparian forests as nutrient filters in agricultural watersheds. Climate, altitudinal gradients, upland impacts, flood regimes, and geomorphic channel processes all have a significant impact on the ecological richness of riparian corridors [3](Naiman, et al., 1993). Factors such as light levels, habitat productivity, water flow, soil moisture, disturbance patterns, and rates of erosion and sediment deposition vary along rivers, influencing the distribution of riparian species ([4]Naiman and Décamps, 1997; [5]Larsen et al., 2019; [6]Pielech and Czortek, 2021)

Degradation of riparian zones not only affects the riparian area but also the surface and ground water resources along with terrestrial ecosystem. Climate change has strongly reduced the number of native species in these ecosystem puffing many of them at risk of extinction

Materials and Methods

Study Area

The research approach involves a taxonomic / vegetational survey of lower stretch of Gayathripuzha River(Fig.1). It is the one of the main tributaries of the second largest river in kerala, the Bharathapuzha river. It passes through Kollengode, Nemmara, Alathur, Padur and

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Pazhayannur before joining the Bharathapuzha at Mayannur. The average rainfall of the area where Gayathripuzha flows is 7348mm.

The main tributaries of Gayathripuzha are Mangalam river, Ayalur river, Vandazhy river, Meenkara river and Chulliyar.We have selected five sites along the Gayathri river to study about its herbeaceous riparian vegetation. These study sites were Pappanchalla and Ootara Vallanghy, Tripallur and Athipotta (Table 1). A series of survey were conducted from July-February. The specimens were collected and the identification of the specimens was made initially with the help of standard floras [7] Bentham & Hooker (1862–1883),[8] Gamble & Fischer (1915–1936).The collected specimens were deposited in the herbarium of NSS College, Nemmara.

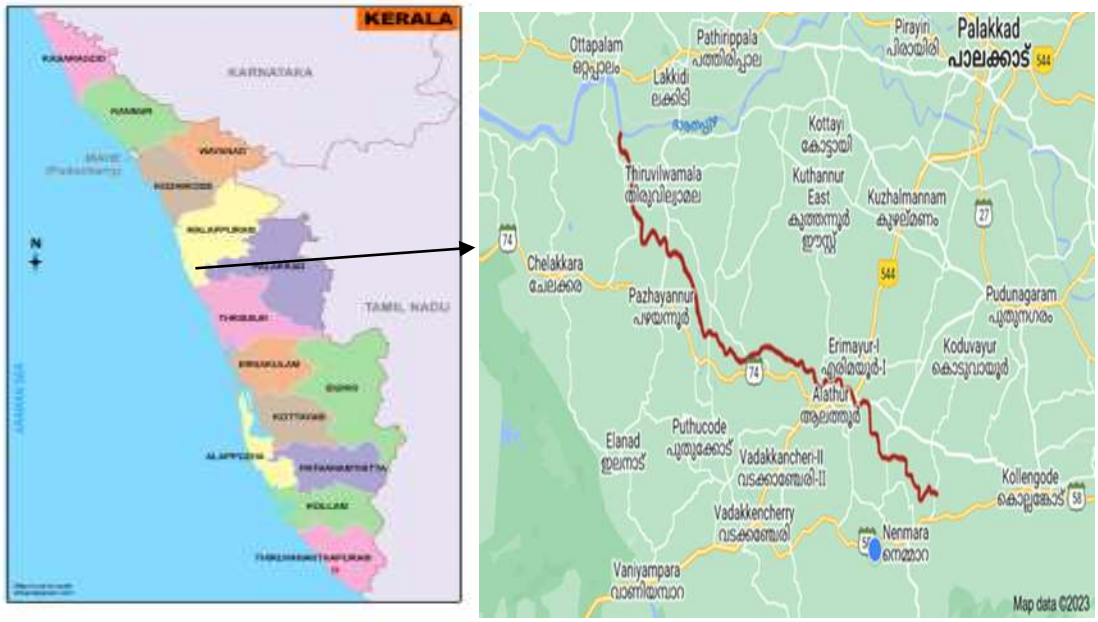


Fig.1 Map showing study area

Table 1. Brief description of study area

S.No.	Name of Sampling Site	GPS Coordinates
1	Pappanchalla	10.619° N and 76.7746° E.
2	Ootara	10.6094° N and 76.7754° E.
3	Vallanghi	10.5967° N and 76.6052° E.
4	Tripalur	10.6441° N and 76.5668° E.
5	Athipotta	10.37435° N and 76.77619° E.

Results and Discussion

During this study of riparian vegetation along the Gayathripuzha river basin, a total of 87 species belonging to 31 families were identified. The Gayathri River's riparian region in the Palakkad district's Chittur thaluk was primarily covered with grasses, shrubs, and herbaceous species. A total of 86 species of angiosperms and 1 species of pteridophytes from 77 genera and 31 families were identified (Table 2.). 74 dicot species and 12 monocot species make up the variety of angiosperm plants. The habit wise analysis of the species suggests that 65% of the plants were herbs, 16% shrubs and 8% climbers and 11% creepers (Fig.3). The ratio of Monocots to Dicots was 1:6.1, Families to Genera is 1: 2.5 Genera to species is1:1.1 Asteraceae family dominated the vegetation analysed (Table3). Among the Dicots the sub class Gamopetalae was represented by 38 species, while Polypetalae had 27 species and Monochlamydeae was represented with 9 species. A total of 25% of the monocot plant species pertain to the Coronarieae series, 66.66% to the Glumaceae series, and 8.33% to the Nodiflorae Series. Coronarieae, Nodiflorae and Glumaceae have a 3:1:8 ratio.

Table 2.Details of enumerated plants, their botanical names, habit, families

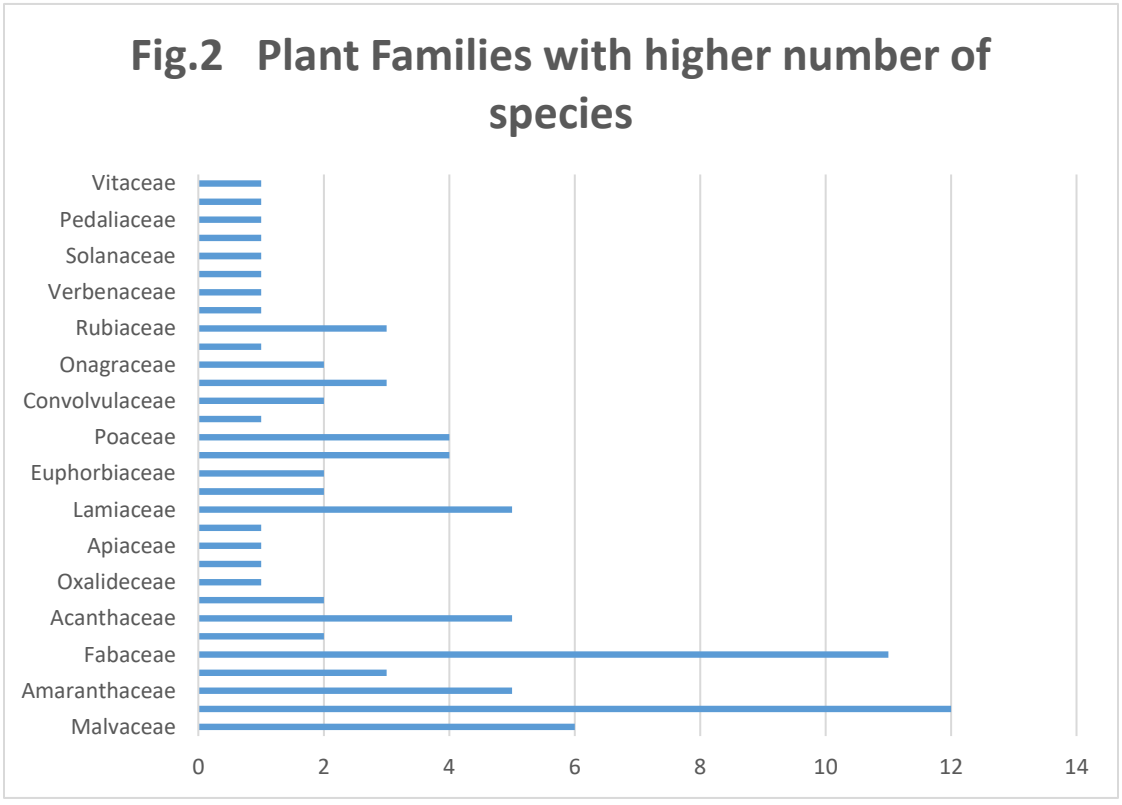
Sl No	Species	Family	Common Name	Habit
1	*Abutilon indicum*	Malvaceae	Velluram	Shrub
2	*Acmella ulignosa*	Asteraceae	Palluvedhana chedi	Herb
3	*Aerva lanata*	Amaranthaceae	Cherula	Herb
4	*Ageratum conyzoides*	Asteraceae	Murianpacha	Herb
5	*Allamanda cathartica*	Apocynaceae	Kolambi	Creeping shrub
6	*Alternanthera sessilis*	Amaranthaceae	Ponamgani	Herb
7	*Alysicarpus vaginalis*	Fabaceae	Nila orila	Herb
8	*Amaranthus spinosus*	Amaranthaceae	Mullencheera	Herb
9	*Amaranthus viridis*	Amaranthaceae	Kuppacheera	Herb
10	*Ammannia baccifera*	Lythraceae	Kallur Vanchi	Herb
11	*Asteracantha longifolia*	Acanthaceae	Vayalchulli	Herb
12	*Asystasia gangetica*	Acanthaceae	Valliupudali	Herb
13	*Bacopa monnieri*	Plantaginaceae	Brami	Creeping herb
14	*Biophytum sensitivum*	Oxalidaceae	Mukkootti	Herb
15	*Blepharis maderaspatensis*	Acanthaceae	Hemakandi	Herb
16	*Blumea axillaris*	Asteraceae		Herb
17	*Calopogonium mucunoides*	Fabaceae		Climber
18	*Calotropis gigantea*	Apocynaceae	Erukku	Shrub
19	*Cardiospermum halicacabum*	Sapindaceae	Uzhinja	Climber
20	*Cassia tora*	Fabaceae	Vattathakara	Herb
21	*Centella asiatica*	Apiaceae	Kudangal	Herbaceous creeper
22	*Centrosema plumieri*	Fabaceae		Climber
23	*Chromolaena odorata*	Asteraceae	Communist Pacha	Herb
24	*Cleome viscosa*	Cleomaceae	Ariavila	Herb
25	*Clerodendrum phlomidis*	Lamiaceae	Kozhiyappa	Shrub
26	*Clinopodium brownei*	Lamiaceae		Creeping herb
27	*Commelina benghalensis*	Commelinaceae	Vazhaplaachi	Herb
28	*Conyza japonica*	Asteraceae		Herb
29	*Corchorus olitorius*	Stericulaceae	Chanam	Herb
30	*Croton hirtus*	Euphorbiaceae		Herb
31	*Cyperus iria*	Cyperaceae		Herb
32	*Cyperus marginatus*	Cyperaceae		Herb
33	*Cyperus rotundus*	Cyperaceae	Perumkora	Herb
34	*Desmodium gangeticum*	Fabaceae	Orila	Shrub
35	*Desmodium paniculatum*	Fabaceae		Herb
36	*Desmodium triflorum*	Fabaceae	Cherupulladi	Herb
37	*Desmostachya bipinnata*	Poaceae	Dharbapull	Herb
38	*Eichhornia crassipes*	Pontederiaceae	Kulavazha	Herb

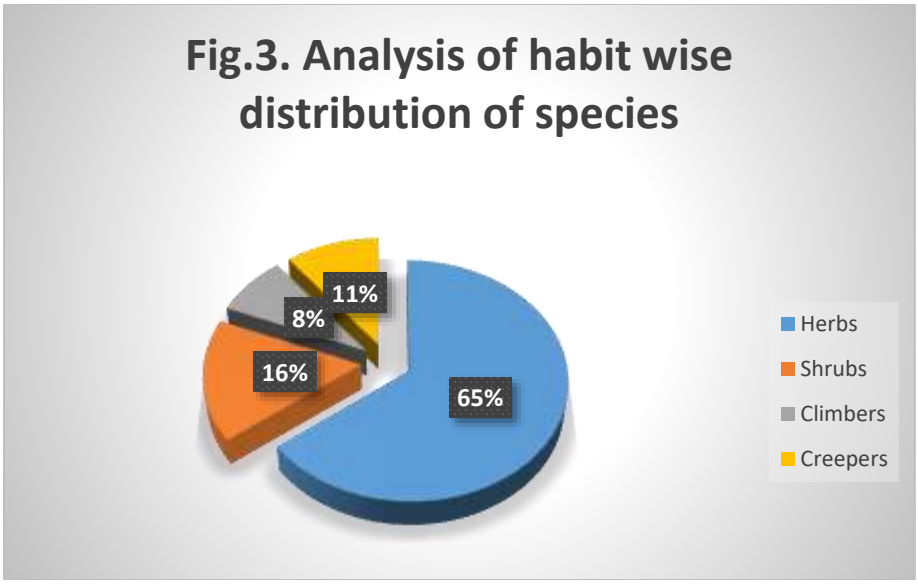
39	*Eleusine indica*	Poaceae		Herb
40	*Emilia sonchifolia*	Asteraceae	Muyalcheviyan	Herb
41	*Eragrotis annulata*	Poaceae		Herb
42	*Euphorbia hirta*	Euphorbiaceae	Attuvattappala	Herb
43	*Gomphrena serrata*	Amaranthaceae	Velutha Vaadamalli	Herb
44	*Heimia salicifolia*	Lythraceae		Shrub
45	*Hemidesmus indicus*	Apocynaceae	Naruneendi	Shrub
46	*Hyptis suaveolens*	Lamiaceae	Nattapoochedi	Herb
47	*Indigofera linnaei*	Fabaceae	Chempulladi	Herb
48	*Ipomoea aquatica*	Convolvulaceae	Ballel Kozhuppa	Climber
49	*Ipomoea cairica*	Convolvulaceae	Udhayamalari	Climber
50	*Justicia tranquebariensis*	Acanthaceae		Sub shrub
51	*Kyllinga brevifolia*	Cyperaceae		Herb
52	*Leucas aspera*	Lamiaceae	Thumba	Herb
53	*Lindernia antipoda*	Linderniaceae		Creeping herb
54	*Lindernia ciliata*	Linderniaceae	Chiravanakk	Herb
55	*Lindernia crustacea*	Linderniaceae	Cherukakkapoo	Herb
56	*Ludwigia polycarpa*	onagraceae		Herb
57	*Ludwigia hyssopifolia*	onagraceae	Neergra	Herb
58	*Marsilea quadrifolia*	Marsileaceae	Nalilakeera	Herb
59	*Mikania micrantha*	Asteraceae	Vayara	Climber
60	*Mimosa pudica*	Fabaceae	Thottalvadi	Creeping shrub
61	*Mitracarpus hirtus*	Rubiaceae	Thaval	Herb
62	*Murdannia semiteres*	Commelinaceae	Nilampull	Herb
63	*Oldenlandia corymbosa*	Rubiaceae	Parppadakam	Herb
64	*Ocimum sanctum*	Lamiaceae	Tulasi	Herb
65	*Pennisetum setaceum*	Poaceae	Pothapullu	Herb
66	*Persicaria attenuata*	Polygonaceae		Herb
67	*Phyla nodiflora*	Verbenaceae	Neerthippali	Creeping Herb
68	*Phyllanthus niruri*	Phyllanthaceae	Keezharnelli	Shrub
69	*Physalis minima*	Solanaceae	Notinotta	Herb
70	*Portulaca oleracea*	Portulacaceae	Koluppa	Creeping herb
71	*Ruellia tuberosa*	Acanthaceae	Shivakaratha	Herb
72	*Scoparia dulcis*	Plantaginaceae	Kallurukki	Herb
73	*Senna occidentalis*	Fabaceae	Mattan Thakara	Shrub
74	*Sesamum indicum*	Pedaliaceae	Ellu	Herb
75	*Sida acuta*	Malvaceae	Malakurumthotti	Shrub
76	*Sida cordifolia*	Malvaceae	Velluram	Shrub
77	*Sida longifolia*	Malvaceae		Shrub
78	*Spermacoce articularis*	Rubiaceae	Kudalchurukki	Herb
79	*Sphagneticola trilobata*	Asteraceae	Amminipoo	Creeping herb

80	*Spilanthes acmella*	Asteraceae		Herb
81	*Synedrella nodiflora*	Asteraceae	Mudiyanpacha	Herb
82	*Tephrosia purpurea*	Fabaceae	Kozhinjil	Shrub
83	*Typha angustifolia*	Typhacea	Aanapullu	Herb
84	*Urena lobata*	Malvaceae	Oorppanam	Shrub
85	*Vernonia cinerea*	Asteraceae	Poovamkurunnila	Herb
86	Vitis berlandieri	Vitaceae		Climber
87	Xanthium strumarium	Asteraceae	Arishta	Herb

Table 3.Dominant families of the study area

Sl.No	Family	No. of Genera	No. of species
1	Asteraceae	12	12
2	Fabaceae	9	11
3	Malvaceae	3	5
4	Amaranthaceae	4	5
5	Acanthaceae	5	5
6	Lamiaceae	5	5
7	Cyperaceae	2	4
8	Poaceae	4	4
9	Lindernaceae	1	3
10	Apocynaceae	3	3
11	Rubiaceae	3	3





The results of the study showed that Poacea and Cyperaceae families are the largest monocotyledon families, with 4 species each. While the Asteraceae family is the largest dicotyledon family, with 12 species and 12 genera(Fig2). The Asteraceae was the largest contribution (12 genera and 12 species) followed Fabaceae family (9 genera and 11species), and Malvaceae (3 genera), Acanthaceae (5 genera), Lamiaceae(5 genera) and Poaceae (4 genera) and cyperaceae (2genera and 4 species). Majority of the plants identified in the current study were either moisture-loving or wetland species. The research area's wetland and terrestrial plant diversity indicates to a close relationship between the two ecosystems as well as the river basin's declining size and sedge and grass predominance. Sedges and grasses make up 10% of the flora's diversity along the Gayathripuzha River's banks. According to [9], the Cyperaceae and Poaceae make up the majority of the wetland flora of the district of Kanyakumari. Riparian vegetation studies conducted earlier along the Chalakudy River [10], the Pamba River[11] and the Benin River [12], revealed Euphorbiaceae (including Phyllanthaceae), Fabaceae and Rubiaceae as the dominant families, in the present study Asteraceae,Fabaceae and Malvacea were the dominant families. The prevalence of these families along riverbanks can be attributed to the thriving of species in flooded, highly humid environments, as well as the enhanced ability of leguminous trees to adapt to waterlogged areas. This adaptation is facilitated by the presence of symbiotic nitrogen-fixing organisms ([13]; [14]; [15].

The aquatic ecosystem is seriously threatened by the invasive species like Eichhornia crassipes and several Ipomoea species, which would obliterate native species. These weeds are a sign that the local vegetation has been disturbed. The primary cause of the proliferation of these weeds outside of their natural habitat is human involvement. The invasion of weeds is the beginning of ecosystem degradation [16].

Threats and its consequences in riparian basin of Gayathripuzha

Grazing is a major threat to the riparian corridor and was recorded throughout the riparian areas of Gayathripuzha. Grazing of cattle and goats has altered the geomorphic riparian ecosystems of the study area. Grazing disrupted the natural riparian vegetation by the removal of herbaceous plants, causing physical damage to plants and changes in fluvial processes that may eliminate germination sites for woody vegetation.

Construction of check dams is another major threat to riparian ecosystem. These dams disconnect rivers from their riparian zones and wetlands. By slowing down the movement of water it prevent the natural downstream movement of sediments to riparian zones, affecting riparian biodiversity and productivity.

Encroachment for agriculture was observed throughout the riparian system of Gayathripuzha this leads to the degradation of natural riparian vegetation. Sand mining had hampered the riparian ecology by causing the river bank to erode, lowering the river bed, and reducing its ability to hold water.

Another important threat to riparian ecosystem is the alien invasive species. Among these, species *Mikania micrantha* is frequently occurring along the riparian system of the study area and it was more widespread and abundant. Invasive exotic species like *Chromolaena odorata, Hyptis suaveolens and Alternanthera tenella* cause considerable threat to native species

Conclusion

The Gayatripuzha river basin is a home to 87 plant species belonging to31 families revealing its rich biodiversity and ecological dynamics.It is home to 12 Asteraceae species ,11 Fabaceae family members, 5 species from each of the families like Malvaceae , Acanthaceae , Lamiaceae and 4 Poaceaen members and 2 Cyperaceae species.The vegetation profile of the Gaysathripuzha riverbasin shows a dynamic mix of life forms and ecological niches. By addressing the threats , conservation and management strategies for Gayathripuzha river basin can be customized to ensure the region's biodiversity is preserved for the future.

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RESEARCH ARTICLE

Endemic Asclepiads in Nilgiri biosphere reserve, India

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Abstract

While conducting field surveys to document gamopetalous flora of Nilgiri Biosphere Reserve, India, a special attention was given to document endemic asclepiad plant species. In addition to field surveys, consultation of herbarium and literature related to the flora of NBR revealed that a total of 38 endemic taxa of have been documented of which some of them categorized as Critically Endangered (5 species) followed by Endangered (5), Vulnerable (4) and Possibly extinct (1) by several authors. Based on habit analysis 18 species are categorized as twiners.

Key words: *Asclepiadaceae, Conservation status, Distribution, Eastern Ghats, Western Ghats.*

Introduction

India has ten biogeographic zones with characteristic habitat and biota, it is the 7th largest country by geographical area, ranks 10th positions of species richness (Singh & al., 2015) and one of the 17 mega biodiversity country in the world (Dash & Mao, 2020). In southern India, Nilgiri Biosphere Reserve (NBR) spread over 5520 km^2 (Daniels, 1996) and shares 4721 km^2 with Western Ghats of around 1,64,280 km^2 (Nayar & al., 2014) and 799 km^2 with Eastern Ghats of around 75,000 km^2 (Pullaiah & al., 2007). The Nilgiri-wayanad-silent valley is well known for its rich biodiversity and varied habitats of life due to its altitudinal variation from 700 to 2637 m (Singh & al., 2015). Asclepiadaceae *sensu stricto* are represented by 45 genera and 301 taxa (292 species, 09 infraspecific) in India (Jayanthi & al., 2020). It is a distinct family and can be easily identified by very unique flower organization and it seems to be very conservative for the family. 'Corona' it is an synorganization of corolla and androecium. 'Gynostegium and pollinaria' s an synorganization of androecium and gynoecium. Similarly, sympetaly, synandry and post-genital fusion of style head and anthers are characteristic to the family.

Materials and Methods

As a part of gamopetalous flora of NBR, botanical explorations were conducted since from 2019 to May 2024. During this exploration a special attention was given to locate and document the endemic asclepiads. As it never found in abundance and most of the species have restricted distribution. They don't have much economic importance but few of them has medicinal value, some are of ornamental value and few are edible. As a result, a total of 38 taxa has been recorded based on collection and consultation of literature and herbarium. Specimen collection and herbarium preparation were done by standard herbarium method (Jain & Rao, 1977). The voucher specimens were identified by using protologues, regional floras, revisions and monographs. The collected plants are arranged alphabetically, accepted names was given by referring databases (POWO, 2025 and IPNI, 2025). Followed by Flowering and fruiting (Fl. & Fr.), Habitat details given by observed field notes, based on earlier literature. Also distribution in NBR (District names mentioned in abbreviations), endemic region, conservation status, specimen examination and if possible a note given to the plant taxa documented based on earlier literature and some observations noted in filed.

Study area

The International Coordination Council (ICC) of UNESCO's first meeting in Paris held during 9–19 November 1971 introduced the designation

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Biosphere Reserve” for natural areas to minimize conflict between development and conservation. The Department of Science and Technology had constituted a committee under the Chairmanship of Professor Madhav Gadgil of the Indian Institute of Science, Bangalore to survey and demarcate the exact limits of the proposed biosphere reserve in the Nilgiris (Sathish, 2014). As result, Govt. of India first identified 7 sites in India, among this NBR declared as first Biosphere Reserve. It was set up on 1.9.1986 vide order number 5.22010/6/86.CSC, Government of India. In 2012 it is declared as the World heritage site by UNESCO. NBR is situated in the tri-junction of Karnataka, Kerala and Tamil Nadu. It encompasses a total area of 5,520 km^2. The Biosphere Reserve is spread over ten districts partly surrounding the Nilgiris District. which contains a mosaic of different forest types and habitats, dense flora and fauna including many endemic and endangered species.

NBR forests constitute an excellent habitat for flora, fauna and other microbial forms. The NBR has a remarkable topographic diversity as a result of this topographic complexity, the NBR encompasses a wide range of rainfall zones which receive between 500 and 7000 mm of precipitation annually. The rainfall is generally heavier in the western side averaging 5000 mm. The wet season is June-September though there is summer and occasional winter rains locally within the biosphere reserve. The length of the dry season varies from about a month in the western hills to over six months in the eastern plateau. April- May is the hottest months. Ground temperature below 0^{o}C (frost) is frequent during December–January in the higher hills of Nilgiris. Hence NBR harbours different vegetation types (Plate I).

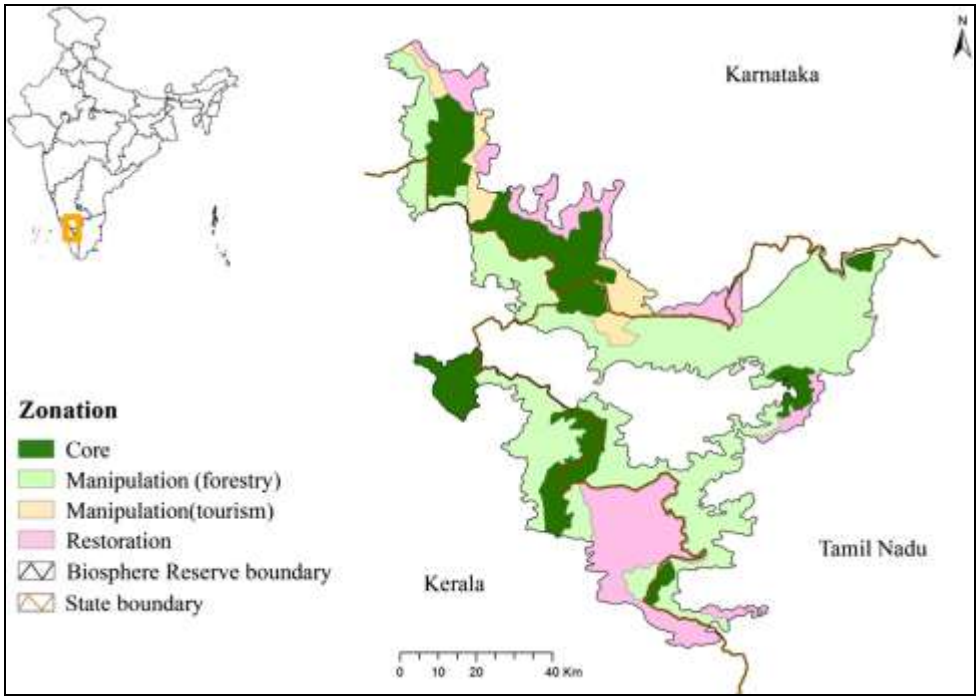

Fig. 1. Map of the study area,The Nilgiri Biosphere Reserve.
(Source: Sathish et. al., 2014)

Checklist of Endemic Asclepiads

1. Boucerosia diffusa Wight, Icon. Pl. Ind. Orient. 4(4): 14, t. 1599. 1850
Habit: Herb
Fl. & Fr.: April–December
Habitat: Rocky localities in hills, 300–600 m
Distribution: NBR: Tamil Nadu (CBE)
Endemic to Southern India
Specimen examined: C.P. Sreemadhavan 957 (MH)
Note: The succulent stems are used as vegetable by local people

2. Boucerosia indica (Wight & Arn.) Plowes, Haseltonia 3: 59. 1995.
Hutchinia indica Wight & Arn. in Wight, Contr. Bot. India: 34. 1834.
Habit: Herb
Fl. & Fr.: November–July
Habitat: Scrub jungles and dry rocky hillslopes, 300–600 m
Distribution: NBR: Tamil Nadu (CBE)
Endemic to Southern India
Specimen examined: K. Ramamurthy 14787 (MH)
Note: The succulent stems are used as vegetable by local people

3. Brachystelma maculatum Hook.f., Fl. Brit. India 4: 65. 1883.
Habit: Herb
Fl. & Fr.: July–September
Habitat: Among clumps of grasses in dry deciduous forests, 500–600 m
Distribution: NBR: Karnataka (MYS); **Tamil Nadu** (ERD)
Endemic to Western Ghats and Eastern Ghats
Critically Endangered (Prasad & Venu, 2020)
Specimen examined: C.E.C. Fischer 61 (CAL)
Note: It is recently rediscovered from type locality after a lapse of 100 years

4. Brachystelma mahajanii Kambale & S.R. Yadav, Kew Bull. 69(1)-9493: 2. 2014.
Habit: Herb
Fl. & Fr.: March–June
Habitat: Soils accumulated on rocks at an elevation of c. 1792 m
Distribution: NBR: Tamil Nadu (NLG)
Endemic to NBR
Critically Endangered (Prasad & Venu, 2020)
Specimen examined: Sharad S. Kambale & S.R. Yadav 49 (MH)
Note: It is described on 2014 by Kambale & al., from Ebbenad in Nilgiris District, Tamil Nadu.

5. Brachystelma rangacharii Gamble, Bull. Misc. Inform. Kew 1922: 120. 1922.
Habit: Herb
Fl.: March–June. **Fr.:** Unknown
Habitat: Rocky hillslopes and crevices in dry deciduous forests, 700–800 m
Distribution: NBR: Tamil Nadu (ERD)
Endemic to NBR
Critically Endangered (Prasad & Venu, 2020)
Specimen examined: K. Rangachari 10654 (MH)
Note: It is known only by its type; not rediscovered yet.

6. Brachystelma swarupa Kishore & Goyder, Kew Bull. 56(1): 210. 2001.
Habit: Herb
Fl. & Fr.: March–July
Habitat: Amidst grasses, open rocky slopes in moist deciduous forests, ± 600
Distribution: NBR: Tamil Nadu (CBE)
Endemic to Western Ghats
Critically Endangered (Prasad & Venu, 2020)
Specimen examined: MM & KB 161803 (MH)
Note: It is an addition to the flora of NBR.

7. Caralluma adscendens (Roxb.) Haw. var. **bicolor** (V.S. Ramach., S. Joseph, H.A. John & Sofiya) Karupp., Ugraiah & Pull., Caralluma Antiobesity Pl.: 116. 2013.
Caralluma bicolor V.S. Ramach., S. Joseph, H.A. John & Sofiya, Nordic J. Bot. 29: 447. 2011.
Habit: Herb
Fl. & Fr.: August–December
Habitat: Open rocky areas in scrub jungles, 300–600 m
Distribution: NBR: Kerala (PLK); **Tamil Nadu** (CBE, NLG)
Endemic to Southern India
Specimen examined: MM & KB 157460 (MH)
Note: The succulent stems are used as vegetable by local people

8. Caralluma attenuata Wight, Icon. Pl. Ind. Orient. 4(4): 15, t. 1268. 1848.
Habit: Herb
Fl. & Fr.: April–December
Habitat: Open rocky areas in scrub jungles, 300–700 m
Distribution: NBR: Karnataka (MYS); **Tamil Nadu** (CBE, NLG)
Endemic to southern India
Specimen examined: K. Subramanyam 246 (MH)
Note: The succulent stems are used as vegetable by local people

9. Ceropegia barnesii E.A. Bruce & Chatterjee, Kew Bull. 3(1): 62. 1948.
Habit: Twiner
Fl. & Fr.: May–August
Habitat: Evergreen forests
Distribution: NBR: Tamil Nadu (NLG)
Endemic to Western Ghats
Endangered (Nayar & Sastry, 1987)

Specimen examined: *s.coll., s.n.* (Acc. No.: 32832) (MH)
Note: It is not rediscovered more than 45 years.

10. Ceropegia ciliata Wight, Icon. Pl. Ind. Orient. 4(1): 15, t. 1262. 1848.
Habit: Twiner
Fl. & Fr.: July–September
Habitat: Grows amidst grasses on hillslopes, 1000–2200 m
Distribution: NBR: Karnataka (KDU); **Kerala** (PLK); **Tamil Nadu** (NLG)
Endemic to Western Ghats
Critically Endangered (Kambale & Yadav, 2019)
Specimen examined: MM & KB 157355 (MH)

11. Ceropegia ensifolia Bedd., Madras J. Lit. Sci., ser. 3, 1:52. 1864.
Habit: Twiner
Fl. & Fr.: August–November
Habitat: Grows amidst grasses and other herbs in grasslands on hillslopes
Distribution: NBR: Kerala (MPM, PLK); **Tamil Nadu** (CBE, NLG)
Endemic to Western Ghats
Specimen examined: E. Vajravelu 48769 (MH)

12. Ceropegia fimbriifera Bedd., Madras J. Lit. Sci., ser. 3, 1: 53. 1864.
Habit: Herb
Fl. & Fr.: June–November
Habitat: Rocky hillslopes in evergreen forests, 1500–1850 m
Distribution: NBR: Karnataka (CMN, MYS); **Kerala** (PLK); **Tamil Nadu** (CBE, NLG)
Endemic to Western Ghats and Eastern Ghats
Vulnerable (Nayar & Sastry, 1987)
Specimen examined: MM & KB 157366 (MH)

13. Ceropegia intermedia Wight, Icon. Pl. Ind. Orient. 4(1): 12, t. 1263. 1848.
Habit: Twiner
Fl. & Fr.: June–January
Habitat: On slopes, roadsides in evergreen forests, 1775–1950 m
Distribution: NBR: Karnataka (KDU, MYS); **Kerala** (PLK); **Tamil Nadu** (CBE, NLG)
Endemic to Western Ghats and Eastern Ghats
Endangered (Nayar & Sastry, 1987)
Specimen examined: G.V. Subba Rao 36643 (MH)

14. Ceropegia manoharii Sujanapal, P.M. Salim, Anil Kumar & Sasidh., J. Bot. Res. Inst. Texas 7: 342. 2013.
Habit: Twiner
Fl. & Fr.: August–February
Habitat: Grasslands, 1500–1850 m
Distribution: NBR: Kerala (WND).
Endemic to NBR
Specimen examined: P. Sujanapal & P.M. Salim 0428 (MSSH)

15. Ceropegia metziana Miq., Anal. Bot. Ind. 3: 11. 1852.
Habit: Twiner
Fl. & Fr.: September–December
Habitat: On roadsides and open places in evergreen forests, ± 900 m
Distribution: NBR: Kerala (PLK); **Tamil Nadu** (CBE, NLG).
Endemic to Western Ghats
Specimen examined: MM & KB 157114 (MH)

16. Ceropegia pusilla Wight & Arn. in Wight, Contr. Bot. India: 81. 1834.
Habit: Herb
Fl. & Fr.: June–October
Habitat: Grows amidst grasslands on hillslopes, 1500–2400 m
Distribution: NBR: Karnataka (MYS); **Tamil Nadu** (CBE, NLG)
Endemic to Western Ghats
Specimen examined: MM & KB 157598 (MH)

17. Ceropegia spiralis Wight, Icon. Pl. Ind. Orient. 4(1): 15, t. 1267. 1848.
Habit: Herb
Fl. & Fr.: August–February
Habitat: On hillslopes amidst grasses
Distribution: NBR: Kerala (PLK)
Endemic to Western Ghats and Eastern Ghats
Vulnerable (Nayar & Sastry, 1987)
Specimen examined: R. Wight, *s.n.* (K000894294, image!)

18. Ceropegia thwaitesii Hook., Bot. Mag. 80: t. 4758. 1854.
Habit: Twiner
Fl. & Fr.: February–November
Habitat: Shola forests
Distribution: NBR: Kerala (PLK); **Tamil Nadu** (NLG)
Endemic to Western Ghats
Specimen examined: N.C. Nair 64452 (MH)

19. Ceropegia vincifolia Hook., Bot. Mag. 66: t. 3740. 1839.
Habit: Twiner

Fl. & Fr.: August–September
Habitat: Lateritic soil and on hillslopes in evergreen forests
Distribution: NBR: Kerala (PLK)
Endemic to Western Ghats
Endangered (Nayar & Sastry, 1987)
Specimen examined: A.R. Kulavmode & S.S. Kambale 3132 (SUK)

20. Cryptolepis grandiflora Wight, Icon. Pl. Ind. Orient. 3(1): 4, t. 831. 1845.
Habit: Twiner
Fl. & Fr.: August–March
Habitat: Margins of dry deciduous forests, 600–1000 m
Distribution: NBR: Karnataka (MYS); **Tamil Nadu** (CBE, NLG)
Endemic
Specimen examined: C.P. Sreemadhavan 420 (MH)
Note: The latex yields good quality rubber comparable to Hevea-rubber (Jayanthi & al., 2022)

21. Cynanchum sahyadricum (Ansari & Hemadri) Liede & Khanum, Taxon 65(3): 480. 2016.
Seshagiria sahyadrica Ansari & Hemadri, Indian Forester 97: 126. 1971.
Habit: Twiner
Fl. & Fr.: May–December
Habitat: Scrub jungles and moist deciduous forests
Distribution: NBR: Tamil Nadu (NLG)
Endemic to WG
Note: It is included here based on earlier report by Jayanthi & al. ()
22. Cynanchum sarcomedium Meve & Liede, Kew Bull. 67: 753. 2012.
Habit: Twiner
Fl. & Fr.: June–December
Habitat: Scrub jungles, 300–500 m
Distribution: NBR: Tamil Nadu (CBE, NLG)
Endemic
Specimen examined: K. Subramanyam 1984 (MH)

23. Decalepis hamiltonii Wight & Arn. in Wight, Contr. Bot. India: 64. 1834.
Habit: Climber
Fl. & Fr.: March–October
Habitat: Dry deciduous forests, 600–700 m
Distribution: NBR: Karnataka (MYS); **Tamil Nadu** (ERD, NLG)
Endemic to Western Ghats and Eastern Ghats
Endangered (Nayar & al., 2014)
Specimen examined: G.V. Subba Rao 37329 (MH)
Note: Tubers pickled and eaten, also traded in crude drug markets (Narasimhan & Sheeba, 2021).

24. Decalepis nervosa (Wight & Arn.) Venter, Taxon 46: 712. 1997.
Brachylepis nervosa Wight & Arn., Contr. Bot. Ind. 63. 1834.
Habit: Climber
Fl. & Fr.: March–September
Habitat: Margins of evergreen forests, 1500–2200 m
Distribution: NBR: Kerala (PLK); **Tamil Nadu** (CBE, NLG).
Endemic to NBR
Specimen examined: MM & KB 157713 (MH).

25. Decalepis salicifolia (Bedd. ex Hook.f.) Bruyns, Taxon 65: 499. 2016.
Utleria salicifolia Bedd. ex Hook.f., Fl. Brit. India 4: 7. 1883.
Habit: Subshrub
Fl. & Fr.: April–October
Habitat: Grasslands, 1500–1800 m.
Distribution: NBR: Kerala (PLK).
Endemic to Western Ghats
Endangered (Nayar & al., 2014).
Note: It is included here based on the earlier report by Anilkumar (2015).

26. Gymnema decaisneanum Wight, Icon. Pl. Ind. Orient. 4(1): 16, t. 1271. 1850.
Habit: Climber
Fl. & Fr.: March–September
Habitat: Moist deciduous and evergreen forests, 500–1000 m.
Distribution: NBR: Karnataka (MYS); **Tamil Nadu** (NLG).
Endemic.
Specimen examined: K.M. Sebastine 3321 (MH).

27. Gymnema indicum (M.A. Rahman & Wilcock) Karthik. & Moorthy, Fl. Pl. India: 170. 2009.
Bidaria indica M.A. Rahman & Wilcock, Blumea 34: 99. 1989.
Habit: Climber
Fl. & Fr.: June–December
Habitat: Moist deciduous and evergreen forests.
Distribution: NBR: Kerala (PLK)
Endemic to Western Ghats
Specimen examined: MM & KB 150073 (MH).

28. Gymnema montanum (Roxb.) Hook.f., Fl. Brit. India 4: 31. 1883.
Asclepias montana Roxb., Hort. Bengal.: 85. 1814.
Habit: Climber
Fl. & Fr.: May–March
Habitat: Dry deciduous and semi-evergreen forests, 600–1200 m.
Distribution: NBR: Kerala (PLK); **Tamil Nadu** (NLG).
Endemic to southern India
Specimen examined: E. Vajravelu 38204 (MH).

29. Heterostemma beddomei (Hook.f.) Swarupan. & Mangaly, Bot. J. Linn. Soc. 101: 254. 1989.
Oianthus beddomei Hook.f., Fl. Brit. India 4: 49. 1883.
Habit: Twiner
Fl.: August–September
Habitat: Moist deciduous forests.
Distribution: NBR: Kerala (WND).
Endemic to NBR
Possibly extinct (Nayar, 1997).
Note: It is included here based on the earlier report by Gamble (1923). It is known only by its type (illustration); not rediscovered yet.

30. Heterostemma deccanense (Talbot) Swarupan. & Mangaly, Bot. J. Linn. Soc. 101(2): 255. 1989.
Oianthus deccanensis Talbot, Forest Fl. Bombay 2: 260. 1911.
Habit: Twiner
Fl. & Fr.: August–January
Habitat: Moist deciduous forests, 500–800 m.
Distribution: NBR: Tamil Nadu (CBE).
Endemic to southern India
Specimen examined: MM & KB 150025 (MH).
Note: Based on the above cited collection it was reported as an addition to the flora of Tamil Nadu by Murugesan & al. (2023).

31. Hoya wightii Hook.f., Fl. Brit. India 4: 59. 1883.
Habit: Twiner
Fl. & Fr.: March–December
Habitat: Grows on tree trunks and branches in evergreen forests, streamsides, 1200–2200 m.
Distribution: NBR: Karnataka (KDU, MYS); **Kerala** (PLK, WND); **Tamil Nadu** (CBE, NLG).
Endemic to Western Ghats
Specimen examined: MM & KB 157740 (MH).

32. Marsdenia raziana Yogan. & Subr., Proc. Indian Acad. Sci., B 83: 147. 1976;
Habit: Climber
Fl. & Fr.: January–March
Habitat: Semi-evergreen forests.
Distribution: NBR: Kerala (WND).
Endemic to Western Ghats
Vulnerable (Nayar, 1997).
Note: It is included here based on earlier report by Narayanan (2009).

33. Toxocarpus concanensis Hook.f., Fl. Brit. India 4: 14. 1883.
Habit: Climber
Fl. & Fr.: March–July
Habitat: Semi-evergreen forests.
Distribution: NBR: Karnataka (KDU).
Endemic to Western Ghats
Note: It is included here based on earlier report by Manikandan & Lakshminarasimhan (2013).

34. Toxocarpus palghatensis Gamble, Bull. Misc. Inform. Kew 1922: 119. 1922.
Habit: Climber
Fl. & Fr.: September–March
Habitat: Evergreen forests.
Distribution: NBR: Karnataka (KDU, MYS); **Kerala** (PLK).
Endemic to Western Ghats
Vulnerable (Nayar, 1997).
Specimen examined: E. Vajravelu 33255 (MH).

35. Vincetoxicum balakrishnanii (P.M. Salim & J. Mathew) Kottaim., Int. J. Curr. Res. Biosci. Pl. Biol. 6(10): 37. 2019.
Tylophora balakrishnanii P.M. Salim & J. Mathew, NeBIO 8(3): 130. 2017.
Habit: Twiner
Fl. & Fr.: June–September
Habitat: Margins of evergreen forests, 1400–1800 m.
Distribution: NBR: Kerala (WND); **Tamil Nadu** (CBE).
Endemic to NBR.
Specimen examined: MM & KB 157284 (MH).
Note: Based on the above citated collection it was reported as an addition to the flora of Tamil Nadu by Murugesan & al. (2023).

36. Vincetoxicum capparidifolium (Wight & Arn.) Kuntze, Revis. Gen. Pl. 2: 424. 1891.
Tylophora capparidifolia Wight & Arn. in Wight, Contr. Bot. India: 51. 1834.

Habit: Twiner
Fl. & Fr.: May–January
Habitat: Roadsides in evergreen forests, 1000–1800 m.
Distribution: NBR: Karnataka (MYS); **Kerala** (WND); **Tamil Nadu** (CBE, NLG).
Endemic to Western Ghats and Eastern Ghats
Specimen examined: MM & KB 157720 (MH).

37. Vincetoxicum dalzellii (Hook.f.) Kuntze, Revis. Gen. Pl. 2: 424. 1891.
Tylophora dalzellii Hook.f., Fl. Brit. India 4: 43. 1883.
Habit: Twiner
Fl. & Fr.: Febuary–December
Habitat: Moist deciduous forests.
Distribution: NBR: Karnataka (KDU, MYS); **Kerala** (WND); **Tamil Nadu** (NLG).
Endemic to Peninsular India.
Note: It is included here based on earlier report by Manikandan & Lakshminarasimhan (2013) who reported it from Kodagu district.

38. Vincetoxicum rotundifolium (Buch.-Ham. ex Wight) Kuntze, Revis. Gen. Pl. 2: 425. 1891.

Tylophora rotundifolia Buch.-Ham. ex Wight, Contr. Bot. India: 50. 1834.

Habit: Twiner
Fl. & Fr.: April–December
Habitat: Dry and moist deciduous forests, 700–1000 m.
Distribution: NBR: Karnataka (MYS); **Kerala** (WND); **Tamil Nadu** (ERD, NLG).
Endemic to Peninsular India
Specimen examined: V. Narayanaswami 3459 (MH).

Results and discussion

Floristic analysis shows that NBR harbours 38 endemic plant taxa, belonging to 13 genera and 37 species and 1 infraspecific taxa (Plate II to IV). Among these 27 (47%) were Twiners 18 (17%) Climbers 8 (21%), Herb 11 (29%), subshrubs, 1 (3%) (Fig.2)

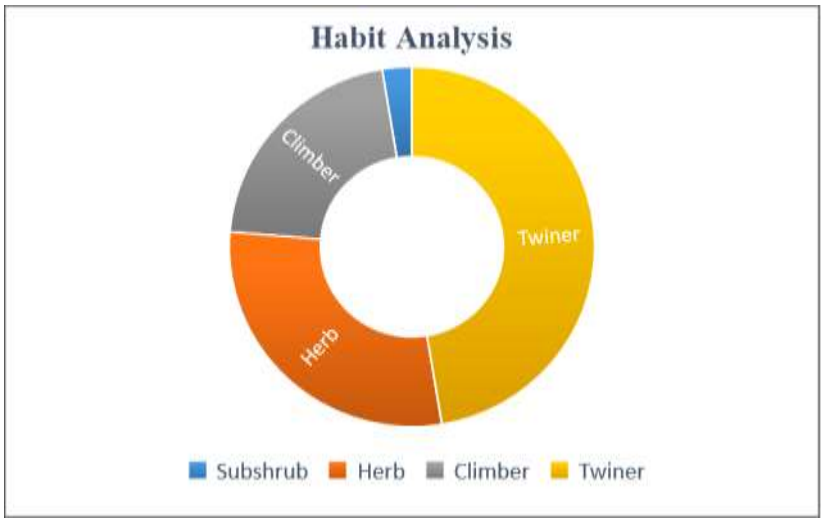

Fig.2. Habit analysis

From these documented 13 genera *Ceropegia* has high number of species diversity with 11 species, followed by *Brachystelma* and *Vincetoxicum* each with 4 species, *Decalepis* and *Gymnema* each with 3 species. The endemic diversity was observed to be highest in the moist deciduous and semi-evergereen forests, followed by evergreen and scrub jungles. Endemic plants are further categeriosed as per the locality where it is distributed, 2 species are present throughout India, 2 species are restricted to peninsular India, 6 are restricted to southern India these may present in hills as well as in scrub jungles. 15 are only distributed in Western Ghats, 15 are shares

its distribution in Western and Eastern Ghats. 6 are species strictly restricted to NBR namely, *Brachystelma mahajanii* Kambale & S.R. Yadav, *Brachystelma rangacharii* Gamble, *Ceropegia manoharii* Sujanapal, *Decalepis nervosa* (Wight & Arn.) Venter, *Heterostemma beddomei* (Hook.f.) Swarupan. & Mangaly and *Vincetoxicum balakrishnanii* (P.M. Salim & J. Mathew) Kottaim. Documented plants are categeriosed based on its conservation or threat status (Fig.3) mentioned by various authors previously in their studies, it resulted as, 5 are Critically Endangered, followed by 5, Endangered, 4 Vulnerable and 1 Possibly extinct namely, *Heterostemma beddomei* (Hook.f.) Swarupan. & Mangaly. This plant and *Brachystelma rangacharii* Gamble not rediscovered after its type collection.

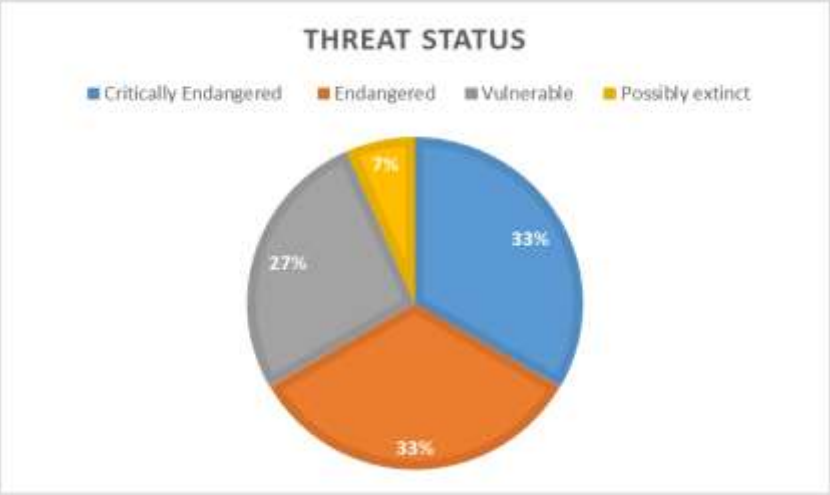

Fig.3. Threat status

Conclusion

Members of Asclepiadaceae are sparsely distributed and do not form pure vegetation. Asclepiads flowers are very difficult to study after getting dry because of its fleshy flowers and floral organs and dried flowers do not provide clear three dimensional picture of flower structure.

Hence photography is very important to study it in details and because of its slender habit sometimes it may overlook during surveys, Among the total 84 taxa of Asclepiadaceae in NBR 38 are endemic, and many of them are Rare or occasionally distributed in forests. 15 endemic plants are categerised under threatened categeory. So, it is very important to conserve it and need further study for better understanding.

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