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Study on Antioxidant and Antimicrobial Efficacy of *Cyphostemmasetosum* (Roxb.) Alston, Leaves: Implications for Natural Therapeutics

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ABSTRACT

Plants undergo significant abiotic and biotic stresses, resulting in heightened formation of reactive oxygen species (ROS) that can oxidize cellular components and disrupt physiological functioning. In response, plants have evolved intricate antioxidant systems, comprising enzymes and non-enzymatic substances, to neutralize reactive oxygen species and avert oxidative damage. This research assessed the antioxidant, antibacterial, and antifungal properties of C.setosum leaf extracts.Cyphostemmasetosum (Roxb.) is a succulent climber clothed with scattered grandular bristly hairs; it is belonging to the family Vitaceae. The antioxidant activity was evaluated by the DPPH and phosphomolybdenum tests. In the DPPH experiment, Vitamin C exhibited the maximum activity (98%) at 80 µg/ml, succeeded by methanol (92%), chloroform (82%), and petroleum ether extracts (72%), while ethyl acetate extract displayed the lowest activity (48%). The phosphomolybdenum experiment indicated that Gallic Acid and Ascorbic Acid serve as standards with elevated total antioxidant capacities (TACs) of 92% and 85%, respectively. The methanol extract exhibited the highest total antioxidant capacity (TAC) among plant extracts at 82%, followed by chloroform at 73%, petroleum ether at 62%, and ethyl acetate at 59%, underscoring the significance of solvent polarity in antioxidant extraction. The methanol extract demonstrated the highest antibacterial activity against Pseudomonas fluorescens, E. coli, Staphylococcus aureus, and Bacillus subtilis, exhibiting inhibition zones of 8-9 mm, followed by petroleum ether (6-7 mm), chloroform (5-7 mm), and ethyl acetate (3-6 mm). Ampicillin exhibited enhanced antibacterial efficacy, with inhibition zones ranging from 8 to 15 mm. Correspondingly, the antifungal efficacy against F.oxysporum, S.rolfsii, and P.infestans demonstrated that the methanol extract was the most potent, with inhibition rates of 60%, 52%, and 60%, respectively. Chloroform, ethyl acetate, and petroleum ether extracts demonstrated moderate efficacy, but the fungicide Fluconazole exhibited inhibition rates of 50-55%. The findings highlight the potential of methanol extracts of C.setosum as a substantial source of natural antioxidants, antibacterial, and antifungal agents, positioning it as a prospective candidate for health and functional food applications.

Keywords: *Antioxidant, Total anti-oxidantAnti-bacterial, Antifungal, Cyphostemmasetosum and Vitaceae.*

INTRODUCTION

Plants contain numerous bioactive compounds that demonstrate notable biological activity. Bioactive compounds, also known as secondary metabolites, are naturally generated through the standard metabolic processes in medicinal plants [1-5]. Since ancient times, people have used medicinal plantswhich contain bioactive compoundsto treat a variety of illnesses and medical issues. From ancient times to modern healthcare systems, medicinal plants have been used because they have significant therapeutic effects and have been included into both conventional and alternative medical practices. [6-8]. In addition to protecting traditional history, the development of traditional medicine while taking safety, effectiveness, and quality into account would also help to rationalize the use of herbal medicine in the field of human healthcare. Many people believe that nature provides a complete source of templates for creating new molecules. Using modern scientific methods, the study of therapeutic plants mentioned in several ancient texts from around the globe can produce more encouraging findings in the field of medicine. Drugs made from medicinal plants are notable in the field because of their unique chemical and biological properties. Their capacity to offer natural treatments for illnesses and advance healthcare is the reason for their growing international prominence[9,10].

In order to contribute significantly to general health and disease prevention, the potential of a plant should be properly recognized and investigated, taking into account both its nutritional value and its therapeutic qualities [10,11].An antioxidant is a substance that can stop or reduce oxidative damage to the body's tissues and cells. Free radicals are very reactive chemicals that can harm DNA, proteins, and cells. They are the source of oxidative damage. These free radicals are neutralized by antioxidants, which lessens their negative effects. Antioxidants are important because they can shield the body from a variety of illnesses and conditions linked to oxidative stress. Cellular damage, inflammation, and the emergence of chronic illnesses including diabetes, heart disease, and cancer can result from this imbalance [12-14]. A threat to world health, antimicrobial resistance calls for the creation of innovative treatments. Antibiotic resistance in organisms has emerged as a result of the overuse of antibiotics and their careless release into the environment [15-17]. Numerous pharmaceuticals originate from plant extracts and have been the subject of extensive pharmacological screening [15]A variety of secondary metabolites are found in plants, and these metabolites are essential to their survival and environmental adaption. Alkaloids, phenols, and terpenoids are the three main classes to which these substances belong.

Bioactive molecules can be found in complex combinations of these phytocompounds [16]. Antimicrobial resistance against infectious pathogens may be facilitated by the combination of antibiotics and bioactive chemicals derived from plants. In their quest for novel agents, researchers have turned to conventionally utilized botanical sources [18]. In certain tribal areas of India, traditional medicines are acknowledged as a component of primary healthcare and have been utilized to treat a variety of illnesses. Many individuals use the popular ayurvedic formulation to treat liver diseases, fever, cough, and skin blemishes. The active constituents in triphala are the plants *T. bellirica* and *T. arjuna* [19]. In the past, natural items have been essential to the discovery and creation of antimicrobial agents [20]. For example, most antibiotic medications are made from chemicals derived from microorganisms. About 200 of the estimated 28,000 compounds found throughout decades of purification of antibiotics from microbial sources have been utilized directly as medications, 200–300 more medications were produced via semi-synthetic modifications to these scaffolds, much outnumbering the number of synthetic antibiotics now in clinical use [21]. The majority of plant phytochemicals have antibacterial properties, are abundant in structure, and have few adverse effects. The pharmaceutical industry therefore gave them more attention in an effort to increase the biological activity of currently available antibiotics or as a possible source of new antimicrobial medicines that are effective against a range of diseases, including MDR bacteria [22,23].

Cyphostemmasetosum (Roxb.) Alston., is belongs to the family Vitaceae, it is a prostrate herb distributed in the lower hills of Palani, the Western Ghats, Tamil Nadu is prescribed by the Pullaiah tribal community very commonly for controlling diabetics in this region[24,25], ulcer and wounds[26,27], leaf used as stimulant, indolent tumors and applied externally to assist for the expulsion of guinea worms[28], boils for healing[29], intestinal worms[30,31. Antinociceptive effect[32]. Rheumatism and Dysentery [33] Spinal pains [34], antioxidant and antiulcer activity [35,36], antiplasmodial[37], the leaves are used to cure jaundice[38]; and also used for washing cattle[39].

MATERIAL AND METHODS

Collection and Authentication of Plant Material

The leaves of *Cyphostemmasetosum* were carefully collected from the Kawal Wildlife Sanctuary (Kawal Tiger Reserve), a renowned nature preserve located in the Jannaram Mandal of Mancherial District (formerly Adilabad district), Telangana, India. The collection took place during the months of July and August 2023, ensuring optimal plant material quality for the intended study.

The plant was authenticated by the Botanical Survey of India (BSI), Deccan Regional Centre, Hyderabad, Telangana, under the voucher number BSI/DRC/2023-24/Identification/404. The authenticated specimen has been preserved at the Herbarium, Hyderabadensis, housed within the Department of Botany, Osmania University, Hyderabad, Telangana, India, for future reference and verification.

This authentication ensures the accurate taxonomic identification of the plant, providing a reliable foundation for subsequent research and analysis.

Drying Process

The collected leaves of *Cyphostemmasetosum* were thoroughly cleaned and cut into uniform pieces measuring approximately 0.6–1.4 × 1 × 0.3 cm^3 using sterilized scissors and knives. The cut pieces were initially subjected to shade drying by spreading them evenly on clean newspapers for ten days, ensuring minimal exposure to direct sunlight to preserve their phytochemical integrity.

Subsequently, just before the extraction process, the partially dried leaf pieces were further dried in a hot air oven at 40 °C for one hour. This step ensured the complete removal of residual moisture, optimizing the material for efficient extraction.

DPPH Radical Scavenging Activity Assay

The antioxidant activity of the leaf and bark extracts was assessed using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay, as described by Kibiti and Afolayan (2015). A stock solution of 0.135 mM DPPH was prepared in methanol.

For the assay, 1 mL of the DPPH solution was mixed with 1 mL of the plant extracts (leaf and bark) or standard antioxidants such as Butylated Hydroxytoluene (BHT) and Vitamin C at varying concentrations (5 µg/mL to 80 µg/mL). A control was prepared containing only the DPPH solution in methanol to serve as a baseline.

The mixtures were vortexed thoroughly and incubated in the dark at room temperature for 30 minutes to prevent light-induced degradation. After incubation, the absorbance was recorded at 517 nm using a spectrophotometer. Methanol was used as the blank to eliminate any background interference.

Each experiment was performed in triplicate, ensuring accuracy and reproducibility of results. This method allowed for a reliable evaluation of the DPPH radical scavenging capacity of the plant extracts [40].

$$\text{DPPH Scavenging activity (\%)} = \left\{ \frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \right\} \times 100$$

Total Antioxidant Capacity (Phosphomolybdenum Assay)

The Total Antioxidant Capacity (TAC) was determined using the phosphomolybdenum method by Olugbami et al. (2015). 0.3 mL of each plant fraction or standard (ascorbic acid, gallic acid; 25–400 µg/mL) was mixed with 3 mL of a reagent solution (0.6 M sulphuric acid, 4 mM ammonium molybdate, 28 mM sodium phosphate).

Samples were incubated at 95°C for 95 minutes, cooled, and absorbance was measured at 695 nm. A control with distilled water was included. Higher absorbance indicated greater antioxidant potential.

$$\text{The percentage inhibition (\%)} = \left\{ \frac{(\text{absorbance of sample} - \text{absorbance of control})}{(\text{absorbance of sample})} \right\} \times 100$$

Antibacterial activity

Nutrient agar plates were prepared and inoculated with pathogenic bacterial laboratory cultures, including *Pseudomonas fluorescens* (MTCC 9768), *E. coli* (MTCC 424), *Staphylococcus aureus* (MTCC 96), and *Bacillus subtilis* (MTCC 3053). Following inoculation, activated samples were applied to the agar plates using the paper dip method. The plates were then incubated for 24 hours.

After the 24-hour incubation period, clear zones of bacterial inhibition were observed surrounding certain samples. The diameter of these inhibition zones was measured and recorded. Samples demonstrating antibacterial activity, as evidenced by the presence of inhibition zones, were selected for further investigation [42].

Antifungal activity

Anti-fungal activity was assessed using the dual culture method. Fungal strains including *Fussariumoxysporum* NCIM1008, *Sclerotium rolfsii* NCIM 1084, and *Phytophtherainfestans* MTCC 8707 were cultured on PDA medium. Agar blocks (5 mm in diameter) from actively growing fungal cultures (96 hours old) were placed on fresh agar medium in the center of Petri plates. Paper discs soaked in the respective samples were positioned at various locations on 90 mm diameter Petri plates, with plates then incubated at 30 ± 2 °C. After 5 days of incubation, the inhibition zones between the fungal cultures were measured. Percentage of inhibition was calculated accordingly the formula. Fluconazole was utilized as a positive control (fungicide) for comparison [43].

$$I\% = \frac{(C-T) \times 100}{C}$$

Results and Observations

Plant profile

Cyphostemmasetosum (Roxb.) Alston (Family: Vitaceae) Cooke, 1:254 (1903); Anon. 6:408(2008b); Kirtikar&Basu, 3: 852(2012).

Table:1. Taxonomic classification of Cyphostemmasetosum (Roxb.) Alston

Kingdom	Plantae
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Vitales
Family	Vitaceae
Genus	Cyphostemma
Species	*Cyphostemmasetosum*

Morphological description of *Cyphostemmasetosum*

The plant is clothed with scattered glandular bristly hairs, featuring a herbaceous, prostrate, weak, and succulent stemthat is striate and sulcate. The tendrils are leaf-opposed, forked, and long.

Leaves are succulent and 3-foliolate (with lower ones sometimes simple), with sub-fleshy leaflets measuring 5–7.5 × 3.8–5 cm, shortly petiolulate (the terminal leaflet has the longest petiolule). Leaflets are elliptic to obovate-oblong, obtuse, irregularly bristle-toothed or laciniate, glabrous above, and glandular-hispid along the nerves beneath. Stipules are broadly ovate and acute.

Flowers, approximately 2 mm long, are contracted in the middle and arranged in leaf-opposed or terminal dichotomous/ trichotomous glandular cymes. Peduncles measure 3–8.75 cm, glandular-hispid, with short pedicels. Calyx is cup-shaped and subtruncate, while petals are hooded at the apex and reflexed. The style is subulate.

Berries are ovoid, glandular-hispid, scarlet, and measure 6.5–8 mm in diameter (Fig1A-F).

Fig:1A-F. Habitat of C. setosum

Antioxidant Activity of Vitamin C and *C. setosum* Extracts Using DPPH Radical Scavenging Assay

Vitamin C (Ascorbic Acid): At 80 µg/ml, Vitamin C demonstrated the highest DPPH radical scavenging activity, achieving 98%. This exceptional activity is attributed to its well-established ability to donate electrons and stabilize free radicals. Its high efficacy serves as a benchmark for assessing the antioxidant potential of other substances, particularly plant extracts.

Methanol Extract: The methanol extract of *C. setosum* exhibited 92% scavenging activity at 80 µg/ml. This notable performance underscores methanol's effectiveness in extracting potent antioxidants, particularly polar compounds such as polyphenols and flavonoids. These compounds are renowned for their strong free radical neutralization through electron donation.

Chloroform Extract: The chloroform extract achieved 82% activity at the highest concentration. This significant activity suggests the presence of moderately polar bioactive compounds, including specific alkaloids and phenolics, which contribute to its antioxidant properties. These findings indicate the potential of chloroform extracts as a valuable source of natural antioxidants.

Petroleum Ether Extract: With 72% activity at 80 µg/ml, the petroleum ether extract demonstrated substantial antioxidant capacity. This activity is likely due to the presence of lipophilic antioxidants, such as non-polar carotenoids and fat-soluble vitamins. Although these are less potent than polar antioxidants, they still provide meaningful free radical scavenging benefits.

BHT (Butylated Hydroxytoluene): A synthetic antioxidant commonly used in food preservation, BHT showed 65% activity. While its efficacy is lower compared to natural antioxidants like Vitamin C and the methanol extract of *C. setosum*, its stability and cost-effectiveness make it a practical choice for certain applications.

Ethyl Acetate Extract: The ethyl acetate extract exhibited the lowest activity, at 48% scavenging capacity at 80 µg/ml. This result suggests that the compounds extracted with ethyl acetate are either less abundant or less effective in scavenging DPPH radicals compared to those extracted with other solvents.

This study highlights the variability in antioxidant capacities of *C. setosum* extracts depending on the solvent used. Methanol proved to be the most effective solvent for extracting antioxidants, followed by chloroform and petroleum ether, while ethyl acetate showed the least efficacy. These results emphasize the critical role of solvent selection in optimizing the extraction of bioactive compounds.

The findings also underline the potential of *C. setosum* as a rich source of natural antioxidants. Such plant-based antioxidants present safer and more effective alternatives to synthetic options like BHT, offering promising applications in the development of health supplements and functional foods. Future research could focus on the isolation and characterization of individual bioactive compounds from *C. setosum* to better understand their specific contributions to antioxidant activity **(Fig:2).**

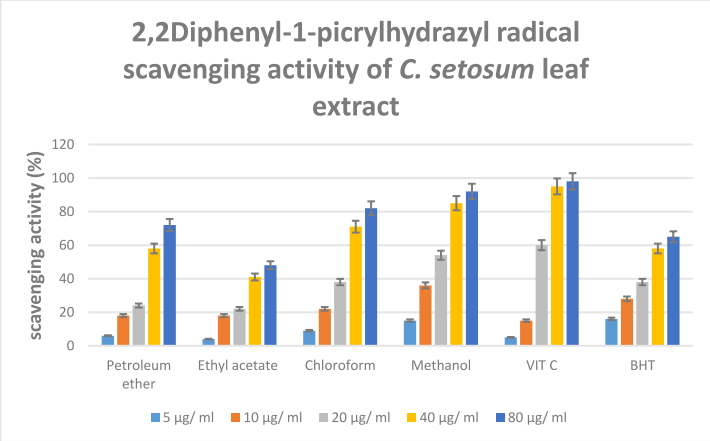

Fig:2. Antioxidant Activity of C. setosumwith DPPH Assay

Total Antioxidant Capacity of *C. setosum*

Gallic Acid and Ascorbic Acid Standards

Gallic Acid: At 400 µg/ml, Gallic Acid exhibited the highest antioxidant capacity of 92%. This high percentage reflects the potent reducing power of Gallic Acid, a polyphenolic compound known for its strong antioxidant properties due to its multiple hydroxyl groups that can donate electrons to neutralize free radicals.

Ascorbic Acid: As a standard antioxidant, Ascorbic Acid showed 85% TAC at 400 µg/ml. Ascorbic Acid, or Vitamin C, is renowned for its electron-donating ability, which makes it highly effective in reducing and neutralizing oxidative species.

***C. setosum* Extracts**

Methanol Extract: The methanol extract demonstrated a substantial TAC of 82% at 400 µg/ml. This significant activity indicates that methanol effectively extracts a wide range of potent antioxidant compounds from *C. setosum*. The high polarity of methanol enables it to dissolve and extract highly polar antioxidants, such as polyphenols and flavonoids, which contribute significantly to the overall TAC.

Chloroform Extract: The chloroform extract showed 73% activity at the highest concentration. The moderate polarity of chloroform suggests it extracts a range of moderately polar antioxidant compounds. The good TAC of the chloroform extract points to the presence of compounds like alkaloids and some phenolic compounds that have moderate antioxidant capacity.

Petroleum Ether Extract: With 62% TAC at 400 µg/ml, the petroleum ether extract reflects the contribution of non-polar antioxidant compounds, which, while generally less potent than their polar counterparts, still play a role in reducing oxidative stress.

Ethyl Acetate Extract: The lowest activity was observed in the ethyl acetate extract, with 59% TAC at 400 µg/ml. The moderate polarity of ethyl acetate might have resulted in the extraction of fewer or less potent antioxidant compounds compared to methanol or chloroform extracts. This suggests that the compounds soluble in ethyl acetate, although beneficial, are less effective in reducing the Mo (VI) to Mo(V) in the phosphomolybdenum assay

The assessment of Total Antioxidant Capacity using the phosphomolybdenum method reveals significant insights into the antioxidant potential of *C. setosum* leaf extracts across different solvents. The results clearly show that solvent polarity significantly impacts the extraction efficiency and the subsequent antioxidant capacity of the extracts. Methanol, with its high polarity, proved to be the most effective solvent, yielding extracts with the highest TAC, followed by chloroform, petroleum ether, and ethyl acetate. The study underscores the importance of selecting appropriate solvents to maximize the extraction of potent antioxidant compounds, thus enhancing the potential health benefits of plant-based antioxidants. Future research should focus on isolating and characterizing the specific antioxidant compounds within each extract to further understand their individual contributions to the overall antioxidant capacity **(Fig:3).**

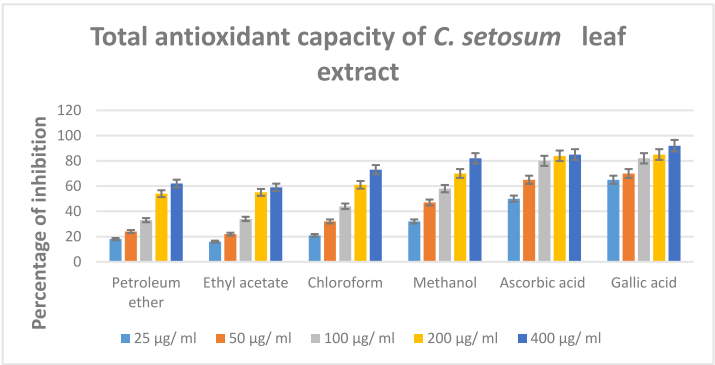

Fig: 3. Total antioxidant Activity of C. setosum

Antibacterial activity of *C. setosum*

***Pseudomonas fluorescens* (MTCC 9768):** The methanol extract of *C. setosum* produced an inhibition zone of 8 mm, demonstrating a moderate antibacterial effect. This activity is indicative of the presence of bioactive compounds that are effective against gram-negative bacteria like P. fluorescens. Methanol, a polar solvent, is efficient at extracting a broad spectrum of polar bioactive compounds, such as polyphenols and flavonoids, which likely contribute to its antibacterial properties.

***Escherichia coli* (MTCC 424):** For E. coli, the methanol extract showed a slightly larger inhibition zone of 9 mm.

This suggests that the extract contains compounds capable of disrupting the bacterial cell membrane or inhibiting essential cellular functions in this gram-negative bacterium, which is known for its resilience against many antibacterial agents.

***Staphylococcus aureus* (MTCC 96):** The methanol extract displayed an inhibition zone of 8 mm against S. aureus, a gram-positive bacterium. The comparable zone of inhibition to that against P. fluorescens suggests a broad-spectrum antibacterial effect, possibly due to the methanol extract's ability to interfere with peptidoglycan synthesis or protein synthesis in bacterial cells.

***Bacillus subtilis* (MTCC 3053):** The inhibition zone of 9 mm for *B. subtilis* indicates a significant antibacterial effect. Given that B. subtilis is a gram-positive bacterium with a thicker peptidoglycan layer, the methanol extract's efficacy suggests the presence of compounds that can effectively penetrate and disrupt these robust bacterial cell walls.

Performance of *C. setosum* chloroform extract

***Pseudomonas fluorescens* (MTCC 9768):** The chloroform extract exhibited a 7 mm zone of inhibition, reflecting a moderate antibacterial effect. Chloroform extracts compounds such as alkaloids and certain terpenoids, which may exert antibacterial effects through mechanisms such as disrupting the cell membrane or inhibiting key bacterial enzymes.

***Escherichia coli (MTCC 424)*:** With a 5 mm inhibition zone, the chloroform extract's efficacy against E. coli is limited compared to the methanol extract. This suggests that the extracted compounds have less impact on this gram-negative bacterium or that the concentration of active compounds was lower.

***Staphylococcus aureus* (MTCC 96):** The inhibition zone of 7 mm against S. aureus demonstrates a reasonable antibacterial effect. Chloroform's extraction of moderately polar compounds that can target the gram-positive bacterial cell wall or protein synthesis could explain this activity.

***Bacillus subtilis* (MTCC 3053):** The 6 mm inhibition zone for B. subtilis indicates moderate activity. The effectiveness against this gram-positive bacterium suggests that chloroform extracts components that can interfere with cell wall synthesis or bacterial metabolism.

Efficacy of *C. setosum* Ethyl Acetate Extract

Pseudomonas fluorescens (MTCC 9768): The ethyl acetate extract showed a 6 mm inhibition zone, suggesting a modest antibacterial effect. Ethyl acetate extracts a range of moderately polar compounds, which may have a limited capacity to penetrate and disrupt the gram-negative bacterial cell membrane of P. fluorescens.

***Escherichia coli* (MTCC 424):** Similarly, a 6 mm zone of inhibition was observed for E. coli. The moderate efficacy indicates the presence of bioactive compounds with limited effectiveness against this gram-negative bacterium.

***Staphylococcus aureus* (MTCC 96):** The inhibition zone of 3 mm indicates a low antibacterial activity against S. aureus. This could be due to the inability of ethyl acetate to effectively extract potent antibacterial compounds that can target gram-positive bacteria.

***Bacillus subtilis* (MTCC 3053):** With a 4 mm inhibition zone, the ethyl acetate extract shows minimal antibacterial activity against B. subtilis. The low efficacy suggests that the extracted compounds are less effective in disrupting the cellular structures or metabolic processes of this gram-positive bacterium.

Activity of *C. setosum* Petroleum Ether Extract

***Pseudomonas fluorescens* (MTCC 9768):** The petroleum ether extract showed a 6 mm inhibition zone, indicating a modest antibacterial effect. Petroleum ether, being a non-polar solvent, extracts lipophilic compounds, which may not be very effective against the gram-negative bacteria's outer membrane structure.

***Escherichia coli* (MTCC 424):** The inhibition zone of 7 mm suggests a slightly better antibacterial activity against E. coli compared to P. fluorescens. The presence of lipophilic bioactive compounds might have a marginally higher impact on disrupting the cell membrane of this gram-negative bacterium.

***Staphylococcus aureus* (MTCC 96):** With a 6 mm inhibition zone, the petroleum ether extract shows moderate activity against S. aureus. Non-polar compounds might interact with the cell membrane or protein synthesis pathways, but their overall effect is relatively limited compared to polar compounds.

***Bacillus subtilis* (MTCC 3053):** The 7 mm inhibition zone indicates moderate efficacy against B. subtilis. The antibacterial activity suggests that non-polar compounds in the petroleum ether extract can affect the integrity of the cell membrane or interfere with bacterial metabolism in gram-positive bacteria.

Ampicillin: Serving as a standard reference, Ampicillin exhibited strong antibacterial activity, with inhibition zones of 15 mm for Pseudomonas fluorescens, 12 mm for Escherichia coli, 13 mm for Staphylococcus aureus, and 8 mm for Bacillus subtilis. Ampicillin's broad-spectrum efficacy is attributable to its mechanism of inhibiting bacterial cell wall synthesis, which is crucial for the survival of both gram-positive and gram-negative bacteria. The higher zones of inhibition compared to the *C. setosum* extracts highlight the potent and well-established antibacterial properties of Ampicillin.

The antibacterial activity of *C. setosum* leaf extracts, evaluated using different solvents, demonstrates the plant's potential as a source of natural antibacterial agents. The methanol extract showed the highest efficacy against a broad range of bacteria, including both gram-positive and gram-negative strains, suggesting that it contains a diverse array of potent antibacterial compounds. While the activity of other solvent extracts varied, the results highlight the significant role of solvent polarity in extracting bioactive compounds with varying antibacterial properties. The comparison with Ampicillin underscores the potential of *C. setosum* extracts as natural alternatives or supplements to conventional antibiotics, warranting further investigation into their specific bioactive components and mechanisms of action **(Fig 4).**

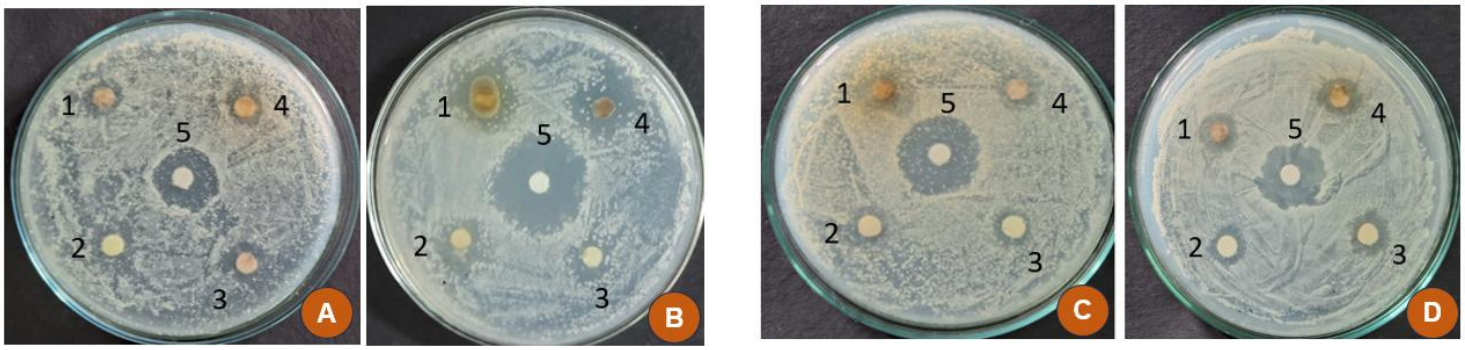

Fig:4. (Here A.P. fluorescens; B. E. coli; C.S. aureus; D.B. subtilis
1: C. setosumleaf extract with methanol, 2: C. setosumleaf extract with chloroform, 3: C. setosumleaf extract with ethyl acetate 4: C. setosumleaf extract with petroleum ether and 5 indicate Antibiotic (Ampicillin)

Table :2 Antibacterial activity of C. setosum

S. No	Sample	*P. fluorescens* (MTCC 9768)	*E. coli* (MTCC 424)	*S. aureus* (MTCC 96)	*B. subtilis* (MTCC 3053)
		Zone of inhibition in mm			
1	*C. setosum*leaf extract with methanol	08	09	08	09
2	*C. setosum*leaf extract with chloroform	07	05	07	06
3	*C. setosum*leaf extract with ethyl acetate	06	06	03	04
4	*C. setosum*leaf extract with petroleum ether	06	07	06	07
5	Antibiotic (Ampicillin)	15	12	13	08

Antifungal activity of *C. setosum*

The antifungal activity of *C. setosum* leaf extracts against three significant plant pathogens—*Fusarium oxysporum* NCIM1008, *Sclerotium rolfsii* NCIM 1084, and *Phytophthora infestans* MTCC 8707—was evaluated using different solvents: methanol, chloroform, ethyl acetate, and petroleum ether. The standard fungicide, Fluconazole, was used for comparison. The percentage of inhibition for each extract was measured to assess their effectiveness against the fungi. The results, presented in Table, highlight the varying degrees of antifungal efficacy exhibited by the different solvent extracts of *C. setosum*.

The methanol extract of *C. setosum* demonstrated the highest antifungal activity across all three fungi, with 60% inhibition for both *Fusarium oxysporum* and *Phytophthora infestans*, and 52% inhibition for *Sclerotium rolfsii*. Methanol, being a polar solvent, is effective in extracting a wide range of bioactive compounds, including polyphenols and flavonoids, which are known for their strong antifungal properties. These compounds likely contribute to the significant inhibitory effect observed against the pathogens. The high activity against *Fusarium oxysporum* and *Phytophthora infestans* suggests that the methanol extract contains compounds that can interfere with the fungal cell wall synthesis or membrane integrity, crucial for their survival and proliferation.

The chloroform extract showed moderate antifungal activity, with 54% inhibition for *Fusarium oxysporum*, 40% for *Sclerotium rolfsii*, and 52% for *Phytophthora infestans*. Chloroform, which is less polar than methanol, extracts compounds such as alkaloids and certain terpenoids, which have been documented for their antifungal effects. The chloroform extract's substantial inhibition against *Fusarium oxysporum* and *Phytophthora infestans* indicates that these compounds are particularly effective against these fungi, potentially by disrupting their cell membranes or interfering with critical metabolic processes.

The ethyl acetate extract exhibited lower antifungal activity, with inhibition percentages of 50%, 40%, and 38% for *Fusarium oxysporum*, *Sclerotium rolfsii*, and *Phytophthora infestans*, respectively. Ethyl acetate is moderately polar, extracting a mix of polar and non-polar compounds.

The reduced efficacy of the ethyl acetate extract may be due to the presence of fewer or less potent antifungal compounds compared to the methanol and chloroform extracts. The moderate inhibition of *Fusarium oxysporum* suggests some efficacy, likely due to the extraction of polyphenolic compounds, but the lower activity against *Sclerotium rolfsii* and *Phytophthora infestans* indicates a limited range of effective compounds against these fungi.

The petroleum ether extract showed the least antifungal activity, with 46% inhibition for *Fusarium oxysporum*, 30% for *Sclerotium rolfsii*, and 40% for *Phytophthora infestans*. As a non-polar solvent, petroleum ether primarily extracts lipophilic compounds such as fatty acids and non-polar terpenoids, which tend to have weaker antifungal activity compared to polar compounds. The relatively low inhibition percentages suggest that the non-polar compounds in the petroleum ether extract are less effective in combating fungal pathogens, likely due to their inability to penetrate fungal cell walls or interfere with crucial cellular processes.

Fluconazole, a widely used fungicide, showed inhibition percentages of 50% for *Fusarium oxysporum*, 55% for *Sclerotium rolfsii*, and 50% for *Phytophthora infestans*. The effectiveness of Fluconazole highlights its broad-spectrum antifungal activity, likely due to its mechanism of inhibiting the synthesis of ergosterol, an essential component of fungal cell membranes. While the methanol and chloroform extracts of *C. setosum* showed comparable or even superior inhibition against certain fungi, the overall effectiveness of Fluconazole against *Sclerotium rolfsii* indicates its continued relevance in managing fungal infections. However, the results suggest that the methanol extract, in particular, could be a potential natural alternative to synthetic fungicides, especially in combating *Fusarium oxysporum* and *Phytophthora infestans*

The evaluation of antifungal activity of *C. setosum* leaf extracts using different solvents demonstrates the significant potential of plant-based antifungal agents. The methanol extract, in particular, showed promising results with high inhibition percentages against multiple fungal pathogens, indicating the presence of potent bioactive compounds.

The study underscores the importance of solvent choice in extracting effective antifungal compounds and highlights the potential of *C. setosum* as a source of natural antifungal agents. Further research is warranted to isolate and identify the specific compounds responsible for the observed antifungal activity, which could lead to the development of new, natural antifungal treatments **(Fig 4).**

Fig:4. (Here 1: C. setosumleaf extract with methanol, 2: C. setosumleaf extract with chloroform, 3: C. setosumleaf extract with ethyl acetate, 4: C. setosumleaf extract with petroleum ether and 5: Fungicide (Fluconazole)

Table:3. Anti-fungal activity of C. setosum

S. No	Sample	*F.oxysporum* NCIM1008	*S.rolfsii* NCIM1084	*P.infestans* MTCC 8707
	Percentage of inhibition (%)			
1	*C. setosum*leaf extract with methanol	60%	52%	60%
2	*C. setosum*leaf extract with chloroform	54%	40%	52%
3	*C. setosum*leaf extract with ethyl acetate	50%	40%	38%
4	*C. setosum*leaf extract with petroleum ether	46%	30%	40%
5	Fungicide (Fluconazole)	50%	55%	50%

Discussions

Cyphostemmasetosum (Roxb.) Alston.is an herbaceous plant known for its folkloric treatment of ailments and belongs to the family Vitaceae [44-46]. The review of literature reveals, no earlier information on antioxidant, total antioxidant activity of *C.setosum*. The present study showed The DPPH radical scavenging activity was highest at 98% for Vitamin C at 80 µg/ml, then at 92% for the methanol extract of *C. setosum*, 82% for the chloroform extract, and 72% for the petroleum ether extract. The activity level of the ethyl acetate extract was 48%, but synthetic BHT showed a level of 65%. Antioxidants were most effectively extracted from *C. setosum* using methanol, demonstrating the plant's potential as a natural source of safer and more powerful antioxidants than synthetic ones like BHT.At a concentration of 400 µg/ml, Gallic Acid had the highest total antioxidant capacity (TAC) at 92%, with Ascorbic Acid coming in second at 85%. The total alkaloids content (TAC) was highest in the methanol extract (82%) followed by the chloroform extract (73%), the petroleum ether extract (62%), and the ethyl acetate extract (59%). The results show that the polarity of the solvent is important; methanol is the best solvent for extracting powerful antioxidants like flavonoids and polyphenols, which boost the antioxidant capacity overall. The review of literature reveals lack of information in*C. setosum*of antibacterial studies. However, few reports available accordioning to [47-48] demonstrated significant antimicrobial activity of *C. setosa* ethanol extract against bacterial and fungal pathogens, including *E. coli, Salmonella typhi, Bacillus subtilis, Candida albicans*, and *Fusarium solani*, using the well diffusion method. These findings highlight the potential of plant-based extracts as natural antimicrobial agents [48]. Present study observed the antibacterial efficacy of *C. setosum* extracts differed according to the solvent used.

The methanol extract exhibited the largest inhibition zones of 9 mm for *E. coli* and *B. subtilis*, and 8 mm for *P. fluorescens* and *S. aureus*. The chloroform extract exhibited inhibition zones of 7 mm for *P. fluorescens* and *S. aureus*, 6 mm for *B. subtilis*, and 5 mm for *E. coli*. The ethyl acetate extract exhibited moderate activity, measuring 6 mmagainst *P. fluorescens* and *E. coli,* 4 mm against *B. subtilis*, and 3 mm against *S. aureus*. The petroleum ether extract exhibited inhibition zones of 7 mm for *E. coli* and *B. subtilis*, and 6 mm for P. fluorescens and *S. aureus*. Ampicillin demonstrated enhanced efficacy, with inhibition zones measuring 15 mm for *P. fluorescens*, 12 mmfor *E. coli*, 13 mm for *S. aureus*, and 8 mm for *B. subtilis*. The results indicate that the methanol extract is the most potent natural antibacterial agent, exhibiting variable effectiveness depending on the solvent used.The antifungal efficacy of *C. setosum* extracts differed by solvent, with the methanol extract exhibiting the greatest inhibition: 60% against *F.oxysporum* and *P. infestans*, and 52% against *Sclerotium rolfsii*. The chloroform extract exhibited modest action, achieving 54%, 52%, and 40% inhibition against *F. oxysporum*, *P. infestans*, and *Sclerotium rolfsii*, respectively. The ethyl acetate extract had reduced efficacy, with inhibition rates of 50%, 38%, and 40%, but the petroleum ether extract displayed the least activity, with rates of 46%, 40%, and 30% against the same fungi. Fluconazole, the conventional fungicide, demonstrated 50%, 55%, and 50% inhibition, underscoring its broad-spectrum effectiveness. The findings establish the methanol extract as a potential natural antifungal agent, especially effective against *Fusarium oxysporum* and *Phytophthora infestans*.

Conclusions

Cyphostemmasetosum shows great promise as a natural source of antifungal, antibacterial, and antioxidant compounds.

With 92% DPPH radical scavenging activity, 82% total antioxidant capacity, and better antibacterial and antifungal activities, the methanol extract continuously demonstrated the highest efficacy throughout experiments. The polarity of the solvent was important; methanol efficiently extracted bioactive substances including flavonoids and polyphenols, boosting the plant's antibacterial and antioxidant qualities. According to these results, *C. setosum* is a viable candidate for the development of natural medicinal medicines, and more research is necessary to identify and describe its bioactive constituents.

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