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COMPARATIVE ANALYSIS OF ANTIBACTERIAL ACTIVITY OF *TINOSPORA CORDIFOLIA* GROWING ON HOST PLANTS *AZARDIRACHTA INDICA*, *SARACA ASOCA*, AND *CITRUS LIMON.*

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To check the antimicrobial activity of *Tinospora cardifolia* (Giloy) growing on host plants *Azadirachta indica* (Neem), *Saraca asoca* (Ashoka), *Citrus limon* (lemon), on common endodontic pathogens like *Escherichia coli*, *Salmonella*, *Staphylococcus aureus*, *Streptomyces*, *Enterococcus faecalis*, and *Listeria*. The agar diffusion test was used to check the antimicrobial activity of the Methanolic extracts of the medicinal plants with different concentrations. The values of the Zone of Inhibition were tabulated according to the concentration. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were also recorded. Extracts of all the *Tinospora cordifolia* growing on host plants showed considerable antimicrobial activity against selected pathogens. At a minimum 50mg/ml conc. all hosted and normal giloy shows zone of inhibition like- against *E. coli.- Saraca asoca,* against *Enterococcus- Citrus limon* (*Azadirachta indica also*), against *Listeria- Azadirachta indica*, against *Streptomyces -Azadirachta indica,* against *Salmonella- Azadirachta indica* (*Saraca asoca*)*,* and S.*aureus- Azadirachta indica* (*Saraca asoca* and *Citrus limon* also, but less than (*Azadirachta indica*) shows maximum zone of inhibition. The Methanolic extract of *Tinospora cardifolia* (Giloy) grows on host plants *Azadirachta indica* (Neem), *Saraca asoca* (Ashoka), *Citrus limon* (lemon), and normal giloy has considerable antimicrobial activity against *E. coli*, *Salmonella*, *Staphylococcus aureus, Streptomyces, Enterococcus faecalis,* and *Listeria*.

Keywords: Agar well diffusion method, Antibacterial activity, *Azadirachta indica*, Host plant dependency, Host plant dependency, *Tinospora cordifolia.*

Introduction

Herbal remedies have been employed in medical systems for the treatment and management of different diseases. Natural products are known to play an important role in human life. Various parts of the plants, like the root, bark, seed, and leaves, have been an important source of medicine for thousands of years. In recent years, a predominant interest has been observed in evaluating different plant extracts for their antimicrobial properties against bacteria causing dental caries and peri-radicular pathology. The plant finds an important place in the indigenous system of medicine, and its various parts are used in the treatment of various systemic diseases, including dental problems. It has shown significant anti-inflammatory, analgesic, and antipyretic activity[1]. Every part of the tree has been used as traditional medicine for household remedies against various human

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ailments. The tree is still regarded as a "Village dispensary" in India. Most of the parts of the plant, such as fruits, seeds, leaves, bark, and roots, contain compounds with proven antiseptic, antiviral, antipyretic, anti-inflammatory, antiulcer, and antifungal properties.

The plant *Tinospora cordifolia* has been used in different systems of traditional medicine for the treatment of diseases and ailments of human beings[2]. Antibiotics refer to the secondary metabolites produced by microorganisms or higher animals and plants during life that have anti-pathogen or other activities and can interfere with the development of other living cells[3]. According to research findings, antibiotics can promote cancer apoptosis, inhibit cancer growth, and prevent cancer metastasis[4]. For these reasons, antibiotics are increasingly being used to assist in the treatment of cancer[5]. There is a great need for the development of new antibiotics[6]. Application of phytoconstituents is frequently considered nowadays because plant constituents have high potential to develop a drug[7]. The development of antibiotic agents, their preclinical evaluation on an animal paradigm, is essential[8].

Tinospora cardifolia is a large deciduous climbing shrub found throughout India. The ayurvedic name of the plant is Guduchi, Giloy, or Amrita. In India, the extract of the plant is used as a remedy for many diseases, including diabetes, hepatitis, etc. The plant finds a special mention for its use in tribal or folk medicine in different parts of the country. The drug has been subjected to extensive phytochemical, pharmacological, and clinical investigations, and many interesting findings have been reported.

Lemon (*Citrus limon*) juice stimulates bile production, aiding digestion and alleviating indigestion. Rich in Vitamin C boosts the immune system and combats oxidative stress[9]. It also shows Antimicrobial Properties, traditionally used with honey to soothe sore throats and coughs[9].

Neem (*Azardirachta indica*) has many Antibacterial & Antifungal properties. Effective against various skin infections, including acne and eczema. Assists in managing diabetes by lowering blood glucose levels. Promotes faster healing due to its antimicrobial properties[10].

Ashoka (*Saraca asoca*) shows Anti-inflammatory properties. Protects cardiac tissues and reduces inflammation, particularly beneficial for heart health. Renowned for treating menstrual disorders and uterine problems, and used to treat skin diseases and improve complexion[11].

This study aimed to compare the antimicrobial efficacy of various concentrations of selected medicinal plants like *Azardirachta indica* (Neem), *Saraca asoca* (Ashoka), *Citrus limon*(lemon), and *T. cardifolia* (Giloy) used as a control plant against common endodontic pathogens such as *E. coli*, *Salmonella*, *Staphylococcus aureus*, Streptomyces, *Enterococcus faecalis,* and *Listeri*a.

Material and Method

Sample collection: The present investigation involved the collection of plant samples, such as the stem of *Tinospora cardifolia* (Giloy) growing on host plants *Azadirachta indica* (Neem), *Saraca asoca* (Ashoka), *Citrus limon*(lemon) and their specific parts from HUDA, Narnaul, Haryana, India, during March. The entire study was carried out over 15 months, encompassing all stages from sample acquisition to final data interpretation. Plant materials were washed with distilled water and dried in the shade for 10-12 days. All the material was ground in an electric grinder to produce a powder.

Preparation of extracts: The powdered material was again dried in an oven at 40°C for 4 h and used for extraction. Accurately

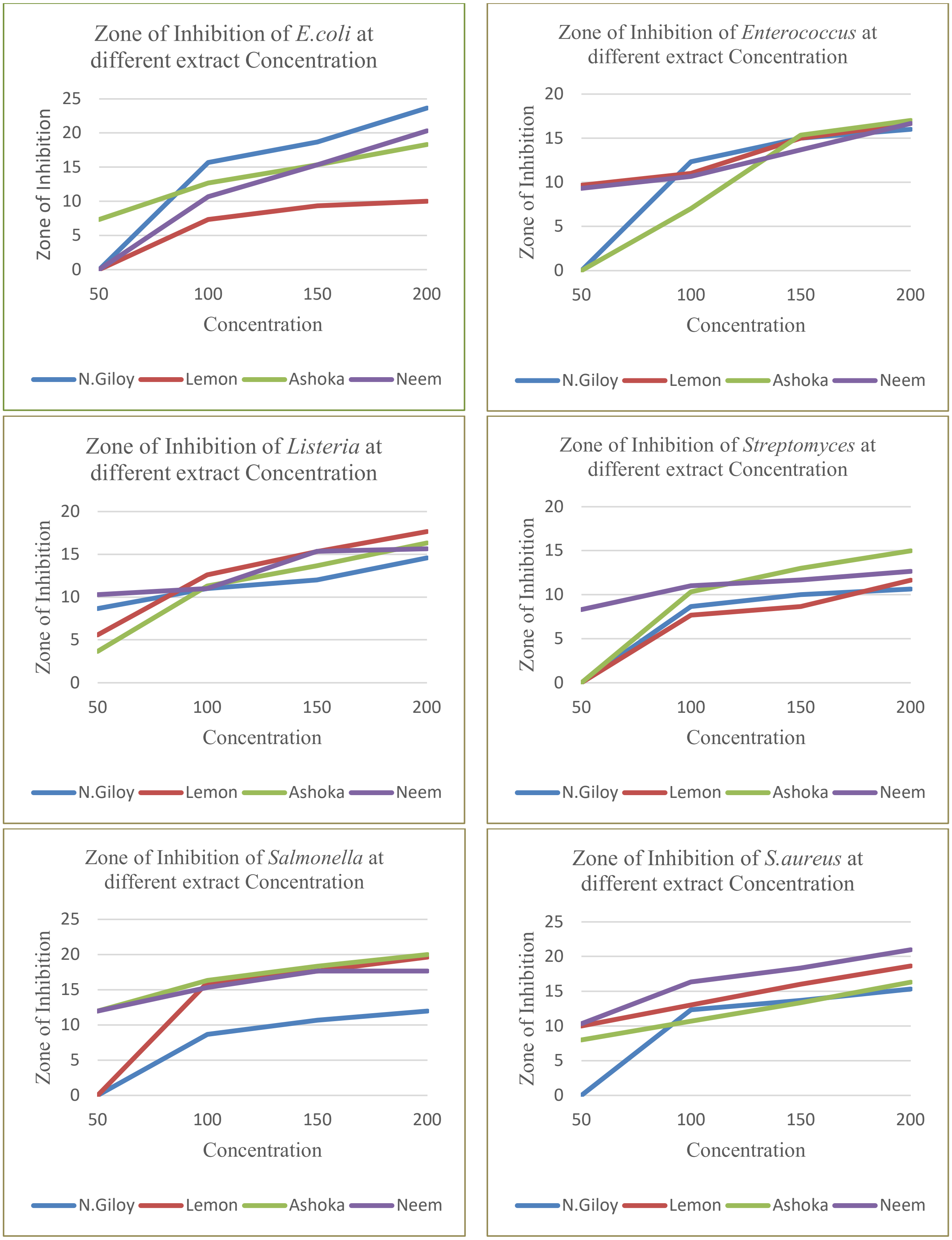

Figure 1: Zone of inhibition on different concentrations of different host plants against bacteria.

weighed 5 g of powdered stem sample was extracted with 50 ml methanol. This process was repeated until the residual marc got exhaustively extracted and finally extracts were pooled and evaporated in a rotavapor. The extracts were concentrated under partial vacuum at 80°C to dryness, leaving behind thick semi-solid residue.

Procurement of microorganisms: The microbial strains investigated in the study were obtained from Imtech-Chandigarh, India. The strains are *Escherichia coli (MTCC443)*, *Salmonella enterica typhimurium (MTCC98)*, *Staphylococcus aureus (87)*, Streptomyces *(MTCC4734)*, *Enterococcus faecalis (MTCC439),* and *Listeri*a *monocytogenes* (MTCC657).

Test for antibacterial assay

Agar well-diffusion assays: The test microorganisms were subcultured on specific media procured by HI Media Laboratory Pvt. Ltd., Mumbai, India, and incubated aerobically at 37°C for 24 h. The antibacterial activity of plant extracts was evaluated using the agar well diffusion method. Mueller-Hinton Agar plates were prepared and inoculated with a standardized bacterial suspension. Wells of 6 mm diameter were punched aseptically into the agar and filled with 100 µL of plant extract. Plates were incubated at 37°C for 24 hours, and zones of inhibition were measured in millimeters. After overnight incubation, the plates were observed for the zone of inhibition, and the diameters of the inhibition zone in millimeters were measured using a scale. Each extract was tested three times, and mean values were recorded. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined using the micro broth dilution method.

Statistical analysis

The data collected was analyzed using the following statistical test (SPSS version 17, Inc., Chicago, USA)

1. Mean value and standard deviation
2. One-way analysis of variance

Results and Discussion

In the present study, extracts of *Tinospora cordifolia* growing on three different host plants (Ashoka, Lemon, Neem) were evaluated for antibacterial activity against gram-positive and gram-negative bacteria. Overall, the extract from the host plant Ashoka demonstrated the highest inhibitory effect, followed by the host plants Lemon and Neem.

(A) *E.coli.* - Normal Giloy > Neem > Ashoka > Lemon in antibacterial strength against *E. coli*. There is a clear dose-dependent increase in inhibition across all samples. The host plant significantly affects antibacterial potency by altering the phytochemical composition of *T. cordifolia*.

(B) *Enterococcus*-The antimicrobial activity of *Tinospora cordifolia* extracts against *Enterococcus* increased progressively with concentration (50–200 µg/mL) for all host-based samples. Lemon-hosted Giloy showed the highest inhibition overall**,** reaching approximately 17 mm at 200 µg/mL, followed closely by Neem-hosted and Normal Giloy, both showing zones in the range of 16–17 mm**.** Ashoka-hosted Giloy initially exhibited the lowest inhibition at 50 µg/mL**,** but its activity increased steadily with concentration and became comparable to the other extracts at higher doses.Overall, the results indicate that all extracts possess significant anti-Enterococcus activity, with Lemon > Neem ≈ Normal > Ashoka at peak concentration, demonstrating a host-dependent variation in antibacterial potency.

(C) *Listeria*- All extracts showed a concentration-dependent increase in activity against *Listeria*. Lemon-hosted Giloy exhibited the highest inhibition at all higher concentrations (up to ~18 mm at 200 µg/mL), followed by Neem and Normal Giloy, which showed moderate activity (~15–16 mm). Ashoka-hosted Giloy showed the lowest activity but still increased steadily with concentration. Overall: Lemon > Neem ≈ Normal > Ashoka**.**

(D) *Streptomyces*- The zone of inhibition of *Streptomyces* increased with rising extract concentration for all samples. Among the four extracts, Ashoka-hosted Giloy showed the highest antibacterial activity**,** followed by Neem**,** while Lemon-hosted Giloy exhibited the lowest inhibition at all concentrations.

(E) *Salmonella*-The antibacterial activity against *Salmonella* increased with higher extract concentrations for all Giloy samples. Ashoka-hosted Giloy showed the maximum zone of inhibition, followed closely by Neem and Lemon, while normal Giloy exhibited the lowest activity throughout all concentrations.

(F) *S.aureus*- All Giloy extracts showed increasing antibacterial activity against *S. aureus* with rising concentrations. Neem-hosted Giloy demonstrated the highest zone of inhibition at all concentrations, followed by Lemon and Ashoka, while normal Giloy showed the lowest activity throughout.

At a minimum 50mg/ml conc. against *E. coli*, *Saraca asoca*, against *Enterococcus*, *Citrus limon* (*Azardirachta indica* also), and against Listeria *Azardirachta indica*, against *Streptomyces Azardirachta indica*, against *Salmonella Azardirachta indica* (*Saraca asoca*), and, *S.Aureus Azadirachta indica* (*Saraca asoca* and *Citrus limon* also but less than *Azardirachta indica*) shows maximum zone of inhibition.

Table 1 shows that the antimicrobial activity of *T. cordifolia* extracts increased with rising concentrations for all host plants. Giloy grown on Ashoka showed the maximum inhibition (15 mm at 200 mg/ml)**,** followed by Neem-hosted Giloy, which showed strong activity even at the lowest concentration (8.33 mm at 50 mg/ml). Lemon-hosted and normal Giloy showed comparatively lower inhibition. Overall, Ashoka-hosted Giloy exhibited the strongest antibacterial effect against *Streptomyces*.

Table 2 shows that all extracts of *T. cordifolia* showed increasing antibacterial activity against *Listeria* with rising concentrations. At lower concentration (50 mg/ml), Neem-hosted Giloy showed the highest inhibition**,** while Ashoka-hosted extract showed the least. At higher concentrations (150–200 mg/ml), Lemon-hosted Giloy exhibited the maximum inhibition (17.67 mm at 200 mg/ml)**,** followed closely by Ashoka- and Neem-hosted extracts. Overall, Lemon-hosted Giloy demonstrated the strongest antibacterial effect against *Listeria* at higher doses.

Table 3 shows that all host-dependent extracts of *T. cordifolia* showed strong and increasing antibacterial activity against *Salmonella* with rising concentrations. At lower concentration (50 mg/ml), Ashoka- and Neem-hosted Giloy showed the highest inhibition (12 mm)**,** while normal and Lemon extracts showed no activity. At higher concentrations (100–200 mg/ml), Ashoka-hosted Giloy consistently exhibited the strongest antibacterial effect**,** reaching 20 mm at 200 mg/ml**,** followed closely by the Lemon and Neem - hosted

Table-1. Antimicrobial activity of *T. cordifolia* stem growing on host plant *Azardirachta indica*, *Saraca asoca*, and *Citrus limon* extract against *Streptomyces* determined by agar well diffusion method.

Concentration of extract (mg/ml)	Zone of inhibition (mm) Average± standard deviation			
	T. cordifolia stem	*T. cordifolia* stem growing on the host plant, lemon	*T. cordifolia* stem growing on the host plant Ashoka	*T. cordifolia* stem growing on the host plant Neem
50mg/ml	0	0	0	8.33±1.53
100mg/ml	8.67±0.58	7.67±0.58	10.33±2.52	11.00±0.00
150mg/ml	10±0	8.67±0.58	13.00±2.00	11.67±0.58
200mg/ml	10.67±0.58	11.67±0.58	15.00±2.00	12.67±1.15

Table 2. Antimicrobial activity of *T. cordifolia* stem growing on host plant *Azardirachta indica*, *Saraca asoca*, and *Citrus limon* extract against *Listeria* determined by agar well diffusion method.

Concentration of extract (mg/ml)	Zone of inhibition (mm) Average± standard deviation			
	Extract of *T. cordifolia* stem	Extract of the *T. cordifolia* stem growing on the host plant, lemon	Extract of *T. cordifolia* stem growing on the host plant Neem	Extract of the *T. cordifolia* stem growing on the host plant Ashoka
50mg/ml	8.67±1.53	5.67±1.15	10.33±0.58	3.67±0.58
100mg/ml	11.00±1.00	12.67±1.53	11.00±1.00	11.33±0.58
150mg/ml	12.00±1.00	15.33±0.58	15.33±1.53	13.67±1.53

Table 3. Antimicrobial activity of *T. cordifolia* stem growing on host plant *Azardirachta indica*, *Saraca asoca*, and *Citrus limon* extract against *Salmonella* determined by agar well diffusion method.

Concentration of extract (mg/ml)	Zone of inhibition (mm) Average± standard deviation			
	Extract of *T. cordifolia* stem	Extract of *T. cordifolia* growing on the host plant Lemon	Extract of *T. cordifolia* stem growing on the host plant Ashoka	Extract of *T. cordifolia* stem growing on the host plant Neem
50mg/ml	0	0	12.00±2.00	12.00±1.00
100mg/ml	8.67±1.15	16.00±1.00	16.33±1.53	15.33±0.58
150mg/ml	10.67±1.15	17.67±0.58	18.33±1.53	17.67±0.58
200mg/ml	12.00±2.00	19.67±0.58	20.00±0.00	17.67±2.52

extracts. Overall**,** Ashoka-hosted Giloy demonstrated the maximum potency against *Salmonella*.

Table 4 shows that the antibacterial activity of *T. cordifolia* extracts against *S. aureus* increased progressively with concentration. At 50 mg/ml, only the Neem-hosted extract showed strong inhibition (13.67 mm), while the normal extract showed no activity. As concentration increased, all extracts demonstrated improved inhibition. The Neem-hosted *T.*

Table 4. Antimicrobial activity of *T. cordifolia* stem growing on host plant *Azardirachta indica*, *Saraca asoca*, and *Citrus limon* extract against *S.aureus* determined by agar well diffusion method.

Concentration of extract (mg/ml)	Zone of inhibition (mm) Average± standard deviation			
	Extract of *T. cordifolia* stem	Extract of *T. cordifolia* stem growing on host plant Lemon	Extract of *T. cordifolia* stem growing on host plant Ashoka	Extract of *T. cordifolia* stem growing on host plant Neem
50mg/ml	0	10.00±1.00	8.00±1.00	13.67±1.15
100mg/ml	12.33±0.58	13.00±1.00	10.67±0.58	16.33±1.53
150mg/ml	13.67±0.58	16.00±1.00	13.33±1.15	18.33±1.53
200mg/ml	15.33±0.58	18.67±1.15	16.33±0.58	21.00±1.00

Table 5. Antimicrobial activity of *T. cordifolia* stem growing on host plant *Azardirachta indica*, *Saraca asoca*, and *Citrus limon* extract against *Enterococcus* determined by agar well diffusion method.

Concentration of extract (mg/ml)	Zone of inhibition (mm) Average± standard deviation			
	Extract of *T. cordifolia* stem	Extract of *T. cordifolia* stem growing on the host plant Lemon	Extract of *T. cordifolia* stem growing on the host plant Ashoka	Extract of *T. cordifolia* stem growing on the host plant Neem
50mg/ml	0	9.67±0.58	0	9.33±1.15
100mg/ml	12.33±0.58	11.00±1.00	7.00±1.00	10.67±1.15
150mg/ml	15.00±1.00	15.00±1.00	15.33±0.58	13.67±1.15
200mg/ml	16.00±1.00	16.67±1.53	17.00±0.00	16.67±1.53

Table 6. Antimicrobial activity of *T. cordifolia* stem growing on host plant *Azardirachta indica*, *Saraca asoca*, and *Citrus limon* extract against *E.coli* determined by agar well diffusion method.

Concentration of extract (mg/ml)	Zone of inhibition (mm) Average± standard deviation			
	Extract of *T. cordifolia* stem	Extract of *T. cordifolia* stem growing on the host plant Lemon	Extract of *T. cordifolia* stem growing on the host plant Ashoka	Extract of *T. cordifolia* stem growing on the host plant Neem
50mg/ml	0	0	7.33±0.58	0
100mg/ml	15.67±1.15	7.33±0.58	12.67±2.52	10.67±1.15
150mg/ml	18.67±1.53	9.33±0.58	15.33±0.58	15.33±0.58
200mg/ml	23.67±1.53	10.00±1.00	18.33±1.15	20.33±0.58

cordifolia consistently exhibited the highest antibacterial activity**,** reaching 21 mm at 200 mg/ml**,** followed by the Lemon-hosted extract (18.67 mm). Normal and Ashoka-hosted extracts showed moderate but increasing activity. Overall, Neem-hosted Giloy displayed the maximum potency against *S. aureus* at all concentrations.

Table 5 shows that the antimicrobial activity of *T. cordifolia* extracts against *Enterococcus* increased with concentration across all host plants. At 50 mg/ml, only Lemon- and Neem-hosted extracts showed inhibition (≈9–9.6 mm), while normal and Ashoka extracts showed no activity. As the concentration increased to 100–150 mg/ml, all extracts demonstrated moderate inhibition. At 200 mg/ml, all four extracts showed strong and comparable activity**,** with inhibition zones between 16–17 mm**.** The Ashoka-hosted extract showed the highest activity (17 mm) at 200 mg/ml, followed closely by Lemon and Neem extracts. Overall, all host-dependent extracts exhibited potent antibacterial action against *Enterococcus* at higher concentrations**,** with Ashoka showing a slight edge at peak concentration.

Table 6 shows that the antimicrobial activity of *T. cordifolia* extracts against *E. coli* increased steadily with concentration. At 50 mg/ml, only the Ashoka-hosted extract showed inhibition (7.33 mm), while all other extracts were inactive. From 100 mg/ml onward, all extracts displayed measurable activity. The normal *T. cordifolia* extract showed the highest overall activity**,** reaching 23.67 mm at 200 mg/ml, followed by Neem-hosted extract (20.33 mm). The Ashoka extract showed moderate but consistent activity (up to 18.33 mm), whereas the Lemon-hosted extract exhibited the weakest response across all concentrations. Overall, *T. cordifolia* demonstrated strong antibacterial potential against *E. coli*, with activity influenced significantly by the host plant.

Figure 1 and Tables 1 to 6 show that the antimicrobial activity of *Tinospora cordifolia* stem extracts showed clear host-dependent variation across all six tested organisms, demonstrating that the climbing host plant significantly influences the plant's phytochemical accumulation and therapeutic efficacy. Overall, extracts from Neem-hosted *T. cordifolia* exhibited the strongest and most consistent broad-spectrum activity, showing superior inhibition against *Streptomyces*, *Staphylococcus aureus*, *Enterococcus*, *Listeria*, and *E. coli*, which may be attributed to the absorption of Neem's bioactive compounds such as azadirachtin and nimbidin. Ashoka-hosted extracts displayed enhanced activity, particularly against *Salmonella*, *Listeria*, and *Enterococcus*, suggesting increased levels of glycosides and phenolic compounds when growing on this host[12]. Lemon-hosted extracts showed organism-specific enhancements, especially against *Listeria* and *Salmonella*, likely due to the acidic and flavonoid-rich environment of Citrus plants. Although the normal extract (not grown on any host) demonstrated strong natural antibacterial effects—especially the highest inhibition against *E. coli*—host-dependent vines frequently outperformed it, proving that phytochemical enrichment occurs through host interaction. Across increasing concentrations (50–200 mg/ml), all extracts generally exhibited a dose-dependent increase in activity, with the highest zones of inhibition at 200 mg/ml. Overall, the results confirm that the antimicrobial potential of *T. cordifolia* is significantly enhanced by the host plant, with the order of effectiveness varying by organism but generally following the pattern: Neem-hosted > Ashoka-hosted > Lemon-hosted > Normal extract**,** validating traditional claims that the medicinal strength of Giloy depends strongly on the plant it climbs and draws nourishment from.

The present study demonstrates that the antimicrobial activity of *Tinospora cordifolia* varies significantly depending on the host plant on which it grows[13]. This host-dependent variation in bioactivity is likely attributed to differences in the phytochemical composition absorbed or influenced through the host plant, a phenomenon documented in previous studies on parasitic and symbiotic medicinal plants[14,15].

This study evaluated the antibacterial activity of *Tinospora cordifolia* (Giloy) growing on different host plants against selected Gram-positive and Gram-negative bacteria. The results demonstrated that the host plant significantly influenced the antibacterial potency of *T. cordifolia*, with extracts from Host Ashoka exhibiting the strongest inhibitory effect, followed by Host Lemon and Host Neem. This variation supports earlier research indicating that the phytochemical profile of *T. cordifolia* is modulated by its host plant due to differential uptake of secondary metabolites, nutrients, and growth conditions[16,17].

The stronger activity observed against Gram-positive bacteria compared with Gram-negative strains aligns with previous findings on plant-based antimicrobials. Gram-negative bacteria possess an additional outer membrane containing lipopolysaccharides, which limits the penetration of many phytochemicals[18].

In the current study, *T. cordifolia* growing on *Azardirachta indica* exhibited the most potent and broad-spectrum antibacterial activity. This may be attributed to synergistic interactions with neem-derived compounds such as azadirachtin, nimbin, and quercetin, which are well known for their antimicrobial effectiveness[19]. Host Neem–derived Giloy showed significantly higher antibacterial activity, likely due to increased concentrations of bioactive compounds such as tinosporaside, cordifolide, berberine, palmatine, and phenolic compounds. Previous phytochemical analyses have confirmed that *T. cordifolia* climbing on nutrient-rich or medicinal host plants accumulates higher levels of alkaloids, lignans, diterpenoids, and flavonoids—molecules known for their antimicrobial properties. Our findings align with earlier reports indicating that *T. cordifolia* can modify its secondary metabolite profile based on environmental and host-related factors, thereby altering its pharmacological properties. Such synergism has been previously reported in host-influenced medicinal plants, where the phytochemical uptake from the host enhances the bioactivity of the climber or parasite[20-23]. The strong inhibitory response against *Listeria monocytogenes*, *Streptomyces* spp., *Salmonella* spp., and *Staphylococcus aureus* supports the hypothesis that neem-associated *T. cordifolia* develops an enriched phytochemical profile with improved antibacterial potency.

The extract from *Saraca asoca* showed the highest inhibition against *Escherichia coli*, which may be linked to the presence of polyphenols and tannins in *S. asoca* bark and leaves. These compounds are known to contribute to broad antimicrobial activity and may influence the metabolite expression in *T. cordifolia* climbing on this host[21]. Moderate activity against *Salmonella* spp. also reflects this trend, indicating selective enhancement of antibacterial pathways.

Interestingly, *Citrus limon*-associated *T. cordifolia* showed the maximum inhibition against *Enterococcus*, which could be attributed to the absorption of citrus-derived flavonoids such as hesperidin and limonoids, known for their activity against Gram-positive cocci[22]. This demonstrates that host-specific metabolites influence the antibacterial spectrum of *T. cordifolia* in distinct ways.

The enhanced activity against *S. aureus, Salmonella, Enterococcus,* and *E.coli* supports existing literature where methanolic extract of *T. cordifolia* produced zones of inhibition ranging from 12–20 mm depending on solvent and concentration[24].

The relatively lower activity against *streptomyces,* and *Listeria* is also consistent with reports that this organism possesses efflux pumps and biofilm-forming abilities that reduce susceptibility to plant extracts[25].

Overall, the results highlight that host-dependent variation in phytochemistry markedly influences the antibacterial performance of Giloy. These findings support the traditional Ayurvedic concept that the therapeutic value of *Guduchi* is enhanced when it grows on certain host plants, especially those with medicinal potential[26].

Overall, the results emphasize that *A. indica*-associated *T. cordifolia* provides the broadest and strongest antibacterial response. These findings agree with prior observations that neem provides a phytochemically rich platform for enhancing the medicinal potential of associated plants[27]. The study supports the broader concept that host-driven phytochemical variation may play a crucial role in determining the pharmacological properties of medicinal climbers. Recognizing these differences is important for standardizing plant-based antimicrobial formulations, as host plant selection may significantly influence therapeutic efficacy[28].

The study further emphasizes the potential of host-specific *T. cordifolia* extracts as natural antibacterial agents and encourages further phytochemical analysis and in-vivo evaluation.

The demonstrated host-dependent variability opens new possibilities for selecting optimal host–climber combinations to maximize antimicrobial strength. Further phytochemical profiling of extracts from different hosts could help identify the specific compounds responsible for enhanced activity.

Conclusion

The findings of this study reveal that *Tinospora cordifolia*, when growing on different host plants (*Azardirachta indica*, *Saraca asoca*, and *Citrus limon*), exhibits varying degrees of antimicrobial activity against selected pathogenic bacterial strains. At a minimum concentration the extract derived from host plant *Saraca asoca* demonstrated the highest zone of inhibition against *Escherichia coli*. Against *Enterococcus*, the extract from host plant *Citrus limon* showed the most effective inhibition, followed by *Azardirachta indica*. The extract from host *Azardirachta indica* exhibited the strongest antibacterial effect against *Listeria monocytogenes*, *Streptomyces*, and *Salmonella* spp., with moderate activity observed from *Saraca asoca* against *Salmonella*. For *Staphylococcus aureus*, *Azardirachta indica*-associated *T. cordifolia* extract showed the maximum zone of inhibition, while extracts from *Saraca asoca* and *Citrus limon* demonstrated comparatively lower activity.

Overall, *T. cordifolia* growing on *Azardirachta indica* displayed the broadest spectrum and highest magnitude of antibacterial activity across most tested organisms, supporting its ethnomedicinal relevance. These results suggest the potential of host-dependent variation in phytochemical composition, offering insights for the development of novel plant-based antimicrobial formulations.

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