

Original Research

***In vitro* Antibacterial Activity and Phytochemical Profiling of Selected Medicinal Plants**

Mohamed SHAFIL^{S1}, Sriram R², Syed RAFFIC ALI S², Md. AZMATHULLAH N², Ghani BASHA K¹, Sultan MOHIDEEN A. K^{*2}.

AUTHOR AFFILIATIONS:

¹Department of Biotechnology, The New College, Chennai -600014, Tamil Nadu, India.

²PG & Research Department of Zoology, The New College, Chennai -600014, Tamil Nadu, India.

ABSTRACT This study aimed to investigate the phytochemical composition and antimicrobial activity of five Indian medicinal plants: *Azadirachta indica*, *Mangifera indica*, *Ocimum tenuiflorum*, *Solanum trilobatum*, and *Piper betle*, which were selected for their traditional medicinal use and therapeutic potential. Ethanolic extracts from the leaves were analyzed using standard qualitative methods. The antimicrobial efficacy of these extracts was evaluated against *Escherichia coli* and *Staphylococcus aureus* through the well diffusion method, determining the minimum zone of inhibition concentration (MIC) using Mueller-Hinton agar. The findings indicated that antimicrobial activity was observed across all the plant extracts at different concentrations (100 mg/ml, 50 mg/ml, 25 mg/ml, and 12.5 mg/ml). The *P. betle* showed a notable inhibitory activity against *E. coli* (10 mm), while *A. indica* expressed an inhibitory effect against *S. aureus* (12 mm). The varying inhibitory effects observed are attributed to the diverse phytochemical components found in different plant species. Some plants demonstrated minimal inhibitory action against specific pathogens. The observed positive inhibitory effects in certain plants are traced to the presence of active bioactive compounds, which have potential applications as antimicrobial or therapeutic agents.

KEYWORDS: *Antimicrobial activity, antimicrobial sensitivity Test, Ethanolic extract, Medicinal plants, Phytochemicals.*

The biggest challenge facing our generation is antimicrobial resistance. The ability to microorganisms to persist in growing in opposition of antibiotics is known as antimicrobial resistance. Multiple drug resistance has emerged in recent times which resulted in the extensive and careless use of modern antimicrobial drugs to treat infectious diseases. Given this circumstance, fresh research is required to find antibacterial drugs that can successfully fight off harmful microbes that are resistant to current antibiotics (Han et al., 2022; Ahmed et al., 2024). Medicinal plants are essential for preserving people's and communities' health. Chemical compounds that cause certain physiological reactions in the human body are the source of these plants' therapeutic qualities. Alkaloids, flavonoids, tannins, and phenolic compounds are the



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Reviewers Dr. Manisha Sharma, Amity University, Noida, U.P. India

Dr. N. Roopa, Periyar University, Salem, Tamil Nadu, India

Address correspondence to Sultan Mohideen A. K
smdbio@yahoo.co.in

ORCID ID: <https://orcid.org/0000-0001-7064-2963>

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most important bioactive substances present in plants (Noor et al., 2022). Various parts of plants have established antimicrobial potential toward specific pathogens. For instance, different solvent extracts from plants have shown effectiveness against microbes such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* (Parekh & Chanda, 2007; Lubis, et al., 2020). In the quest to address the current challenges posed by antimicrobial-resistant strains, medicinal plants that have demonstrated efficacy in managing various infections should be considered as potential leads. Due to the diverse array of compounds present in a single plant part, medicinal plants may pose a challenge for microorganisms to develop resistance (Osei-Djarbeng et al., 2020). There is a dire need to explore alternative sources for developing neo-antimicrobial drugs to treat infections. One potential approach to achieve this goal is the systematic identification of bioactive phytochemicals. Alkaloids exhibit various therapeutic properties, including antiviral, antiarrhythmic, antimalarial, and anticancer effects (Faizal et al., 2023; Heinrich, Mah, & Amirkia, 2021). Plant extracts containing tannins serve as astringents, remedies for diarrhea, diuretics, treatments for stomach and duodenal tumors (Karim et al., 2019), and possess anti-inflammatory, antiseptic, antioxidant, and hemostatic qualities (Dolara et al., 2005). Research has shown that flavonoids demonstrate multiple biological functions, such as antimicrobial, cytotoxic, anti-inflammatory, and antitumor activities (Liga et al., 2023). Phenolic acids have been found to have diverse biological effects, including increasing bile production, lowering blood cholesterol and lipid levels, and exhibiting antimicrobial action against *S. aureus* (Khan et al., 2022). In plants, phytosterols function as substrates for secondary metabolite synthesis, regulate cell membrane permeability and fluidity, and serve as biogenic precursors for growth factors (Begum et al., 2022). These compounds also impede cholesterol absorption in the intestines (Nyamai et al., 2016). Saponins are thought to increase immunoglobulin production and stimulate the cell-mediated immune system by acting as adjuvants. These substances reportedly interact with antigen-presenting cells and trigger interferon and interleukin production, thereby mediating immunostimulant effects (Kunnumakkara, 2014). Saponins induce cell death in blood cancer cell lines, and the cell cycle will be inhibited in breast cancer cell lines, thus inhibiting tumor cell growth (Dastmalchi et al., 2007). Additionally, they have been observed to eliminate protozoans and molluscs, act as antioxidants, hinder protein digestion and vitamin and mineral uptake in the gut, cause hypoglycemia, and function as antifungal and antiviral agents (Saxena et al., 2013; Venkatesan et al., 2019).

The main objective of this investigation is to assess the antibacterial sensitivity of ethanolic extracts of *A. indica*, *M. indica*, *O. tenuiflorum*, *S. trilobatum*, and *P. betle* against *S. aureus* and *E. coli*. To determine its bioactive elements that give the plant extracts their antibacterial properties, the phytochemical details of the plant extracts were also examined.

MATERIALS AND METHODS

Collection of plants

The fresh plant leaves of *A. indica* and *M. indica* were collected from The New College campus, Chennai, Tamil Nadu, India, and *O. tenuiflorum*, *S. trilobatum*, and *P. betle* were obtained from a local market in Chennai. They were identified and authenticated at PG

Extraction of Plant material

The plant specimens underwent cleaning with tap water and were subsequently allowed to dry in the air. Once air-dried, the samples were pulverized into a homogeneous powder. To create the ethanol extract for each specimen, 10 g of the dried powdered sample was immersed in 100 ml of ethanol for 24 hours and placed in a shaker. The plant extracts were sieved using Whatman No. 1 filter paper to get the filtrate (Al-Salt, 2012).

Phytochemical Screening

A qualitative phytochemical analysis (alkaloids, tannin, flavonoid, saponin, quinones, phenolic compounds, and phytosterols) was carried out to detect some bioactive constituents in the plant extract according to (Banu & Cathrine, 2015; Shaikh & Patil, 2020).

Antimicrobial Assay

Test Organisms:

The antimicrobial susceptibility tests were conducted by taking two common bacterial strains: the gram-positive bacteria, like *S. aureus*, and the gram-negative bacteria, like *E. coli*.

Extract Dilution:

Stock solutions of each crude extract were made at a 100 mg/ml concentration. From these, additional concentrations (50, 25, and 12.5 mg/ml) were prepared through serial dilution, as described by Andrews (2001).

Antimicrobial Testing:

The well-diffusion technique was used to perform the assay. Bacterial isolates were cultured in peptone water for 18-24 hours, with the broth turbidity adjusted to match the 0.5 McFarland standard. The inoculum was then evenly spread on Muller-Hinton agar (MHA) plates. Utilizing a sterile cork borer, 3 mm diameter wells were prepared in the agar. Each well received 5 µl of extract at varying concentrations. Following this, the plates underwent incubation for 24 hours at a temperature of 37°C. Subsequently, the zones of inhibition were evaluated and recorded in millimeters. (mm). (Diso et al., 2017). Antimicrobial activity was assessed in a preliminary investigation, with single replicates for most concentration, except for 100 mg/ml, where two additional replicates were performed to validate the experiment.

RESULTS

The phytochemical profiles of the five ethanolic plant extracts are tabulated (Table 1). The results showed that all plant samples contain phenols and phytosterols. Alkaloids were detected in *P. betel* sample, Instead of other samples *A.indica*, *M.indica*, *O. tenuifolium* and *S. trilobatum* trace amount of alkaloids are found which was evidenced by formation of minimal brown colored precipitate. *A. indica* and *P. betel* exhibited the presence of hydrolyzable tannins. Flavonoids were found in *P. betel*, while *A. indica*, *M. indica*, and *O. tenuifolium* showed slight colour changes, indicating trace amounts. *S. trilobatum* lacked flavonoids. Saponins were exclusively present in *A. indica* and *S. trilobatum*. Quinones were detected in all samples except *S. trilobatum*.

The plant extracts of various concentrations were used to assess their antimicrobial efficacy against bacterial samples like *S. aureus* and *E. coli* (Table 2). The results

Shafil S et al; Exon 2(2) :165-172 (2025)
indicated differences in antimicrobial effectiveness across the plant extracts. *P. betel* demonstrated the highest antimicrobial activity against *E. coli* with a supreme inhibition zone of 10 mm at 100 mg/ml, suggesting potent antibacterial properties. *A. indica* was most efficient towards *S. aureus*, showing an inhibition zone of 12 mm at 100 mg/ml, outperforming the other plant extracts.

Table 1: Qualitative analysis of Phytochemicals in selected plant species.

Phyto Chemicals	Alkaloid	Tannins	Flavonoid	Saponins	Quinones	Phenolic compound	Phytosterols
<i>A.indica</i>	Trace	+	Trace	+	+	+	+
<i>M.indica</i>	Trace	-	Trace	-	+	+	+
<i>O.tenuiflom</i>	Trace	-	Trace	-	+	+	+
<i>S.trilobatum</i>	Trace	-	-	+	-	+	+
<i>P.betle</i>	+	+	+	-	+	+	+

M. indica, *O. tenuiflorum* and *S. trilobatum* showed moderate to low efficacy, with inhibition zones ranging between 4-8 at highest concentration. At 50 mg/mL, the inhibition zones reduced, with *P. betle* showing 6 mm against *E. coli* and *A. indica* showing 10 mm against *S. aureus*. At the lowest concentration (12.5 mg/mL), only *P. betle*, *A. indica*, and *M. indica* showed minimal inhibition. Gentamicin (40 mg/mL) produced inhibition zones of 36 mm for *E. coli* and 42 mm for *S. aureus*, serving as the positive control. Ethanol (negative control) showed no inhibition, confirming that the antimicrobial activity was due to the plant extracts (Figure 1-4).

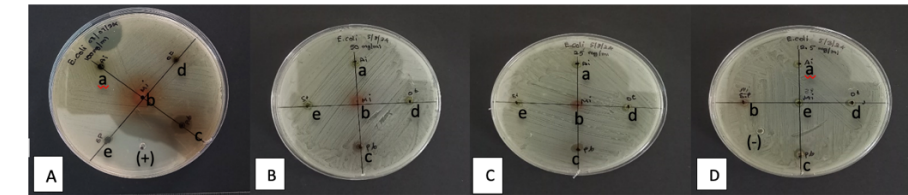


Figure 1: Antimicrobial activity of different plant extracts against *E. coli* in different concentrations. [Image A: 100 mg/ml, B: 50 mg/ml C: 25 mg/ml and D: 12.5 mg/ml. Wells labeled as; a= *A. indica*, b= *M. indica*, c= *P. betle*, d= *O. tenuiflorum*, e= *S. trilobatum*, (+) = Positive control (Gentamycin 40mg/ml), (-) = Negative control (Ethanol).]

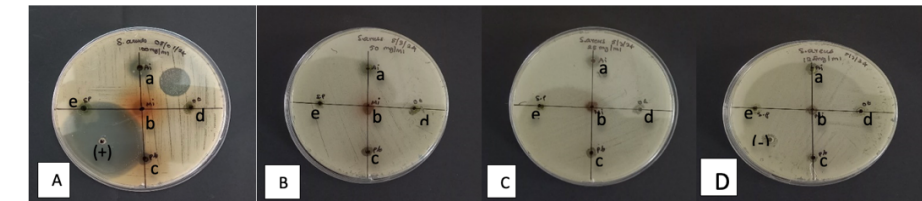


Figure 2: Antimicrobial activity of different plant extracts against *S. aureus* in different concentrations. [Image A: 100 mg/ml, B: 50 mg/ml C: 25 mg/ml and D: 12.5 mg/ml. Wells labeled as; a= *A. indica*, b= *M. indica*, c= *P. betle*, d= *O. tenuiflorum*, e= *S. trilobatum*, (+)=Positive control (Gentamycin 40mg/ml), (-)=Negative control (Ethanol).]

Table 2: Antimicrobial Sensitivity Test of plant extracts against *E. coli* and *S. aureus* using varying concentrations.

Strain	Concentration (mg/ml)	Zone of Inhibition (mm)					Positive control (Gentamycin 40mg/ml)	Negative Control (Ethanol)
		<i>A.indi</i>	<i>M.indica</i>	<i>P.betle</i>	<i>O. tenuiflom</i>	<i>S.trilobatum</i>		
		<i>ca</i>						
<i>E. coli</i>	100	4	7	10	5	5	36	-
	50	-	5	6	5	4		
	25	-	4	5	4	-		
	12.5	-	-	4	-	-		
<i>S.aureus</i>	100	12	8	7	6	5	42	-
	50	10	6	5	5	4		
	25	7	5	4	4	-		
	12.5	6	4	-	4	-		

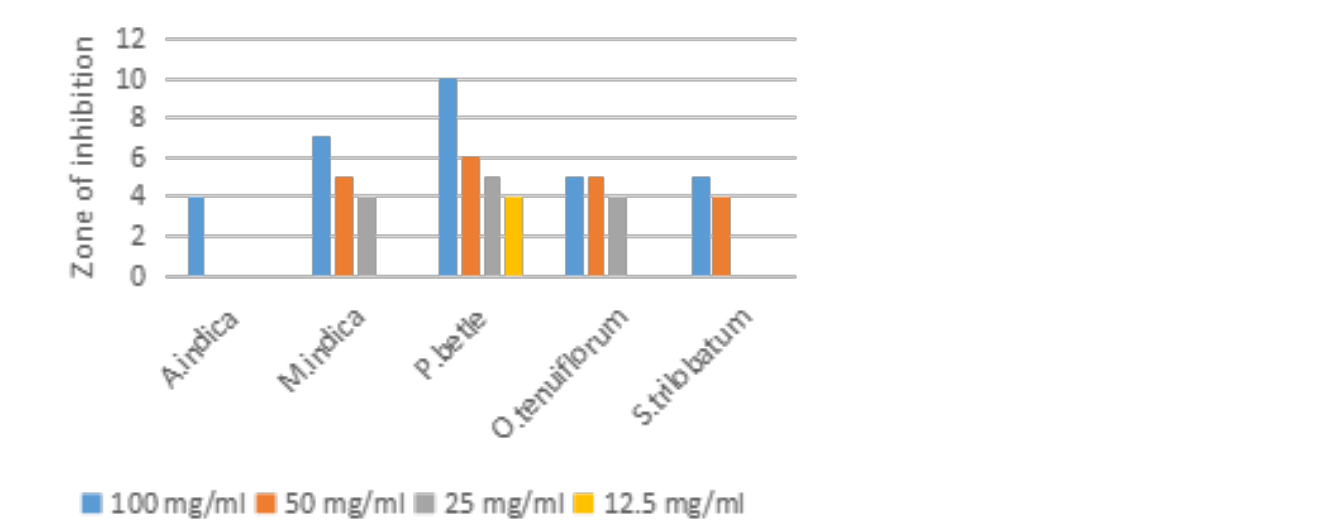


Figure 3: Zone of Inhibition at different concentrations against *E. coli*.

DISCUSSION

The present study investigated the antimicrobial potential of five medicinal plants native to India: *A. indica*, *M. indica*, *O. tenuiflorum*, *S. trilobatum*, and *P. betle*. Analysis of ethanolic extracts revealed various bioactive compounds, such as alkaloids, flavonoids, tannins, saponins, quinones, phenolic compounds, and phytosterols. These compounds are known for their antimicrobial properties, which encompass damaging microbial cell membranes, suppressing enzymatic activity, and disrupting the process of DNA replication (Xu et al., 2023). *P. betle* demonstrated the most potent antimicrobial

activity, particularly against *E. coli*, with a 10 mm inhibition zone at 100 mg/mL. The high efficacy of *P. betle* is likely due to its alkaloid and flavonoid content, as alkaloids destabilize microbial membranes and flavonoids interfere with bacterial DNA synthesis and protein production (Pruteanu et al., 2020). These findings support the traditional use of *P. betle* for treating bacterial infections and suggest its potential in drug development. Similar antibacterial activity of *P. betle* against some human pathogen with various extracts has been reported in earlier studies (Ermawati et al., 2021; Al-Gburi, et al. 2024)

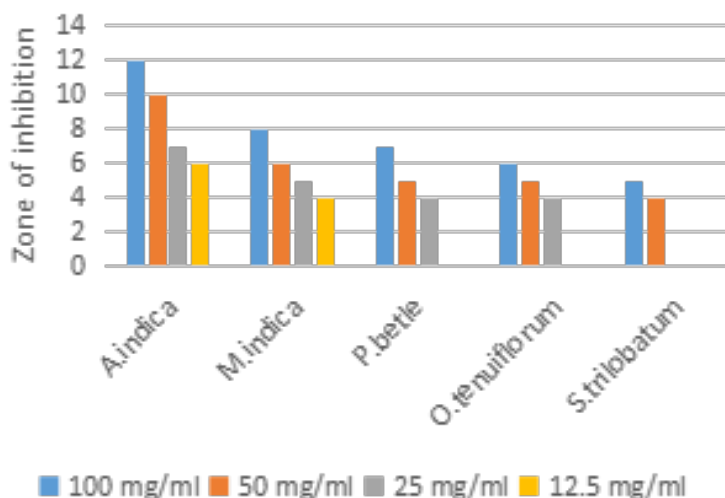


Figure 4: Zone of Inhibition at different concentrations against *S. aureus*.

In contrast, *A. indica* exhibited the strongest activity against *S. aureus*, a gram-positive bacterium, with a 12 mm inhibition zone at 100 mg/mL. The effectiveness of *A. indica* may be attributed to its phenolic compounds, tannins, and quinones, as phenolic compounds damage bacterial cell walls and disrupt metabolism (Sun & Shahrajabian, 2023), while tannins and quinones impede microbial enzyme activity and cell wall synthesis. This is in accordance to the previous finding which showed *A. indica* leaf extracts to possess high antibacterial activity, particularly against gram-positive strains (Patil et al., 2017; Somchit et al., 2010). *M. indica*, *O. tenuiflorum*, and *S. trilobatum* showed moderate to weak antimicrobial activity. *M. indica* produced inhibition zones ranging from 4-8 mm, depending on the ethanolic extract with various concentration against *E. coli* and *S. aureus*, which may be due to its lower bioactive compound content. *O. tenuiflorum*'s limited effectiveness is probably a result of trace phytochemicals, while *S. trilobatum* displayed minimal activity, especially against *E. coli*, indicating a weaker phytochemical profile. In fact, earlier studies on *O. tenuiflorum* and *S. trilobatum* indicated inconsistent antimicrobial activity which relies on the solvent type and plant part used (Muniyan et al., 2022; Kumar et al., 2018). Higher extract concentrations (100 mg/mL) yielded larger inhibition zones, whereas lower concentrations (12.5 mg/mL) showed little to no activity. For example, *P. betle* and *A. indica* demonstrated significant inhibition at 100 mg/mL but reduced efficacy below 25 mg/mL, emphasizing the importance of optimal dosage in therapeutic applications. Compared to gentamicin, which produced inhibition zones of 36 mm against *E. coli* and 42 mm against *S. aureus*,

the plant extracts exhibited lower but promising activity. The negative control (ethanol) showed no inhibition, confirming the effects of the bioactive components. Further research aims at retrieving and finding out the specific compounds that are responsible for the antimicrobial efficacy and examining their synergistic interactions to enhance efficacy and therapeutic potential. Additionally, in vivo studies are necessary to assess the safety, effectiveness of these plant extracts in clinical settings. Addressing these areas could position medicinal plants like *P. betle* and *A. indica* as valuable resources in the fight against antimicrobial resistance.

CONCLUSION

The research reveals the antimicrobial potential of five plant species: *A. indica*, *M. indica*, *O. tenuiflorum*, *S. trilobatum*, and *P. betle*. Demonstrated the strongest inhibitory effect against *E. coli*, while *A. indica* showed notable activity in combating *S. aureus*. These findings indicate their effectiveness against both Gram-positive also negative bacteria. The study observed that antimicrobial efficacy increased with concentration, emphasizing the need to determine optimal dosages for maximum therapeutic benefit. The results validate the traditional medicinal applications of these plants and suggest their potential in developing natural antibacterial agents. However, future research, including isolation of active compounds and investigation of their mechanisms of action, is crucial to fully elucidate their therapeutic capabilities and enhance their use in addressing antimicrobial resistance.

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