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Evaluation of Antibacterial Activity of Ethno-Gynecological Medicinal Plants against *Escherichia coli* and *Staphylococcus aureus*

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This study aimed to evaluate the antibacterial activity of crude methanolic extracts from twelve medicinal plants traditionally used to treat gynecological diseases. An ethno-gynecological survey identified plants commonly used for treating reproductive ailments, and their extracts were screened against common clinical pathogens, Escherichia coli ATCC 23716 and Staphylococcus aureus ATCC 6538, using the agar well diffusion method. Extracts were tested at four different concentrations (0.25 mg/mL–1 mg/mL) and compared with the standard antibiotic amikacin. A concentration-dependent increase in antibacterial activity was observed for several plants. Pterocarpus marsupium showed the highest inhibition against E. coli ATCC 23716 (6±0 mm), whereas Buchanania cochinchinensis, Dregea volubilis, Pueraria tuberosa, and Piliostigma malabaricum exhibited no activity. Against S. aureus ATCC 6538, Buchanania cochinchinensis displayed the strongest inhibition (9±0 mm), while Ougeinia dalbergioides showed minimal activity (2±0 mm). The findings support the traditional use of selected ethnomedicinal plants and highlight species with promising antibacterial potential. These results justify further research on the phytochemical components, mechanisms of action, and potential development of plant-based antibacterial agents targeting reproductive tract pathogens.

Keywords: Agar well diffusion; Antibacterial; *Escherichia coli*; Ethno-gynecology; Medicinal plants; *Staphylococcus aureus.*

The study of ethnobotany, or how people use plants in their traditional cultures, remains beneficial for identifying plants with medicinal properties. Tribal and indigenous communities in India have traditionally utilized medicinal plants to treat diseases and infections. By documenting this traditional knowledge, researchers can systematically identify plant species with potential antibacterial properties. This information increases the possibility of discovering bioactive compounds with pharmacological potential because it is grounded in extensive practical use. This is supported by recent research from India. For instance, Himalayan ethnomedicinal herbs can support sustainable livelihoods in addition to treating diseases.[1] A comprehensive investigation

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of plants used in Indian traditional medicine revealed that several of them have antibacterial properties against drug-resistant infections caused by multidrug-resistant (MDR) pathogens. These studies show that ethnobotanical knowledge remains an essential part of drug discovery.[2]

Plants naturally contain chemical molecules called phytochemicals that give them their color, flavor, and disease resistance. It has been demonstrated that phytochemicals provide an extensive range of health benefits and are important for human health.[3] Considering the concerning increase in antimicrobial resistance (AMR), infectious diseases caused by pathogenic microorganisms continue to pose a serious threat to global health. Novel antimicrobial drugs with different modes of action are urgently needed, as many bacteria are becoming resistant to traditional antibiotics.[4] In this regard, because of their chemical diversity and long history of use in traditional medical systems worldwide, plants have become a promising source of novel bioactive compounds.[5]

Plants used in traditional medicine contain secondary metabolites such as terpenoids, alkaloids, flavonoids, tannins, and phenolic compounds. These compounds possess antibacterial properties that act in several ways, including breaking down microbial cell membranes, inhibiting nucleic acid synthesis and deactivating microbial enzymes. Plant-based antimicrobials may be less likely to cause resistance than conventional antibiotics because they target multiple pathways.[6] The integration of ethnobotanical knowledge with modern microbiological methods has become increasingly significant as a strategic approach to plant-based antimicrobial screening in recent years. This method facilitates the protection of indigenous knowledge and biodiversity while supporting the scientific validation of traditional practices.

Gynecological infections, including urinary tract infections (UTIs) and bacterial vaginosis, are commonly associated with pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus*. These infections significantly impact women's reproductive health and require effective antimicrobial management, highlighting the need to explore plant-based therapeutic alternatives. *E. coli* is a major causative agent of UTIs, while *S. aureus* is frequently linked to vaginal and opportunistic infections; therefore, these pathogens were selected as representative organisms to evaluate the antibacterial potential of the selected medicinal plants.

The evaluation of antimicrobial activity is crucial for the discovery of new therapeutic agents, particularly in the face of increasing antibiotic resistance. Antimicrobial assays test the ability of a compound, extract or substance to inhibit the growth of or kill pathogenic microorganisms such as bacteria. The antimicrobial effects of medicinal plants traditionally used by tribal populations in Dharampur Taluka of Gujarat, India, are the focus of this study. To treat gynecological diseases, these communities rely on herbal remedies. Plant samples were selected based on interviews with traditional healers and ethnobotanical surveys. Standard antimicrobial assays, including the agar well diffusion method, were used to evaluate the antibacterial activity of crude methanolic extracts against *E. coli* and *S. aureus* bacterial strains.[7]

MATERIALS AND METHODS

Collection of Plant Material

Medicinal plants traditionally used by tribal communities for the treatment of gynecological diseases were studied and documented. A total of twelve plant species belonging to different families were collected in the winter season (January) from Dharampur (20.5401° N, 73.1792° E), Gujarat, India. For phytochemical analysis, the collected plant samples were thoroughly washed with distilled water and dried under shade conditions. The shade-dried material was then used for the extraction of crude phytochemicals using the Soxhlet extraction method.[8]

Extraction of crude phytochemicals

The crude methanolic extract was prepared by extracting 10 g of dried plant powder in 100 mL of methanol (Loba Chemie Pvt. Ltd., India) using a Soxhlet apparatus (Borosil Scientific Ltd., India) for 24 hours. The extract was filtered and dried, and the dried residue was stored at 4°C in airtight bottles.[9]

Well diffusion assay

Luria Bertani (LB) agar (Himedia Laboratories) containing 3% agar was prepared in distilled water and poured into sterile Petri plates. The plates were allowed to cool for agar

solidification. Top agar was prepared separately with 1% agar using the same medium. Culture suspensions of *E. coli* ATCC 23716 and *S. aureus* ATCC 6538 were activated in LB broth (Himedia Laboratories).[10] Then, 100 ìL of each culture was mixed with the top agar and poured over the LB plates, which were allowed to solidify. Wells were punched (4 mm diameter) in the agar plates using a sterile cork borer and 50 ìL of each plant extract at four different concentrations (0.25, 0.50, 0.75, and 1.00 mg/mL) were loaded into the wells. The plates were incubated at 37°C overnight in an incubator (REMI, India). The next day, the zones of inhibition (ZOI) observed around the wells were measured in millimeters (mm). The same procedure was followed using amikacin as the standard antibiotic for comparison. All experiments were performed in triplicate, and results are expressed as mean ± standard deviation. Statistical analysis was performed using one-way analysis of variance (ANOVA) to evaluate differences between concentrations. A p-value of less than 0.05 was considered statistically significant.[11,12]

RESULTS

Methanolic extracts of medicinal plants traditionally used for the treatment of gynecological diseases were evaluated for antibacterial activity against *E. coli* ATCC 23716 and *S. aureus* ATCC 6538. The antibacterial potential of the selected medicinal plant extracts against both *E. coli* and *S. aureus* was confirmed using the agar well diffusion method.

Table 1 presents the antibacterial activity of twelve plant extracts evaluated at four different concentrations against *E. coli*. A concentration-dependent increase in antibacterial activity was observed in the tested strains. The methanolic extract of *Pterocarpus marsupium* (Roxb.) exhibited the highest antibacterial activity, with a zone of inhibition of 6±0 mm against *E. coli* ATCC 23716 (Figure 2). In contrast, *Buchanania cochinchinensis*, *Dregea volubilis*, *Pueraria tuberosa*, and *Piliostigma malabaricum* showed no inhibition even at higher concentrations. The antibacterial activity of the plant extracts remained lower than that of the standard antibiotic amikacin, which showed ZOI ranging from 9-14 mm against *E. coli* ATCC 23716 (Figure 1). One-way ANOVA revealed that the increase in antibacterial activity with increasing concentrations was statistically significant ($p < 0.05$) for the active plant extracts (*Pterocarpus marsupium*, *Schleichera oleosa*, and *Prosopis cineraria*).

Table 1. Antibacterial activity of methanolic extracts of selected medicinal plants against *E. coli* ATCC 23716 at different concentrations determined by agar well diffusion method

Sr. no	Name of Plant	Concentration in mg/mL				
		Control (Methanol)	0.25	0.50	0.75	1
		Inhibition in mm (mean ± SD (n = 3))				
1	Amikacin (Antibiotic)	0	9±0	12±0.6	13±0	14±0.6
2	*Pterocarpus marsupium* (Roxb.)	0	1±0	3±0	5±0.6	6±0
3	*Boerhavia verticillate* Poir.	0	1±0	2±0	2±0.6	2±0
4	*Schleichera oleosa* (Lour.)	0	3±0	3±0.6	4±0	5±0
5	*Prosopis cineraria* (L.)	0	1±0	2±0	3±0	5±0.6
6	*Buchanania cochinchinensis* (Lour.)	0	0	0	0	0
7	*Dregea volubilis* (L.f.) Benth. ex-Hook f.	0	0	0	0	0
8	*Pueraria tuberose* (Roxb.)	0	0	0	0	0
9	*Piliostigma malabaricum* (Roxb.)	0	0	0	0	0
10	*Ougeinia dalbergioides* (Benth.)	0	0	1±0	1±0	1±0
11	*Millettia racemose* (Roxb.) Benth.	0	0	0	1±0	2±0
12	*Helicteres isora* (Linn.)	0	0	0	2±0	3±0
13	*Sterculia villosa* Roxb.	0	0	0	1±0	2±0

Note: Values are expressed as mean ± SD (n = 3). Statistical significance was determined using one-way ANOVA ($p < 0.05$).

Table 2 presents the antibacterial activity of plant extracts against *S. aureus* ATCC 6538. A concentration-dependent increase in antibacterial activity was also observed against this strain. The methanolic extract of *Buchanania cochinchinensis* exhibited the highest antibacterial activity, with a 9±0 mm zone of inhibition (Figure 4), whereas *Boerhavia verticillata* Poir. and *Ougeinia dalbergioides* showed the lowest activity, with a 2±0 mm zone of inhibition. The antibacterial activity of all plant extracts remained lower than that of the standard antibiotic amikacin, which exhibited zones of inhibition ranging from 10-19 mm against *S. aureus* (Figure 3). One-way ANOVA analysis indicated that the antibacterial activity increased significantly with increasing concentrations for most of the tested plant extracts, including *Buchanania cochinchinensis*,

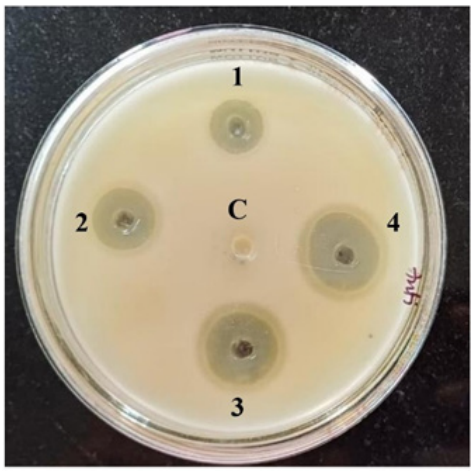

Fig. 1. Inhibition of *E.coli* ATCC 23716 by amikacin using the agar well diffusion assay

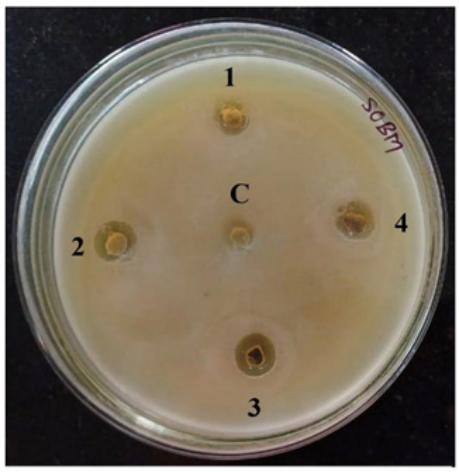

Fig. 2. Antibacterial activity of Pterocarpus marsupium (Roxb.) against *E.coli* ATCC 23716

Table 2. Antibacterial efficacy of methanolic extracts from selected medicinal plants against *S. aureus* ATCC 6538 at varying concentrations, evaluated using the agar well diffusion method.

Sr. no	Name of Plant	Concentration in mg/mL				
		Control (Methanol)	0.25	0.5	0.75	1
		Inhibition in mm				
1	Amikacin (Antibiotic)	0	10±0.6	14±0	16±0.6	19±0
2	*Prosopis cineraria* (L.)	0	1±0	3±0	5±0	4±0.6
3	*Boerhavia verticillate* Poir.	0	0	0	2±0	2±0
4	*Buchanania cochinchinensis* (Lour.)	0	4 ±0	5±0.6	7±0	9±0
5	*Dregea volubilis* (L.f.) Benth. ex-Hook f.	0	0	0	2±0	4±0
6	*Helicteres isora* (Linn.)	0	1±0	3±0	5±0	6±0
7	*Millettia racemose* (Roxb.) Benth.	0	0	2±0	3±0	3±0
8	*Ougeinia dalbergioides* (Benth.)	0	0	0	1±0	2±0
9	*Piliostigma malabaricum* (Roxb.)	0	3±0	4±0	5±0	6±0
10	*Pterocarpus marsupium* (Roxb.)	0	3±0	4±0	4±0	5±0
11	*Pueraria tuberose* (Roxb.)	0	0	2±0	3±0	5±0
12	*Schleichera oleosa* (Lour.)	0	2±0	3±0	5±0	7±0
13	*Sterculia villosa* Roxb.	0	1±0	3±0	4±0	5±0

Note: Values are expressed as mean ± SD (n = 3). Statistical significance was determined using one-way ANOVA ($p < 0.05$).

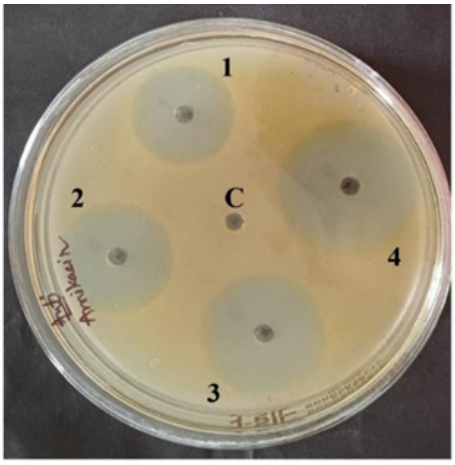

Fig. 3. Antibacterial effect of amikacin against *S. aureus* ATCC 6538

Fig. 4. Evaluation of the antibacterial activity of Buchanania cochinchinensis (Lour.) against *S. aureus* ATCC 6538

Helicteres isora, *Schleichera oleosa*, *Prosopis cineraria*, *Pterocarpus marsupium*, *Piliostigma malabaricum*, *Sterculia villosa*, and *Pueraria tuberosa* ($p < 0.05$).

DISCUSSION

The observed antibacterial activity of the methanolic plant extracts may be attributed to the presence of bioactive compounds such as flavonoids and phenolics, as identified in the phytochemical analysis. The strong antibacterial effect of *Pterocarpus marsupium* (Roxb.) supports its traditional use in treating bacterial infections.

These findings are consistent with previous reports. One study demonstrated that methanolic extracts of *Pterocarpus marsupium* exhibited antimicrobial activity against *Salmonella typhi* and *Enterococcus faecalis*, with MIC values of 12.5 µg/mL and 25 µg/mL, respectively.[13] Similarly, another study reported inhibitory effects of methanolic bark extracts of *Pterocarpus marsupium* against *S. aureus*, with zones of inhibition ranging from 15.86 to 22.50 mm.[14] Comparable antimicrobial activity against *E. coli* has also been reported for zinc oxide nanoparticles synthesized using *Pterocarpus marsupium*.[15] Additionally, stem wood extracts of *Pterocarpus marsupium* have shown activity against multiple pathogens, including *E. coli*.[16]

The antibacterial activity of *Buchanania cochinchinensis* against *S. aureus* observed in the present study aligns with earlier findings. Previous studies have demonstrated the antimicrobial potential of methanolic extracts from *Buchanania* species against *S. aureus*, indicating effective antibacterial properties.[17] A recent study further reported the significant inhibition of *S. aureus* by bark extracts of *Buchanania cochinchinensis*, with inhibition zones around 15 mm, supporting its traditional medicinal use.[18]

The concentration-dependent increase in antibacterial activity, supported by statistical analysis (one-way ANOVA, $p < 0.05$), indicates a dose-responsive effect of the plant extracts. This suggests that the observed activity is not random but is associated with the increasing concentration of bioactive phytochemicals. The variation in antibacterial activity among different plant extracts may be attributed to differences in the type and concentration of phytochemical constituents.

The persistence of traditional medicinal practices highlights the strong link between indigenous health systems and women's reproductive well-being in rural India. Ethno-gynecological knowledge is commonly transmitted orally through midwives, elderly women, and traditional healers, ensuring the continuity of indigenous healthcare practices in regions with limited access to modern medical facilities. The

inhibition zones observed in this study confirm that the investigated plants possess promising antibacterial activity, validating their traditional use in the treatment of microbial infections. These results are consistent with ethnobotanical and pharmacological studies reporting that Indian medicinal plants contain bioactive phytochemicals such as flavonoids, alkaloids, phenols, and terpenoids responsible for antibacterial effects.[19]

This study provides preliminary insights based on in vitro antibacterial screening using crude methanolic extracts. However, further studies involving the determination of minimum inhibitory concentration (MIC), isolation and characterization of active compounds, and evaluation using clinical isolates and in vivo models are required to validate and extend these findings.

CONCLUSION

Among the twelve medicinal plants evaluated in this study for their antimicrobial activity against *E. coli* ATCC 23716 and *S. aureus* ATCC 6538 using the agar well diffusion method, *Pterocarpus marsupium* and *Buchanania cochinchinensis* emerged as the most effective. The methanolic extract of *Pterocarpus marsupium* exhibited a maximum zone of inhibition of 6 mm against *E. coli*, whereas *Buchanania cochinchinensis* showed a 9 mm inhibition zone against *S. aureus*. These results indicate that both plants possess broad-spectrum antibacterial properties, likely attributable to the presence of phytochemicals such as flavonoids, alkaloids, tannins, and saponins. *Pterocarpus marsupium* and *Buchanania cochinchinensis* represent promising candidates for further investigation, including the isolation of active compounds and evaluation through in vivo and clinical studies. Their antibacterial activity, particularly against clinically relevant pathogens like *E. coli* and *S. aureus*, underscores the value of ethno-gynecological knowledge and highlights the importance of these species in future phytopharmaceutical research.

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Conflict of interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Permission to reproduce material from other sources

Not Applicable.

AUTHOR CONTRIBUTIONS

Samta Mahyavanshi: Writing-Original Draft Preparation, Methodology, Data Collection, Visualization; Saklain Mustak Saiyad: Writing-Review & Editing; Nisha Daxini: Supervision, Data verification; Hiren Soni: Supervision, Validation, Writing-Review & Editing.

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