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COMPARATIVE IN VITRO ANTIBACTERIAL ACTIVITY OF AQUEOUS LEAF EXTRACTS OF THREE MEDICINAL PLANTS AGAINST A PREGNANCY-ASSOCIATED CLINICAL ISOLATE OF PROTEUS VULGARIS

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ABSTRACT

Urinary tract infection (UTI) during the third trimester of pregnancy is frequently caused by multidrug-resistant organisms for which the range of safe antibiotics is narrow. This study evaluated the in vitro antibacterial activity of aqueous leaf extracts of *Magnolia champaca*, *Ehretia laevis* Roxb. And *Santalum album* against a clinical isolate of *Proteus vulgaris* recovered from the urine of symptomatic third-trimester pregnant women. The isolate was identified to genus level by cultural, morphological and biochemical characteristics. Extracts were prepared by decoction, screened qualitatively for alkaloids and flavonoids, and tested by agar well diffusion at 50, 100, 150 and 200 mg/mL against gentamicin as positive control and sterile distilled water as negative control. Alkaloids and flavonoids were detected in all three extracts. Inhibition increased with concentration for every extract; *E. laevis* Roxb. Produced the largest zones at all concentrations, reaching 21 mm at 200 mg/mL, close to the 22 mm zone of gentamicin, followed by *M. champaca* (14 mm) and *S. album* (13 mm). The aqueous leaf extract of *E. laevis* Roxb. Showed the strongest inhibition of this clinical uropathogen

among the three species tested and merits further phytochemical and safety evaluation before any clinical consideration.

KEYWORDS: Medicinal plants, Plant extracts, Phytochemical screening, Alkaloids, Flavonoids, Proteus sp., Antibacterial activity.

INTRODUCTION

Urinary tract infection is one of the most frequent bacterial illnesses of pregnancy, and its grip tightens as pregnancy advances. Hormonal relaxation of the urinary tract and mechanical compression of the bladder slow urine flow, allowing bacteria that would normally be flushed out to colonise the ascending tract (*Bakleezi et al., 2025*). The clinical reality behind this physiology is well documented: a prospective cohort of 536 pregnant women in Jordan recorded a 34.3% prevalence of UTI, with fewer than half of the affected women completing the antibiotics they were prescribed (*Bakleezi et al., 2025*). The pattern repeats on the Indian subcontinent. At a tertiary care centre in northern India, 64.6% of clinically suspected pregnant women were culture positive, and nearly half of the cases fell in the third trimester (*Varun et al., 2026*), while a hospital-based study in Jaipur found that almost three-quarters of culture-positive pregnant women carried asymptomatic bacteriuria, again peaking in the third trimester (*Saini et al., 2026*). The stakes are not merely symptomatic. Untreated bacteriuria in pregnancy is linked to preterm labour and low birth weight; in a Lahore case series, a quarter of infected pregnancies ended in preterm labour and a quarter in low-birth-weight deliveries (*Raza et al., 2025*).

Antibiotic treatment, the obvious answer, is quietly losing its power. A three-year retrospective analysis from Odisha traced a dramatic rise in antimicrobial resistance among uropathogens of pregnant women, from 14.2% in 2020 to 43.2% in 2023, with 71.6% multidrug resistance recorded for the ciprofloxacin-ceftriaxone-cefixime combination (*Sahu et al., 2026*). A tertiary hospital study elsewhere in India reported that nearly half of uropathogens isolated from pregnant women were multidrug resistant, with about three-quarters of isolates resistant to ampicillin and co-trimoxazole (*Garaga et al., 2025*). The picture is the same abroad: ampicillin resistance reached 72.7% among Gram-negative isolates from asymptomatic bacteriuria in Türkiye, prompting the authors to demand culture-guided empirical therapy (*Öztürk et al., 2025*). Pregnancy narrows the pharmacological escape routes further, because drugs such as trimethoprim are discouraged near term and fluoroquinolones are generally avoided (Association of Ontario Midwives, 2025). Together

these pressures leave clinicians in late pregnancy with a shrinking list of safe and still-effective agents, which is precisely the setting in which alternative strategies deserve serious testing.

Within this landscape, Proteus vulgaris deserves more attention than it usually receives. Proteus species cause a wide range of infections, and at a tertiary care centre in Puducherry, 76 Proteus isolates carried a 38.15% multidrug resistance rate, with resistance to cefepime exceeding 78% (*Eganathan & Manavalan, 2026*). Egyptian surveillance showed that Proteus accounted for one in five urinary isolates and that 78.6% of these strains were multidrug resistant, driven substantially by ESBL production (*Hassuna et al., 2025*). Of particular relevance to the present study, a hospital survey in Nepal identified P. vulgaris as the species with the highest multidrug resistance rate of any urinary isolate, at 46.15% (*Jha et al., 2026*). Nigerian work on women with UTI has linked P. vulgaris isolates to adhesion and ESBL-encoding plasmids, an arrangement that favours horizontal spread of resistance (*Muhammad et al., 2026*). Reviews of the genus stress that P. vulgaris is not only a common agent of cystitis and pyelonephritis but is also intrinsically less susceptible to several first-line agents than P. mirabilis (*Periferakis et al., 2025*), and that the species has accumulated beta-lactamase-mediated resistance mechanisms of every major class (*Bedenić et al., 2025*). We argue that a uropathogen with this resistance profile, isolated from a population whose safe drug list is already short, is a legitimate test case for non-antibiotic approaches.

Plant-derived antimicrobials are the most accessible of those approaches, and alkaloids and flavonoids sit at the centre of the evidence. Both classes act through multiple bacterial targets at once, damaging membranes and cell walls, disrupting nucleic acids and proteins, blocking efflux pumps and suppressing biofilm formation (*Akkalwar et al., 2026*). Recent reviews consistently identify alkaloids, flavonoids, tannins and saponins as the most frequently detected and most functionally promising phytochemicals in medicinal plants (Kumar, 2025), and well-designed extract studies now report dose-dependent inhibition of both Gram-positive and Gram-negative organisms (Naseer & Adil, 2025). The literature is also candid about its limits: most of this activity remains preclinical, and reviews of plant metabolites as next-generation antibacterial agents call repeatedly for MIC determination, compound isolation and safety work before clinical claims (Thawabteh et al., 2026; Zhou et al., 2026). Against that backdrop, three plants of the Indian tradition stand out as untested candidates for pregnancy-associated UTI. Magnolia champaca carries a long Ayurvedic record and a phytochemistry rich in alkaloids and flavonoids with demonstrated antimicrobial fractions (*Syahfitri et al., 2026*); Ehretia laevis Roxb. is a folk remedy whose ethyl acetate fraction is

rich in phenols, flavonoids and alkaloids and shows the lowest MIC values in extract screening (*Vivek et al., 2025*); and Santalum album has a traditional association with urinary complaints alongside documented antimicrobial characteristics (*Gürgen et al., 2025*). Earlier work on these species targeted oral flora, wound models or reference strains, not uropathogens recovered from pregnant women.

That gap defines this study. We isolated P. vulgaris from the urine of symptomatic third-trimester women, prepared alkaloid- and flavonoid-rich extracts of the three plants by maceration, infusion, decoction and digestion, and tested them against the clinical isolate. Our position is that locally available plants should be evaluated against the organisms that actually infect local pregnant women, because an extract that works on a laboratory strain tells us little about the pathogen a clinician will face. If these traditional plants can inhibit a multidrug-resistant uropathogen such as P. vulgaris, they earn a place in the pipeline of safe adjuncts for late-pregnancy UTI, where every effective and tolerable option matters.

MATERIAL AND METHOD

Sample collection: This laboratory-based study used urine specimens collected from third-trimester pregnant women presenting with symptoms suggestive of urinary tract infection. Midstream urine was collected aseptically in sterile, leak-proof containers, following the sampling approach used in comparable antenatal studies where symptomatic women supply clean-catch specimens for culture (*Garaga et al., 2025*). Samples were transported to the microbiology laboratory without delay, and where immediate processing was not feasible they were held at 4°C, since short-term refrigeration limits bacterial multiplication and preserves viable counts until plating.

Isolation and identification of the uropathogen: Urine samples were streaked onto MacConkey agar, a selective and differential medium commonly employed for the isolation and preliminary differentiation of Gram-negative bacteria. The bile salts and crystal violet present in the medium inhibit the growth of most Gram-positive organisms, whereas lactose and the pH indicator neutral red facilitate the differentiation of lactose-fermenting and non-lactose-fermenting bacteria. Acid production during lactose fermentation lowers the pH of the medium, resulting in pink to red colonies, while non-lactose-fermenting organisms generally produce colourless or translucent colonies (Jung & Hoilat, 2024; O'Hara *et al.,* 2000). The inoculated plates were incubated aerobically at 35–37°C for 18–24 h. Well-isolated colonies were selected and repeatedly subcultured to obtain pure bacterial cultures. Similar cultural

and isolation procedures have been reported in recent studies involving the recovery and characterization of Proteus species from clinical specimens (Rahman *et al.,* 2025; Jawad *et al.,* 2025).

The presumptive identity of the recovered isolate was established through a combination of macroscopic, microscopic and biochemical examinations. Macroscopic characterization involved recording colony morphology, including colour, size, shape, margin and elevation. Microscopic examination was performed using Gram staining to determine the Gram reaction, cellular morphology and arrangement. These cultural and microscopic characteristics are important preliminary criteria for the recognition of Proteus species (O'Hara *et al.,* 2000; Rahman *et al.,* 2025).

Further biochemical characterization included carbohydrate fermentation tests using glucose, lactose, maltose and fructose, together with Simmons' citrate utilization, urease, and methyl red (MR), Voges–Proskauer (VP), catalase and indole tests. Carbohydrate fermentation was assessed by observing the characteristic changes in the respective fermentation media, while citrate utilization, urease activity and the other biochemical reactions were interpreted according to their specific colour changes or enzymatic responses. The selection of these biochemical tests was based on established phenotypic approaches for differentiating members of the genus Proteus (Senior, 1997; O'Hara *et al.,* 2000). Recent investigations have also employed combinations of Gram staining, carbohydrate fermentation, citrate utilization, urease, catalase, and MR, VP and indole tests during the characterization of Proteus isolates (Rahman *et al.,* 2025; Jawad *et al.,* 2025). The collective cultural, microscopic and biochemical characteristics were considered for the presumptive identification of the recovered isolate as *Proteus vulgaris.*

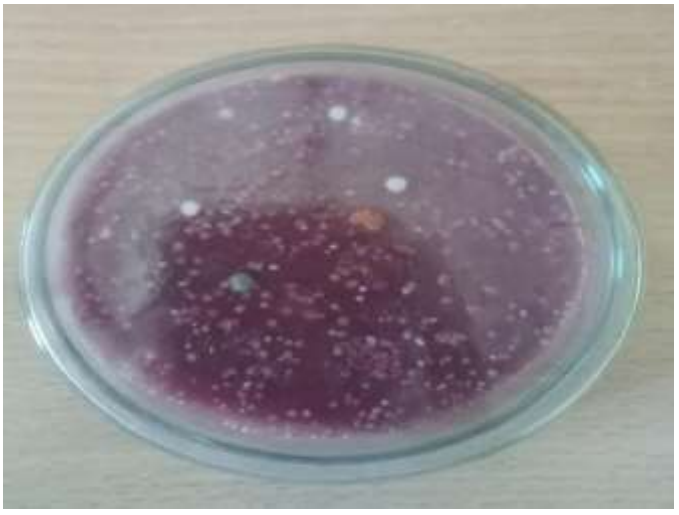

Figure 1. Isolation of bacterial isolates from urine samples on MacConkey agar.



Figure 2. Pure culture of *Proteus vulgaris* isolated from a urine sample and maintained on MacConkey agar.

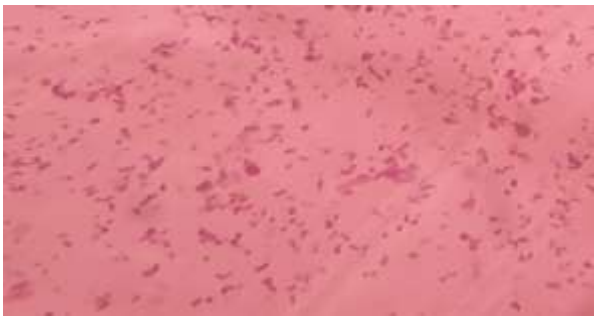

Figure 3. Gram staining characteristics of the isolated bacterial strain showing Gram-negative rod-shaped cells under 100X Lens of Compound Microscope.

Table 1. Cultural, colony morphological and microscopic characteristics of the bacterial isolate.

Size	Shape	Colour	Consistency	Opacity	Margin	Elevation	Gram staining	Motility
0.5 To 0.6 mm	Irregular	Pale	Moist	Opaque	Irregular	Slightly raised	Gram – ve Bacilli	Motile

Table 2. Biochemical Characterization of bacterial isolate.

Biochemical Test	Result
Glucose fermentation	+
Lactose fermentation	–
Maltose fermentation	+
Fructose fermentation	+
Simmons citrate test	–
Urease test	+
Methyl Red (MR) test	+
Voges–Proskauer (VP) test	–
Catalase test	+
Indole test	+
Phenylalanine deaminase test	+
Sucrose fermentation	–
Gelatin hydrolysis test	+
Glucose fermentation	+

Sign: + = Positive, - = Negative

Preparation of plant extracts: **Preparation of plant extracts:** Fresh leaves of *Magnolia champaca*, *Ehretia laevis* Roxb. and *Santalum album* were collected separately, inspected to remove damaged material, washed with running and then distilled water, and shade-dried at room temperature (25–30°C) to constant weight before grinding to a coarse uniform powder, a preparative sequence routinely adopted for leaf-derived extracts (Sayeed & Goyal, 2026). Fifty grams of each powdered leaf were decocted with 500 mL of distilled water (1:10 w/v) in covered conical flasks. The mixtures were gently boiled on a controlled hot plate at approximately 100°C for 30 min, maintaining continuous simmering conditions in line with standard decoction protocols, a method commonly employed to extract water-soluble bioactive constituents such as alkaloids and flavonoids through prolonged heating in an aqueous medium (Royani *et al.,* 2024; Putri *et al.,* 2026). Decoction was chosen deliberately: comparative work on plant material shows that heat-assisted aqueous extraction recovers a markedly higher yield of water-soluble phytoconstituents than cold maceration under the same solvent (Anwar, 2026). Each decoction was allowed to cool to room temperature, first strained through sterile muslin cloth and then filtered through Whatman No. 1 paper, and the
filtrates were concentrated on a water bath at approximately 40°C to obtain a thick aqueous residue, a temperature-controlled concentration step used to minimise thermal degradation of phytochemicals (Sayeed & Goyal, 2026). The crude aqueous extracts were stored in amber airtight containers at 4°C and reconstituted to the required strength with distilled water before each assay.

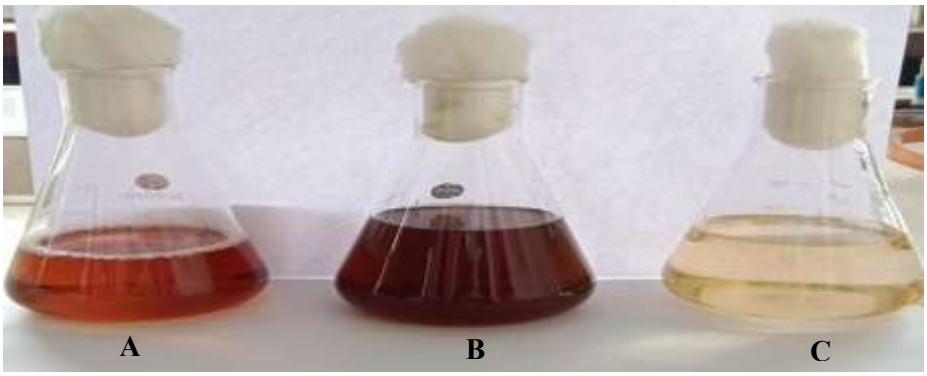

Figure 4. **Preparation of medicinal plant extracts: (A) *Magnolia champaca*, (B) *Ehretia laevis Roxb*. And (C) *Santalum album.***

Phytochemical screening

The concentrated extracts of *Magnolia champaca*, *Ehretia laevis* Roxb. and *Santalum album* were screened qualitatively for alkaloids and flavonoids using established colourimetric and precipitation assays. Each plant extract was analysed independently, and where applicable the reactions were run alongside positive and negative controls to confirm that colour development or precipitate formation arose from the extract constituents rather than from the reagents themselves. A visible colour change or the formation of a characteristic precipitate

was recorded as a positive result for the corresponding phytochemical class, following the reagent-based screening framework applied in recent phytochemical studies (So'ad & Mokthar, 2026; Putri *et al.,* 2026).

Detection of alkaloids

Alkaloids were detected using Dragendorff's reagent. Briefly, 1 mL of the concentrated extract was transferred to a clean test tube and acidified with a small volume of dilute hydrochloric acid to ensure protonation of the alkaloid nitrogen atoms. The mixture was stirred gently and, where turbidity interfered with observation, clarified by filtration. A few drops of freshly prepared Dragendorff's reagent were then added to the filtrate. The appearance of an orange, reddish-orange or orange-brown precipitate was taken as a positive reaction. The reaction proceeds through the formation of relatively insoluble ion-pair complexes between the protonated alkaloids and the bismuth–iodide reagent, which precipitate as the characteristic coloured solid (Sayeed & Goyal, 2026). Because a single colour reaction can occasionally give ambiguous results, the finding was cross-checked with Mayer's test, in which a few drops of Mayer's reagent were added to a further 1 mL aliquot of the acidified extract; the development of a cream or whitish precipitate was interpreted as a second positive indication of alkaloids. Running two independent confirmatory reactions increases confidence in the assignment, since concordant results across different reagents make a false positive less likely (Anwar, 2026; So'ad & Mokthar, 2026).

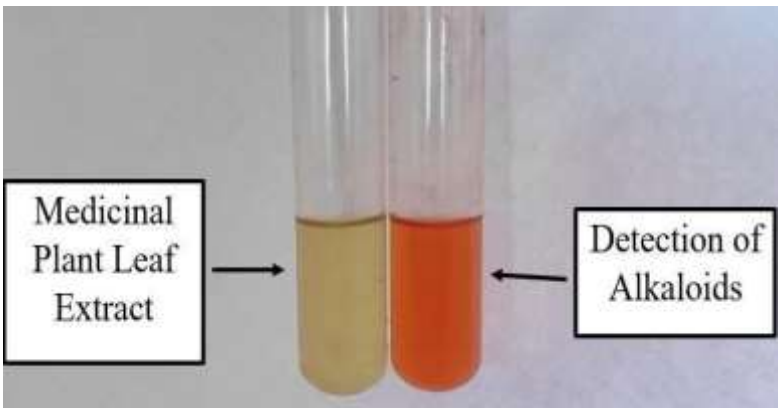

Figure 5. Qualitative phytochemical screening of *Ehretia laevis Roxb.* Showing the presence of alkaloids.

Detection of flavonoids

Flavonoids were evaluated using the Shinoda test. In this assay, 1 mL of plant extract was placed in a clean test tube together with a small quantity of magnesium turnings or magnesium powder, after which a few drops of concentrated hydrochloric acid were added

with care. The appearance of a pink, reddish-pink, crimson or reddish colour was considered a positive reaction. The colour develops because the acidic reducing conditions convert specific flavonoid structures to their corresponding anthocyanidin-like products, which display the characteristic red to pink chromophores (Putri *et al.,* 2026). The presence of flavonoids was further corroborated by the alkaline reagent test. Approximately 1 mL of extract was treated with a few drops of dilute sodium hydroxide solution, producing a yellow colour consistent with flavonoid content. Subsequent acidification with dilute hydrochloric acid caused the yellow colour to fade gradually or disappear entirely, and this reversible colour shift was read as confirmatory evidence for flavonoids, in keeping with the paired alkaline and acidification assays used in current screening protocols (Sayeed & Goyal, 2026; Royani *et al.,* 2024).

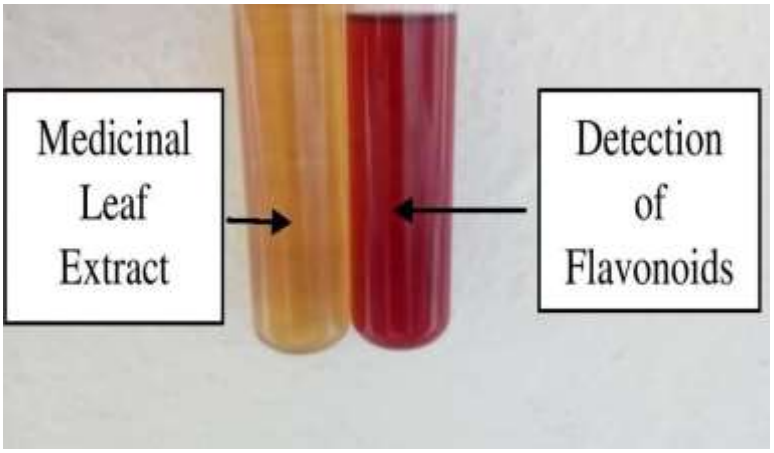

Figure 6. Qualitative phytochemical screening of *Ehretia laevis Roxb*. Showing the presence of flavonoids.

Antibacterial activity assay: The antibacterial activity of each plant extract against the clinical isolate was assessed using the agar well diffusion method, a widely used approach for evaluating plant-derived antibacterial activity against clinical bacterial isolates (Salam *et al.,* 2025; Islam *et al.,* 2026). The aqueous extracts were tested at concentrations of 50, 100, 150 and 200 mg/mL, covering a concentration range comparable to those reported in previous studies on leaf extracts (Udoidiong *et al.,* 2026; Ewa Ibe *et al.,* 2026). Distilled water, used for extract preparation, served as the negative control to assess any possible inhibitory effect of the solvent itself. Following incubation under standard conditions, the inhibition zones produced by each extract were measured in millimetres. The antibacterial activity of all three plant extracts was evaluated at each concentration to examine differences in their inhibitory effects across the tested concentration range (Alghanma *et al.,* 2026).

Table 2. Antibacterial activity of selected medicinal plant extracts against the bacterial isolate at different concentrations, determined by measuring the zone of inhibition (mm).

Sr. No.	Plant Name	Concentration (mg/mL)	Zone of Inhibition (mm)
1	*Magnolia champaca*	50	8
2	*Magnolia champaca*	100	10
3	*Magnolia champaca*	150	12
4	*Magnolia champaca*	200	14
5	*Ehretia laevis Roxb.*	50	12
6	*Ehretia laevis Roxb.*	100	16
7	*Ehretia laevis Roxb.*	150	19
8	*Ehretia laevis Roxb.*	200	21
9	*Santalum album*	50	7
10	*Santalum album*	100	9
11	*Santalum album*	150	11
12	*Santalum album*	200	13

Positive Control Gentamycin: 22 mm Zone of Inhibition Negative Control Sterilized D/W: No Zone Formation

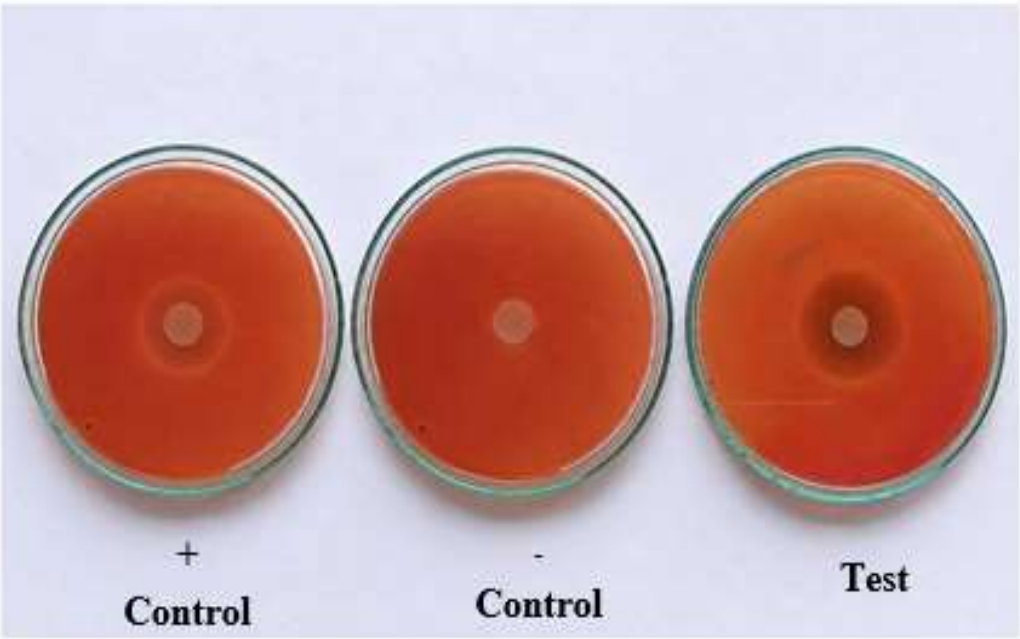

Figure 7. Antibacterial activity of *Ehretia laevis Roxb.* Extracts against Proteus vulgaris at different concentrations, using Gentamycin as Positive Control and distilled water as a negative control.

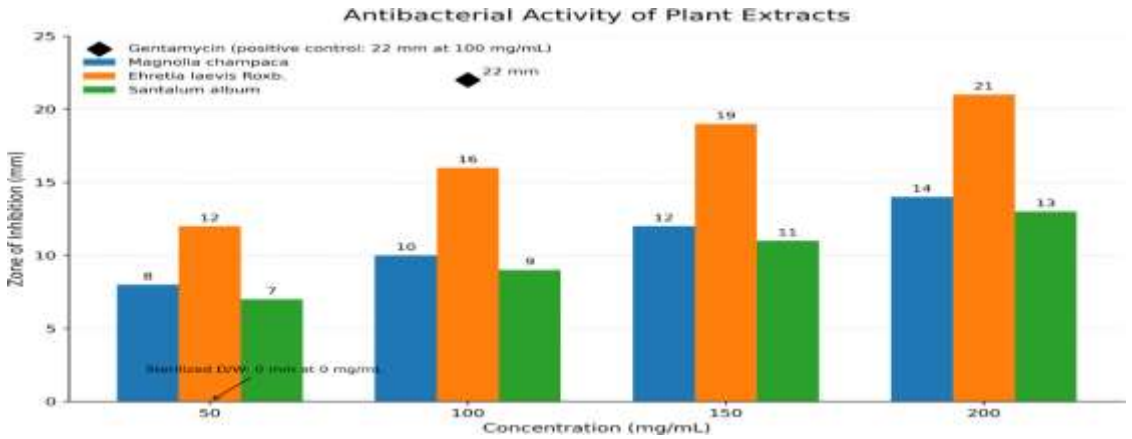

Figure 8. Comparative antibacterial activity of *Magnolia champaca*, *Ehretia laevis* Roxb. and *Santalum album* extracts against the test bacterial pathogen at different concentrations (50, 100, 150 and 200 mg/mL), expressed as zone of inhibition (mm). Gentamycin and sterilized distilled water were used as positive and negative controls, respectively.

Among the three medicinal plants investigated the aqueous leaf extract of *Ehretia laevis* Roxb. Showed the most promising antibacterial activity against *Proteus vulgaris*. At 200 mg/mL, it produced an inhibition zone of 21 mm, whereas the extracts of *Magnolia champaca* and *Santalum album* produced zones of 14 mm and 13 mm, respectively. The comparatively larger inhibition zone observed with *E. laevis* indicates that its aqueous leaf extract was more effective against the test organism under the conditions used in this study. This finding is of interest because recent research has also highlighted the pharmacological potential of plants belonging to the genus *Ehretia*. Jiang et al. (2025), in their review of the genus, described its diverse phytochemical constituents and reported several biological activities, including antibacterial effects. More recently, Mekonnen et al. (2026) demonstrated dose-dependent antibacterial activity of an 80% methanolic leaf extract of *Ehretia cymosa*, another member of the same genus. Although their study involved a different species and extraction solvent, it provides further evidence of the antibacterial potential found within the genus. Similarly, (Dotulong *et al.,* 2026) reported antibacterial activity and flavonoid content in a boiling-water extract of *Sonneratia alba* leaves, supporting the value of aqueous extraction for recovering biologically active plant constituents. The present findings therefore add to the available evidence on the medicinal potential of *Ehretia laevis*, particularly its activity against *P. vulgaris*. Its stronger response compared with the other two plant extracts makes it a suitable candidate for further investigation, including minimum inhibitory concentration testing, chemical characterization and identification of the

compounds responsible for its antibacterial effect.

RESULT

Urine specimens from symptomatic third-trimester pregnant women yielded pale, non-lactose-fermenting, irregular, slightly raised colonies with moist consistency and irregular margins on MacConkey agar (Figure 1). Gram staining showed Gram-negative, motile bacilli (Figure 3; Table 1). Biochemical profiling returned positive reactions for glucose, maltose and fructose fermentation, urease, methyl red, catalase, indole, phenylalanine deaminase and gelatin hydrolysis, and negative reactions for lactose and sucrose fermentation, Simmons citrate and Voges-Proskauer (Table 2). Taken together, these cultural, microscopic and biochemical characteristics support the presumptive identification of the isolate as *Proteus vulgaris*. Confirmatory testing by 16S rRNA sequencing or matrix-assisted laser desorption/ionization time-of-flight mass spectrometry was not performed.

Qualitative screening of the aqueous leaf extracts of *M. champaca*, *E. laevis* Roxb. And *S. album* detected alkaloids and flavonoids in all three extracts. Alkaloids produced an orange-brown precipitate with Dragendorff's reagent and a cream-colored precipitate with Mayer's reagent (Figure 5). Flavonoids produced a pink to reddish coloration in the Shinoda test and a reversible yellow color shift in the alkaline reagent test (Figure 6). Both phytochemical classes were therefore present in every plant tested, although the screening was qualitative and no attempt was made to quantify their abundance.

All three aqueous leaf extracts inhibited the clinical *P. vulgaris* isolate in a concentration-dependent manner between 50 and 200 mg/mL (Table 3, Figure 7). *E. laevis* Roxb. Was the most active extract at every concentration, producing zones of 12, 16, 19 and 21 mm at 50, 100, 150 and 200 mg/mL, respectively *M. champaca* produced zones of 8, 10, 12 and 14 mm, and *S. album* produced zones of 7, 9, 11 and 13 mm over the same range. Gentamicin, included as positive control, produced a 22 mm zone, while sterile distilled water, the negative control, produced no inhibition. At the highest extract concentration, the zone produced by *E. laevis* Roxb. (21 mm) approached that of gentamicin (22 mm) and exceeded the zones of *M. champaca* (14 mm) and *S. album* (13 mm). Even at the lowest concentration tested (50 mg/mL), *E. laevis* Roxb. Produced a zone (12 mm) larger than the maximum zones of *M. champaca* or *S. album* at 200 mg/mL. These values represent single-measurement diameters without replicate means or standard deviations.

DISCUSSION

The aqueous leaf extracts of all three plants inhibited the clinical *P. vulgaris* isolate in a concentration-dependent manner, and *E. laevis* Roxb. Was consistently the most active. The monotonic increase in zone diameter with concentration across all three extracts is consistent with a dose-response relationship between extract concentration and antibacterial effect over the 50 to 200 mg/mL range tested. Even the weaker extracts produced zones at the lowest concentration, indicating that the inhibitory constituents in all three leaves are water-soluble and survive decoction, filtration and the temperature-controlled concentration step used here. The comparatively higher activity of *E. laevis* Roxb. Is the central finding of this work, and several explanations can beadvanced without overclaiming. First, the qualitative phytochemical profile of this species documented in earlier work is broader than that of the other two plants tested here. *E. laevis* is reported to contain pentacyclic triterpenoids, phenolic acids, flavonoids, steroids and alkaloids alongside the two classes screened in the present study (Sharma *et al.,* 2021). In contrast, the antimicrobial activity of *M. champaca* has been attributed largely to specific constituents such as flavonoids, terpenoids and phenolic compounds (Shankar *et al.,* 2023; Anusha & Manogem, 2024), while the biological activity of *S. album* has been linked mainly to its aromatic and phenolic constituents (Gürgen *et al.,* 2025). Second, the ethyl acetate fraction of *E. laevis* leaves has previously shown the strongest antibacterial activity among screened solvent fractions of that species, with strong in vitro and in silico antibacterial signals (Vivek *et al.,* 2025), and related species such as *Ehretia cymosa* have demonstrated dose-dependent in vitro and in vivo antibacterial activity (Mekonnen *et al.,* 2026). Third, the genus *Ehretia* is reported to contain a diverse array of phenylpropanoids, flavonoids and triterpenoids with documented antibacterial potential (Jiang *et al.,* 2025). The larger zones produced by *E. laevis* Roxb. May therefore reflect a richer or more complementary mixture of active constituents within the same aqueous fraction rather than a single uniquely potent compound.

The proximity of the *E. laevis* Roxb. Zone (21 mm at 200 mg/mL) to the gentamicin zone (22 mm) deserves cautious interpretation. Agar well diffusion zones are not directly comparable to disk diffusion zones because diffusion characteristics differ between a liquid extract in a well and an antibiotic impregnated in a disk, and crude aqueous extracts contain a heterogeneous mixture of constituents whose diffusion and stability differ from those of a purified antibiotic. It would therefore be premature to infer equivalence in antibacterial potency from the numerical proximity of the two values. The comparison does, however, indicate that the crude extract inhibited the clinical isolate under the same incubation

conditions, and it provides a within-study benchmark that most plant-extract studies omit. In a recent comparative study of aqueous and alcoholic *Artemisia campestris* extracts against clinical uropathogens, plant extracts produced zones of up to 16.7 mm against *E. coli*, well below the activity of conventional antibiotics tested in parallel against the same isolates (Alghanma *et al.,* 2026). Similarly, a methanol leaf extract of *Sterculia tragacantha* produced zones of 10 to 13 mm against multidrug-resistant clinical isolates (Udoidiong *et al.,* 2026), and an aqueous *Salvia rosmarinus* extract produced only modest inhibition of clinical isolates including Proteus spp. (Al-Shalah *et al.,* 2024). Against these benchmarks the zones observed here for *E. laevis* Roxb., particularly the 21 mm zone at 200 mg/mL and the 12 mm zone at 50 mg/mL, place it in the moderate to strong range among crude plant extracts reported against Proteus spp., although the absence of replicate means and standard deviations in the present dataset limits the statistical weight of that comparison.

The choice of decoction as the extraction method is relevant to interpreting the results. Decoction is a traditional, solvent-free method that recovers water-soluble constituents, and recent comparative work supports its efficiency: decoction of *Ephedra fragilis* yielded higher antioxidant capacity than infusion of the same plant (Abdou-*Allah et al.,* 2026), and a review of decoction as an extraction method concluded that it recovers flavonoids, tannins, polyphenols, alkaloids, saponins and terpenoids efficiently from medicinal plants (Ikfar *et al*., 2026). By contrast, a review of modern extraction technologies notes that non-polar and intermediate-polarity solvents and techniques such as ultrasound-assisted or pressurized extraction can recover a broader or more targeted range of metabolites from the same plant material (Travis *et al.,* 2026). Decoction may therefore under-recover lipophilic constituents that a non-polar or intermediate-polarity solvent would extract, meaning the activity observed here is likely a conservative estimate of what these plants could produce under optimized extraction conditions.

The three plants differ in the extent of prior study, and the present findings add to each in a different way. *M. champaca* has an established ethnomedicinal record, and its leaves have been shown by qualitative and GC-MS/MS analysis to contain alkaloids, flavonoids, terpenes, glycosides, phenols, steroids, tannins and saponins (Shankar *et al.,* 2023), with phytochemical reviews documenting antimicrobial potential in its ethyl acetate and n-butanol fractions (Syahfitri *et al.,* 2026). *S. album* is traditionally associated with urinary complaints, and its antimicrobial and antioxidant properties are documented in recent reviews (Gürgen *et al.,* 2025), with direct evidence of antibacterial activity from sandalwood leaf extracts against *E. coli* and *S. aureus* (Amiroh *et al*., 2025). *E. laevis* is a less-studied folk remedy whose

leaves have been used for oral antisepsis (Kamble et al., 2022) and wound healing (Harne *et al.,* 2025), and whose phytochemistry, although documented, remains incompletely characterized. The activity observed here against a clinical uropathogen adds a new dimension to the existing literature on all three species, which has largely targeted oral flora, wound models or reference strains rather than uropathogens recovered from pregnant women. A limitation of the present study is that the bacterial identification was presumptive, based on cultural, morphological and biochemical characteristics alone, and was not confirmed by molecular methods. A second limitation is that the extracts were tested as crude aqueous preparations without fractionation, so the observed activity cannot be attributed to specific compounds. A third limitation is that the phytochemical screening was qualitative rather than quantitative, so the relative abundance of alkaloids and flavonoids in each extract is unknown. Finally, the study did not evaluate cytotoxicity, hemocompatibility or any in vivo safety profile, and the extracts should not be assumed safe for use during pregnancy on the basis of in vitro antibacterial activity alone. The clinical isolate came from a single laboratory workflow, and susceptibility testing against a broader antibiotic panel was not performed in parallel with the plant extracts.

The findings nevertheless have research significance. They provide preliminary evidence that locally available medicinal plants can inhibit a clinical *P. vulgaris* isolate recovered from a population in which safe and effective antibiotic options are limited, and they identify *E. laevis* Roxb. as the most promising of the three species for follow-up work. The results also add to a growing body of literature indicating that aqueous extracts of traditionally used plants retain measurable antibacterial activity against clinical isolates (Alghanma *et al.,* 2026; Udoidiong *et al.,* 2026). Within these constraints, the study offers a practical template for how locally available medicinal plants can be brought into preclinical evaluation against locally prevalent uropathogens.

CONCLUSION

This study set out to ask a simple but under-examined question: whether three medicinal plants rooted in Indian traditional practice, *Magnolia champaca*, *Ehretia laevis* Roxb. and *Santalum album*, could inhibit a uropathogen recovered not from a culture collection but from the urine of symptomatic women in late pregnancy. The answer, within the limits of an in vitro agar well diffusion assay, is affirmative. All three aqueous leaf extracts, prepared by decoction and screened positive for both alkaloids and flavonoids, suppressed the growth of the clinical *Proteus vulgaris* isolate in a concentration-dependent fashion across the 50 to 200

mg/mL range, while the negative control produced no inhibition and gentamicin, the positive control, produced a 22 mm zone.

The most consequential observation of the work is the consistent superiority of *E. laevis* Roxb. Its extract outperformed the other two species at every concentration tested, produced a 12 mm zone even at the lowest concentration employed, and reached 21 mm at 200 mg/mL, a value that sits within 1 mm of the gentamicin control under identical incubation conditions. This pattern held across the entire concentration series rather than emerging only at the maximum dose, which strengthens the interpretation that the difference between species is genuine and not an artifact of a single measurement point. Placed alongside recent comparative work on plant extracts against clinical uropathogens, where crude extracts commonly yielded zones well below those recorded here, the performance of *E. laevis* Roxb. Stands out among the species examined in this study and justifies its selection as the lead candidate for deeper investigation.

The contribution of this work to the wider literature lies in the population from which the test organism was drawn. Most earlier studies of these three species were conducted against oral flora, wound isolates or reference strains, whereas the isolate used here reflects the resistance background and clinical context of third-trimester pregnancy, a setting in which the list of pharmacologically acceptable antibiotics is unusually short. Testing traditional plants against a pathogen of this provenance, rather than a standard laboratory strain, brings the evaluation closer to the clinical question that motivates such screening in the first place, and it is this framing that the study offers as a reusable template for future work on locally available flora.

Several questions remain open, and they define the path forward. The precise constituents responsible for theobserved inhibition have not been isolated, and quantitative phytochemical profiling, chromatographic fractionation and spectroscopic identification of the active molecules in *E. laevis* Roxb. Are the logical immediate steps. The species-level identity of the isolate rests on phenotypic evidence and would benefit from molecular confirmation, and the safety profile of all three extracts, which remains entirely uncharacterized in this work, must be established before any consideration of therapeutic relevance, with particular caution in pregnancy. Within these boundaries, the present findings position *E. laevis* Roxb., with *M. champaca* and *S. album* as secondary candidates, as worthy subjects of the systematic natural-product characterization that the wider effort against drug-resistant urinary pathogens continues to demand.

REFERENCES

1. Abdou-Allah, F., Hmimid, F., Guenaou, I., Souidek, M., Ait Si, L., Ousaid, F. E., Nait Irahal, I., Errami, A., Kabine, M., & Bourhim, N. (2026). Exploring the phytochemical profile and antioxidant potential of *Ephedra fragilis*: A comparison of decoction and infusion extracts. *Natural Product Research*. https://doi.org/10.1080/14786419.2026.2640161
2. Akkalwar, D., Boriwar, S., & Devnani, D. (2026). Review on pharmacological insights & antimicrobial potential of medicinal plants. *Transformative Medical Materials, 1*(1), 100002. https://doi.org/10.1016/j.trmm.2026.100002
3. Alghanma, H., Agena, M., Saeed, A., Abuifalyjagh, A., & Naas, K. (2026). Comparative antibacterial activity of aqueous and alcoholic *Artemisia campestris* L.
4. extracts against clinical uropathogens. *Libyan Journal of Medical Research, 20*(3). https://doi.org/10.54361/ljmr.20.3.88
5. Al-Shalah, L. A. M., Mohammed, H. A., Omran, A. R., Althahab, A. O., & Gemeel, F. (2024). Evaluation of the active compound and antibacterial activity of a *Salvia rosmarinus* extract against pathogenic bacteria. *Review of Clinical Pharmacology and Pharmacokinetics, International Edition*. https://doi.org/10.61873/ixyc6187
6. Anusha, E., & Manogem, E. M. (2024). Extraction and characterization of bioactive compounds from plant, *Michelia champaca* (L) in polar solvents: GC-MS and antibacterial properties. *Economic Environmental Chemistry*. https://doi.org/10.53550/eec.2024.v30i05s.068
7. Anwar, Y. (2026). Effect of extraction methods on total alkaloid content of *Mimosa pudica* L. leaves. *Medical: Jurnal Kesehatan dan Kedokteran*. https://doi.org/10.69836/medical-jkk.vi.957
8. Association of Ontario Midwives. (2025). *Acute urinary tract infection (UTI) in pregnancy* [Clinical practice algorithm, updated July 2025]. https://www.ontariomidwives.ca
9. Bakleezi, A., Taybeh, E., Binodeh, A., Alsharif, A. A., Alhamed, M., & Naser, A. Y. (2025). Prevalence, risky behaviors, and antimicrobial resistance of urinary tract infections in pregnant women: A study in Jordan. *Medicine, 104*(17), e41986. https://doi.org/10.1097/MD.0000000000041986
10. Bedenić, B., Pospišil, M., Nađ, M., & Bandić Pavlović, D. (2025). Evolution of β-lactam antibiotic resistance in *Proteus* species: From extended-spectrum and plasmid-mediated AmpC β-lactamases to carbapenemases. *Microorganisms, 13*(3), 508.

https://doi.org/10.3390/microorganisms13030508

11. Dotulong, V., Nishani, F., Daliu, P., Damongilala, L. J., Lohoo, H. J., Salindeho, N., Wonggo, D., Santini, A., Romano, R., & De Luca, L. (2026). Aqueous leaves' extract of *Sonneratia alba*: Secondary metabolites with antibacterial activity for functional food applications. *Discover Applied Sciences, 8*, 124. https://doi.org/10.1007/s42452-026-08227-7
12. Eganathan, S., & Manavalan, J. (2026). Antimicrobial resistance patterns of *Proteus* species isolated from various clinical samples at a tertiary care hospital: A retrospective observational study. *Journal of Clinical and Diagnostic Research*. https://doi.org/10.7860/JCDR/2026/87530.24693
13. Ewa Ibe, C., Chukwu, C. N., Achi, N. K., Ibe, O. E., Pipi, O. G., Osodeke, S. C., Okoro-Akpandu, E., & Omodamiro, O. D. (2026). GC-MS profiling and solvent-dependent in vitro antibacterial activity of *Zingiber officinale* Roscoe rhizome extracts against clinical bacterial isolates. *Journal of Advances in Microbiology, 26*(11). https://doi.org/10.9734/jamb/2026/v26i111222
14. Garaga, G. S. V., Madda, D. P., Maninithya, K., & Dayan, A. D. (2025). Antibiotic resistance patterns in uropathogens isolated from pregnant women: A tertiary hospital study. *Journal of Pharmacy and Bioallied Sciences, 17*(Suppl. 4), S3039–S3041. https://doi.org/10.4103/jpbs.jpbs_995_25
15. Gürgen, A., Uysal, I., Ünlü, N., & Sevindik, M. (2025). The power of sandalwood (*Santalum album*): Industrial use, biological activity, and phytochemical contents. *Drewno: Prace Naukowe, Doniesienia, Komunikaty*. https://doi.org/10.53502/wood-202680
16. Harne, K., Tekade, P., Thakre, R., & Rathi, L. (2025). Isolation of effective wound healing compounds from chloroform fraction of ethanol extract of *Ehretia laevis* Roxb. (KhanduChakka/AjanVruksha). *International Journal of Ayurvedic Medicine, 16*(1). https://doi.org/10.47552/ijam.v16i1.5243
17. Hassuna, N. A., Kotb, D., Lami, M., & Abdelrahim, S. S. (2025). Characterization of antimicrobial resistance among *Proteus mirabilis* isolates from catheter-associated urinary tract infections and non-catheter-associated urinary tract infections in Egypt. *BMC Infectious Diseases, 25*(1), 767. https://doi.org/10.1186/s12879-025-11118-8
18. Ikfar, N. F., Aini, F. S. N., Ananta, S. Z., Khusnia, A. K., Mahmud, W., Rahma, N. F., Lestari, S. D., Putri, A. M. C., Chayani, A. M., Hisbiah, A., Klau, I. C. S., & Ningsih, (2026). Artikel review fitokimia: Efektifitas dekokta sebagai metode ekstraksi.

Jurnal Farmasi Syifa, 4(1), 65–72. https://doi.org/10.63004/jfs.v4i1.862

19. Islam, J., Hossain, A.-S., Sultana, S., Mitul, T. F., Alam, S., Karmakar, J., Jabin, T., & Uddin, M. A. (2026). Determination of antibacterial activity of natural products against clinical isolates from renowned diagnostic centers in Dhaka, Bangladesh. *Journal of Preventive, Diagnostic and Treatment Strategies in Medicine*. https://doi.org/10.4103/jpdtsm.jpdtsm_1_26
20. Jha, P., Barma, S., Jha, P. K., Sapkota, B., Chaudhary, S., Ghimire, B., Singh, A., Khakurel, B., Bidari, P., Pathak, P., & Pandey, B. (2026). Urine antibiogram and antimicrobial susceptibility pattern in a provincial hospital of Nepal: A cross-sectional study. *BMC Infectious Diseases, 26*(1). https://doi.org/10.1186/s12879-026-13781-x
21. Jiang, S., Wang, M., Kaur, A., Jiang, L., Cai, Y., Luo, J., Li, M., Wang, H., Wan, D., & Peng, Y. (2025). *Ehretia* genus: A comprehensive review of its botany, ethnomedicinal values, phytochemistry, pharmacology, toxicology and clinical studies. *Frontiers in Pharmacology, 16*, 1526359. https://doi.org/10.3389/fphar.2025.1526359
22. Jawad, M. M., Fayidh, M. A., & Obaid, A. S. (2025). UreC gene detection of *Proteus mirabilis* isolated and identified from patients with UTI. *Microbes and Infectious Diseases, 6*(4), 6689–6698. https://doi.org/10.21608/MID.2025.373132.2671
23. Jung, B., & Hoilat, G. J. (2024). MacConkey medium. In *StatPearls*. StatPearls Publishing. https://www.ncbi.nlm.nih.gov/books/NBK557394/
24. Kamble, S., et al. (2022). *Ehretia laevis* leaves: Potential herbal remedy for mouth microflora. *Indian Journal of Traditional Knowledge, 21*(2). https://doi.org/10.56042/ijtk.v21i2.29345
25. Kumar, A. (2025). Phytochemical screening and antimicrobial properties of medicinal plants. *The Bioscan, 20*(Special Issue 3), 1881–1894. https://doi.org/10.63001/tbs.2025.v20.i03.s.i(3).pp1881-1894
26. Mekonnen, E., Edessa, D., Weldegebreal, F., Aklog, A., & Gashaw, T. (2026). In vitro and in vivo antibacterial activities and phytochemical screening of 80% methanol extract from *Ehretia cymosa* leaves. *PLOS ONE, 21*(7), e0354982. https://doi.org/10.1371/journal.pone.0354982
27. Muhammad, M., Nfongeh, J., Ageba, S. L., & Orole, O. O. (2026). Determinants of virulence and antimicrobial resistance in *Proteus* species from women with urinary tract infections in Lafia, Nigeria. *Innovative Medicines & Omics*. https://doi.org/10.36922/imo025500073

28. Naseer, M. M., & Adil, M. (2025). Phytochemical profiling, HPLC analysis, and antimicrobial potential of *Curio radicans* (L. f.) P.V. Heath. *Scientific Reports, 15*(1), 34753. https://doi.org/10.1038/s41598-025-18323-0
29. O'Hara, C. M., Brenner, F. W., & Miller, J. M. (2000). Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clinical Microbiology Reviews, 13*(4), 534–546. https://doi.org/10.1128/CMR.13.4.534-546.2000
30. Öztürk, C., Kara, M., Akyol, R., & Bolacalı, M. (2025). Antimicrobial resistance and multidrug resistance patterns of uropathogens isolated from pregnant women with asymptomatic bacteriuria. *Journal of Turkish Society of Obstetric and Gynecology, 22*(4), 321–328. https://doi.org/10.4274/tjod.galenos.2025.29938
31. Putri, B. D. A. R., Sabariah, S., Rini, S., & Setiawan, N. (2026). Analysis of secondary metabolite compounds in combination extract of red castor leaves (*Jatropha gossypiifolia* L.) and butterfly pea flowers (*Clitoria ternatea* L.). *Jurnal Biologi Tropis, 26*(1). https://doi.org/10.29303/jbt.v26i1.11559
32. Rahman, M. A., Zereen, F., Halder, D. K., Al-Amin, M., Hossain, M. G., Shimada, M., & Saha, M. (2025). Molecular characterization, antibiogram, and virulence gene profiling of *Proteus vulgaris* isolated from cattle with bovine respiratory disease in Bangladesh. *Veterinary Medicine and Science, 11*(5), e70561. https://doi.org/10.1002/vms3.7056
33. Raza, S., Manzoor, R., Ibrahim, M. W., Fatima, M., Raza, A., & Younas, A. (2025). Frequency and perinatal outcome of UTI in pregnant women coming to a tertiary care hospital Lahore. *The Research of Medical Science Review*.
34. Royani, S., Sugianto, S., & Majid, S. (2024). Uji kualitatif senyawa metabolit sekunder ekstrak etanol 70% daun sukun (*Artocarpus altilis*) [Qualitative test of secondary metabolite compounds of 70% ethanol extract of breadfruit leaves]. https://doi.org/10.47007/ap.v6i2.7927
35. Sahu, S., Behera, J. P., Panigrahy, S. R., Padhi, S., Panda, A., Mahapatra, S., Mishra, P., & Subadarshani, S. (2026). Changing spectrum of antimicrobial drug resistance among uropathogens in pregnant women over three years at a tertiary healthcare facility: A retrospective observational study. *Journal of Clinical and Diagnostic Research*. https://doi.org/10.7860/JCDR/2026/81071.24526
36. Saini, S., Panda, J., Sharma, S., Khayyam, N., & Saini, V. K. (2026). Prevalence and risk factors of asymptomatic bacteriuria among pregnant women attending a tertiary care hospital in Jaipur, India. *Cureus, 18*(6). https://doi.org/10.7759/cureus.111585

37. Salam, H. K., Al-asbahi, M. G. S. S., Omar, D., Alwashli, J. A., Alqarni, H. H., Al-Jama'id, A. M., & Sultan, R. (2025). Phytochemical screening of some medicinal plants and antibacterial activity against bacteria isolated from clinical specimens: Dhamar City, Yemen. *Thamar University Journal of Natural & Applied Sciences, 10*(2). https://doi.org/10.59167/tujnas.v10i2.3014
38. Sayeed, S. Y., & Goyal, A. (2026). Comprehensive phytochemical screening and quantification of bioactive compounds in *Ziziphus spina-christi* for herbal cosmetics.
39. *International Journal of Research in Pharmacy and Allied Science, 5*(10). https://doi.org/10.71431/ijrpas.2026.5106
40. Senior, B. W. (1997). Media and tests to simplify the recognition and identification of members of the Proteeae. *Journal of Medical Microbiology, 46*(1), 39–44. https://doi.org/10.1099/00222615-46-1-39
41. Shankar, R., John, P., Nisha, A. R., Sujith, S., Uma, R., Keerthika, V., & Raj, A. (2023). Qualitative and quantitative phytochemical analysis of methanolic extract of *Magnolia champaca* leaves. *Journal of Veterinary and Animal Sciences, 54*(1), 204–213. https://doi.org/10.51966/jvas.2023.54.1.204-213
42. Sharma, P., Shri, R., Ntie-Kang, F., & Kumar, S. (2021). Phytochemical and ethnopharmacological perspectives of *Ehretia laevis*. *Molecules, 26*(12), 3489. https://doi.org/10.3390/molecules26123489
43. So'ad, S. Z., & Mokthar, N. A. (2026). Phytochemical screening and FTIR analysis for fractions of ethanol extract from *Mitragyna speciosa* leaves. *Journal of Pharmacy, 6*(2). https://doi.org/10.31436/jop.v6i2.527
44. Syahfitri, W., Ananda, Z., & Hasibuan, F. R. (2026). Studi literatur *Michelia champaca* L. dan potensinya sebagai antikanker dan antimikroba. *Jurnal Bioleuser, 10*(1), 1–6. https://doi.org/10.24815/jurnalbioleuser.v10i1.906
45. Thawabteh, A. M., Ma'ali, A., Ibdah, W., Karaman, D., & Karaman, R. (2026). Phytochemical interventions in bacterial resistance: Plant metabolites as next-generation antibacterial agents. *Frontiers in Chemical Biology, 5*. https://doi.org/10.3389/fchbi.2026.1760834
46. Travis, C. R., McMaster, J., & Rivas, F. (2026). Advances in natural product extraction: Established and emerging technologies. *Molecules, 31*(7), 1136. https://doi.org/10.3390/molecules31071136
47. Udoidiong, V. O., Bassey, M., Ekong, U., & Udonkang, M. U. (2026). Antibacterial activity of methanol leaf extract of *Sterculia tragacantha* against selected multidrug-

resistant clinical bacterial isolates. *Global Journal of Pure and Applied Sciences, 32*(5), 805–811. https://doi.org/10.4314/gjpas.v32i5.11

48. Varun, S. K., Gupta, N., Agrawal, P., Gupta, R., Gupta, A., Singhal, A., Dhar, K., Singhal, S., & Kapoor, V. (2026). Bacterial profile and antimicrobial resistance among pregnant women with urinary tract infections in northern India. *SSR Institute of International Journal of Life Sciences, 12*(5), 10781–10788. https://doi.org/10.21276/ssr-iijls.2026.12.5.15
49. Vivek, K., Abdhul, K., Mahendran, D., Poonkothai, M., & Saravanan, K. (2025). Antioxidant and antibacterial potential of phytochemicals extracted from *Ehretia laevis*: An in vitro and in silico analysis. *Research Journal of Chemistry and Environment, 29*(6), 38–50. https://doi.org/10.25303/296rjce038050
50. Zhou, H., Du, J., Jia, M., Li, Y., Zheng, K., et al. (2026). Medicinal plants: A promising therapeutic approach for addressing antimicrobial resistance. *International Journal of Molecular Sciences, 27*(11), 4804. https://doi.org/10.3390/ijms27114804