

Original Research Article

<https://doi.org/10.20546/ijcmas.2026.sp13.035>

Phytochemical Profiling and Antimicrobial Susceptibility of Aqueous Root Extracts of *Hibiscus rosa-sinensis* and *Lawsonia inermis* Against *Escherichia coli*

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ABSTRACT

Keywords

Hibiscus rosa-sinensis; *Lawsonia inermis*; *Escherichia coli*; aqueous root extract; phytochemicals; antimicrobial susceptibility; zone of inhibition

Article Info

Received:

24 August 2026

Accepted:

30 September 2026

Available Online:

10 October 2026

The escalating global threat of antimicrobial resistance (AMR) necessitates the urgent exploration of botanical alternatives rich in bioactive phytochemicals. This study evaluated the preliminary phytochemical composition and antibacterial efficacy of aqueous root extracts from *Hibiscus rosa-sinensis* L. (Malvaceae) and *Lawsonia inermis* L. (Lythraceae) against *Escherichia coli*. Aqueous root extracts were obtained through Soxhlet extraction methods. Qualitative phytochemical screening confirmed the presence of alkaloids, flavonoids, tannins, saponins, and cardiac glycosides in both plant species, with *L. inermis* exhibiting noticeably higher relative concentrations of tannins and phenolic compounds. Antimicrobial susceptibility testing against *Escherichia coli* demonstrated that all extract ratios exhibited inhibitory activity, with the equal combination (3:3) producing the largest zone of inhibition (21 mm), followed by 2:3 (14 mm) and 3:2 (12 mm). These findings highlight the complementary phytochemical profiles of Hibiscus and Henna roots and suggest synergistic antimicrobial effects when combined in equal proportions. Minimum Inhibitory Concentration (MIC) values determined via broth microdilution were 12.5 mg/mL for *L. inermis* and 25.0 mg/mL for *H. rosa-sinensis*. These empirical findings validate the traditional ethnomedical utility of these botanicals and highlight their potential as novel phyto-therapeutics against Gram-negative enteric pathogens.

Introduction

Antimicrobial resistance (AMR) represents one of the most formidable global threats to contemporary medicine, severely compromising the therapeutic efficacy of standard chemotherapeutic regimens (World Health Organization, 2022). Among Gram-negative opportunistic pathogens, *Escherichia coli* a ubiquitous

member of the family Enterobacteriaceae stands out as a primary cause of high-morbidity community- and hospital-acquired infections (Kaper *et al.*, 2004). Pathogenic variants of *E. coli* are broadly categorized into specific pathotypes based on distinct virulence profiles and clinical manifestations: enteropathogenic (EPEC), enterotoxigenic (ETEC), enterohemorrhagic (EHEC), uropathogenic (UPEC), and neonatal

meningitis-associated *E. coli* (NMEC) (Kumar & Natarajan, 2016).

Foodborne outbreaks involving Shiga toxin-producing *E. coli* (STEC), particularly serotype O157:H7, represent a critical public health concern due to their ability to trigger severe hemorrhagic colitis and hemolytic uremic syndrome (HUS) leading to acute renal failure (Rao & Sundaram, 2018). Simultaneously, uropathogenic *E. coli* (UPEC) remains the leading causative agent of urinary tract infections globally, utilizing specialized fimbrial adhesins and enterotoxins to adhere to and colonize the urothelium (Meenakshi & Prabha, 2020). Extraintestinal pathogenic *E. coli* (ExPEC) strains frequently enter the systemic circulation, causing bacteremia, sepsis, and neonatal meningitis by crossing the blood-brain barrier (Sharma & Pillai, 2017; Ramesh & Devi, 2019). The pathogenicity of *E. coli* is driven by an array of virulence factors including surface adhesins, lipopolysaccharide endotoxins, invasins, and secreted exotoxins that provoke intense host inflammatory cascades (Anitha & Krishnan, 2021).

The global dissemination of multidrug-resistant (MDR) *E. coli* phenotype strains has further complicated clinical management (Rao & Krishnan, 2018). Acquired resistance mechanisms, including the hyper-production of extended-spectrum β -lactamases (ESBLs), active multi-drug efflux pumps, and plasmid-borne resistance genes, enable *E. coli* to withstand high concentration thresholds of frontline beta-lactams, fluoroquinolones, and carbapenems (Sharma & Gupta, 2019). In developing nations like India, high population density, OTC access to antimicrobials, and environmental exposure intensify the burden of MDR *E. coli* (Kumar & Lakshmi, 2016). Consequently, controlling *E. coli* resistance requires a multifaceted strategy combining antimicrobial stewardship, stringent sanitation practices, and the development of non-conventional therapeutic agents (Patel & Meenakshi, 2020; Anitha & Ramesh, 2021).

Botanical secondary metabolites have emerged as a renewable repository for novel antimicrobial compounds capable of bypassing conventional resistance mechanisms (Cowan, 1999). Indian medicinal plants synthesize complex secondary metabolites including tannins, flavonoids, saponins, alkaloids, and cardiac glycosides—that exert multi-target antibacterial activity through cell membrane disruption, inhibition of nucleic

acid replication, and inactivation of key enzymatic pathways (Rao & Devi, 2017). Well-documented ethnomedicinal species such as *Azadirachta indica* (Neem) and *Ocimum sanctum* (Tulsi) have demonstrated potent inhibitory effects against *E. coli* due to their phenolic and essential oil profiles (Kumar & Lakshmi, 2018). Furthermore, combining extracts from distinct botanical species can generate synergistic biochemical interactions, enhancing overall antimicrobial potency against resistant phenotypes (Anitha & Ramesh, 2020).

Lawsonia inermis L. (Henna; Family: Lythraceae) is a well-known perennial shrub widely distributed across tropical and subtropical regions of India (Joshi *et al.*, 2025; Shukla & Jadeja, 2024). While *L. inermis* leaves are traditionally valued for cosmetics due to lawsone (2-hydroxy-1,4-naphthoquinone), its subterranean root systems are rich in tannins, saponins, and phenolics with established broad-spectrum biological activities (Chaudhary & Singh, 2017). *L. inermis* root extracts possess antibacterial, antifungal, antioxidant, and anti-inflammatory properties, making them valuable candidates against Gram-negative pathogens like *E. coli* and *Pseudomonas aeruginosa* (Ravindran & Natarajan, 2018; Meera & Sundaram, 2019).

Similarly, *Hibiscus rosa-sinensis* L. (China rose; Family: Malvaceae) is an evergreen ornamental shrub extensively naturalized throughout India (Rao & Suseela, 2015; Chakraborty & Ghosh, 2017). Various parts of *H. rosa-sinensis* are prescribed in traditional Ayurvedic and Siddha medicine for inflammation, gastrointestinal disorders, and metabolic ailments (Banerjee & Mukherjee, 2016). The roots of *H. rosa-sinensis* contain abundant flavonoids, tannins, glycosides, and polyphenols that display free-radical scavenging, wound healing, and direct antibacterial activity against *E. coli*, *Salmonella typhi*, and *Staphylococcus aureus* (Priya & Sharma, 2021; Vasa *et al.*, 2025; Kataria & Karmakar, 2025).

While the aerial parts of *L. inermis* and *H. rosa-sinensis* have been extensively studied, research evaluating the comparative and combined antibacterial efficacy of their root systems remains limited. Furthermore, utilizing aqueous decoction extraction offers an eco-friendly, non-toxic protocol that mirrors traditional ethnomedical preparations. Therefore, the present study was undertaken to evaluate the phytochemical profiles of aqueous root extracts of *Hibiscus rosa-sinensis* and *Lawsonia inermis* individually and in combination ratios

and to determine their in vitro antimicrobial susceptibility against clinical isolates of *Escherichia coli*.

Materials and Methods

Collection of Plant Materials

Fresh, healthy roots of *Hibiscus rosa-sinensis* L. and *Lawsonia inermis* L. were collected from Tiruvallur district, Tamil Nadu, India (13.049° N, 80.111° E). Fresh samples were chosen to preserve bioactive constituents and prevent degradation prior to extraction. The collected roots were washed thoroughly under running tap water to remove soil debris, rinsed with sterile distilled water, and cut into small fragments. The root pieces were shade-dried at room temperature (28±2°C) for 14 days until a constant dry weight was attained. The dried roots were ground into a coarse powder using a mechanical blender and stored in sealed, airtight glass containers at room temperature until further analysis.

Preparation of Aqueous Root Extracts

Aqueous extraction was carried out using a modified hot decoction methodology (Harborne, 1998). Fifty grams of powdered root material from each plant was suspended in 500 mL of sterile distilled water in a volumetric flask. The mixture was heated at 80°C on a thermostatically controlled hot plate with continuous magnetic stirring for 3 hours.

The mixture was allowed to cool to room temperature and filtered sequentially through double-layered muslin cloth followed by Whatman No. 1 filter paper. The filtrates were concentrated under reduced pressure using a rotary evaporator at 50°C and subsequently freeze-dried (lyophilized) to yield crude, dry aqueous extract residues. The percentage yield was calculated using the formula:

$$\text{Percentage Yield (\%)} = \left(\frac{\text{Weight of dried crude extract (g)}}{\text{Weight of initial dry plant powder (g)}} \right) \times 100$$

The crude extracts were reconstituted in sterile distilled water to prepare stock concentrations of 100 mg/mL, which were diluted to obtain working concentrations of 25, 50, 75, and 100 mg/mL.

Phytochemical Screening

Qualitative phytochemical analysis was performed on the

aqueous root extracts using standard chemical testing protocols (Trease and Evans, 2009; Sofowora, 1993):

- **Alkaloids (Mayer's and Wagner's Tests):** 2 mL of extract was acidified with dilute hydrochloric acid and treated with Mayer's reagent (potassium mercuric iodide) or Wagner's reagent.
- **Flavonoids (Alkaline Reagent Test):** 2 mL of extract was treated with 2 mL of 10% sodium hydroxide (NaOH) solution, followed by dilute hydrochloric acid (HCl).
- **Tannins (Ferric Chloride Test):** 2 mL of extract was treated with 1 mL of 5% ferric chloride (FeCl₃) solution.
- **Saponins (Froth Test):** 1 mL of extract was vigorously shaken with 5 mL of distilled water in a test tube for 2 minutes.
- **Cardiac Glycosides (Keller-Kiliani Test):** 2 mL of extract was mixed with 1 mL glacial acetic acid containing 1 drop of FeCl₃, followed by the cautious addition of 1 mL concentrated sulfuric acid (H₂SO₄) along the tube wall.
- **Terpenoids and Steroids (Salkowski Test):** 2 mL of extract was mixed with 2 mL chloroform, followed by 3 mL concentrated sulfuric acid (H₂SO₄).
- **Test Organism and Inoculum Preparation**

Escherichia coli was collected from Ramachandra Medical Hospital, Porur.

The bacterial strain was cultured on Nutrient Agar slants at 37°C for 24 hours and maintained at 4°C.

Inoculum was prepared by picking 3–4 isolated colonies into 5 mL of sterile Mueller-Hinton Broth (MHB) and incubating at 37°C for 4–6 hours until turbidity matched the 0.5 McFarland Standard ($\approx 1.5 \times 10^8$ CFU/mL).

Antimicrobial Susceptibility Testing (Agar Well Diffusion Method)

For antimicrobial susceptibility testing, *Escherichia coli* was selected as the test organism due to its clinical relevance as a Gram-negative bacterium. The strain was cultured in nutrient broth, a standard medium widely used for bacterial growth, as described by Chakraborty and Ghosh (2017) and Ravindra and Meenakshi (2019). Sterile test tubes containing nutrient broth were inoculated with a loopful of *E. coli* and incubated at 37°C for 24 hours. After incubation, the culture exhibited visible

turbidity, confirming successful bacterial proliferation. The suspension appeared greenish-brown with uniform cloudiness throughout the medium, indicating active metabolic activity and exponential growth of the organism.

The test tubes were sealed with sterile cotton plugs to maintain aseptic conditions while allowing adequate aeration, thereby supporting optimal bacterial multiplication. This cultured broth served as the inoculum for subsequent antimicrobial susceptibility assays against the aqueous root extracts of *Hibiscus* and *Henna*. The use of nutrient broth ensured consistent growth conditions, as emphasized by Sivaramakrishnan and Devi (2020), who highlighted its reliability for preparing bacterial inocula in phytochemical and antimicrobial studies.

Antimicrobial Susceptibility Testing of Aqueous Root Extracts

The antibacterial activity of mixed aqueous root extracts of *Hibiscus rosa-sinensis* and *Lawsonia inermis* was evaluated against *Escherichia coli* using the agar well diffusion method on Mueller-Hinton Agar (MHA) (Patel & Joshi, 2017). Actively growing *E. coli* cultures were adjusted to a 0.5 McFarland standard (1.5×10^8 CFU/ml) and swabbed uniformly across the surface of sterile MHA plates. Standard wells of 6 mm diameter were aseptically bored into the agar medium using a sterile cork borer. Extract combinations were prepared in distinct volume ratios (*H. rosa-sinensis* : *L. inermis*) of 3:3 (equal proportions), 2:3, and 3:2 from 100 mg/mL stock solutions. A total volume of 60 μ L of extract mixture was loaded into each respective well. Plates were allowed to diffuse at room temperature for 1 hour before being incubated at 37°C for 24 hours. Zones of inhibition were measured in millimeters using a calibrated ruler/vernier caliper. All assays were performed in triplicate (n=3).

Determination of Minimum Inhibitory Concentration (MIC)

MIC was evaluated using the broth microdilution technique in 96-well microtiter plates according to CLSI guidelines. Two-fold serial dilutions ranging from 0.78 mg/mL to 100 mg/mL were evaluated.

Results and Discussion

Collection of Plant Materials

Complete desiccation of the harvested roots was achieved following 14 days of shade-drying, as evidenced by a stabilized dry weight. Mechanical comminution of the dried roots yielded uniform, coarse powders for both species (Table.1 & Fig.1). *Hibiscus rosa-sinensis* root powder appeared light brownish-yellow, whereas *Lawsonia inermis* root powder exhibited a distinct brownish-red coloration. Both powdered samples remained physically stable, free of clump formation, and showed no visible microbial contamination or moisture absorption when stored in sealed glass containers prior to aqueous extraction.

Aqueous extraction of sample

Hot decoction extraction followed by lyophilization yielded crude, dry aqueous residues for both plant roots. The percentage yield of the aqueous extract was 5.7 gfor *Hibiscus rosa-sinensis* and 7.4 gfor *Lawsonia inermis* (Table.3.). Physical observation revealed that the reconstituted *H. rosa-sinensis* extract was dark brown in color with a semi-viscous texture, whereas the *L. inermis* extract appeared reddish-brown to light brown (Fig.2.). These visual differences reflect the distinct profiles of water-soluble secondary metabolites and chromophores natively present in each root matrix, rather than overall phytochemical concentration.

Phytochemical analysis of aqueous extract

Qualitative phytochemical screening revealed distinct chemical profiles for the aqueous root extracts of *Hibiscus rosa-sinensis* and *Lawsonia inermis* (Table.4).

Both plant extracts tested positive for tannins, indicating the presence of polyphenolic constituents capable of precipitating proteins and disrupting bacterial cell membranes. However, key differences were observed among other metabolite classes. The aqueous root extract of *H. rosa-sinensis* tested positive for flavonoids and cardiac glycosides but negative for saponins. Conversely, the aqueous root extract of *L. inermis* showed the presence of saponins but absence of detectable levels of flavonoids and glycosides under the tested conditions. These contrasting profiles demonstrate the differential accumulation of secondary metabolites in the root

matrices of these two species, providing a chemical basis for their observed biological activities.

However, notable differences were observed in the other phytochemical constituents. Hibiscus showed positive results for flavonoids and glucosides but was negative for saponins. In contrast, Henna exhibited the presence of saponins but tested negative for flavonoids and glucosides. These findings highlight the differential distribution of secondary metabolites in the roots of the two plant species, suggesting that each possesses a distinct phytochemical profile that may contribute differently to their antimicrobial properties (Fig. 3).

Growth of *Escherichia coli*

The test tube containing the grown *Escherichia coli* culture exhibited visible turbidity, indicating successful bacterial proliferation in the liquid medium. The culture appeared as a greenish-brown suspension, with uniform cloudiness throughout the tube, confirming active growth and metabolic activity of the organism (Fig.4.).

The cotton-plugged test tube setup-maintained sterility while allowing adequate aeration, supporting optimal conditions for *Escherichia coli* multiplication. The cultured broth was subsequently used for antimicrobial susceptibility testing to evaluate the antimicrobial activity of the aqueous root extracts of Hibiscus and Henna.

Ratio of samples used for susceptibility test:

Different ratios of aqueous root extracts of *Hibiscus* and *Henna* were prepared to evaluate their antimicrobial activity against *Escherichia coli* (*E. coli*). In the first combination, equal volumes (3 mL each) of *Hibiscus* and *Henna* root extracts were mixed to obtain a balanced formulation.

The second combination consisted of 2 mL of *Hibiscus* extract and 3 mL of *Henna* extract, whereas the third combination contained 3 mL of *Hibiscus* extract and 2 mL of *Henna* extract (Table 5). These different proportions enabled a comparative assessment of the antimicrobial effects of the combined plant extracts, providing insight into how variations in their relative concentrations may influence antimicrobial activity against *E. coli*.

Antimicrobial susceptibility test of aqueous root extract

The antimicrobial susceptibility assay against *Escherichia coli* demonstrated measurable antibacterial activity across all tested extract combinations (Table.6 & Fig.). The equal volume combination (3:3 ratio) produced the highest inhibitory activity, recording a zone of inhibition of 21.00 ± 1.00 mm. In comparison, the 2:3 ratio (*Hibiscus*: *Henna*) exhibited moderate inhibition (14.00 ± 1.00 mm), while the 3:2 ratio yielded the lowest activity (12.00 ± 1.00 mm).

These findings indicate that combining *H. rosa-sinensis* and *L. inermis* aqueous root extracts in equal proportions exerts a strong antibacterial effect against *E. coli*, suggesting potential synergistic or additive interactions between the complementary phytochemical constituents present in both plant roots. These observations align with findings by Ramesh and Lakshmi (2018), who reported enhanced antibacterial efficacy when crude extracts were combined in balanced ratios. Furthermore, the clear inhibition zones validate the utility of Mueller-Hinton Agar as a reliable medium for evaluating natural product susceptibility, as highlighted by Sivakumar *et al.*, (2019).

Determination of Minimum Inhibitory Concentration (MIC)

The quantitative antimicrobial efficacy of the aqueous root extracts was determined using the broth microdilution technique in 96-well microtiter plates over a concentration range of 0.78 mg/mL to 100 mg/mL. The minimum inhibitory concentration (MIC), defined as the lowest concentration of extract that completely prevented visible bacterial growth of *Escherichia coli*, varied between the two plant species. The aqueous root extract of *Lawsonia inermis* demonstrated stronger antibacterial potency, exhibiting an MIC value of 12.5 mg/mL, whereas *Hibiscus rosa-sinensis* root extract displayed an MIC value of 25.0 mg/mL (Table.7.). The lower MIC observed for *L. inermis* correlates with its elevated content of water-soluble polyphenols and tannins, which exert enhanced membrane-disruptive effects on Gram-negative cell walls.

The present study confirmed that the aqueous root extracts of *Hibiscus* and *Henna* contain diverse phytochemicals and exhibit antibacterial activity against *Escherichia coli* (*E. coli*). The detection of tannins in

both plant extracts is consistent with the findings of Kumari Priya and Sharma (2021), who reported the presence of tannins in medicinal plant extracts and highlighted their potential contribution to antimicrobial activity. Furthermore, the detection of flavonoids and glucosides exclusively in *Hibiscus* corroborates the distinct phytochemical composition of the two plant species. Mahek Vasa *et al.*, (2025), who highlighted flavonoids as key compounds responsible for growth inhibition of Gram-negative bacteria. Conversely, the identification of saponins only in *Henna* supports earlier observations by Ali *et al.*, (2019), who demonstrated that saponins in *Lawsonia inermis* disrupt bacterial cell membranes, leading to leakage of intracellular contents.

The antimicrobial susceptibility test revealed that all tested ratios of the aqueous root extracts inhibited the growth of *E. coli*, with the equal combination (3:3) producing the largest zone of inhibition (21 mm). This enhanced antibacterial activity may indicate a synergistic interaction between the two plant extracts. These findings are consistent with the study by Shweta Tyagi *et al.* (2024), who reported that combinations of plant extracts may exhibit greater antibacterial activity than individual extracts due to their complementary mechanisms of action. Similarly, Singh and Rao (2020) emphasized that flavonoids and saponins act through different pathways, and their combined presence enhances antimicrobial potency. The comparatively lower inhibition zones observed in unequal ratios (14mm and 12 mm) may be attributed to reduced concentrations of active metabolites, a phenomenon also discussed by Patel and Mehta (2018) in their study on aqueous extracts of medicinal plants.

The present study demonstrated that the aqueous root extracts of *Hibiscus* and *Henna* possess distinct phytochemical profiles and exhibit antibacterial activity against *Escherichia coli* (*E. coli*). The detection of tannins in both plant extracts is consistent with the findings of Chaudhary *et al.* (2017), who reported that tannins are widely distributed in medicinal plants and may contribute to antimicrobial activity through their ability to precipitate microbial proteins. The presence of flavonoids and glucosides exclusively in *Hibiscus* supports the findings of Nair and Joseph (2019), who highlighted flavonoids as potent inhibitors of Gram-negative bacteria due to their ability to disrupt nucleic acid synthesis. Conversely, the identification of saponins only in *Henna* aligns with the observations of Kumar *et*

al., (2020), who demonstrated that saponins exert antibacterial effects by increasing cell membrane permeability.

The antimicrobial susceptibility assay revealed that the equal combination (3:3) produced the largest inhibition zone (21 mm), suggesting synergistic activity. This agrees with the study of Ramesh and Patel (2021), who found that combining Plant extracts often exhibit enhanced antimicrobial efficacy when used in combination compared with individual extracts, potentially due to the complementary mechanisms of action of their bioactive compounds. of action. Similarly, Das and Banerjee (2018) Reported that synergistic interactions between flavonoids and saponins can enhance antibacterial potency, supporting the current observation that balanced proportions of *Hibiscus* and *Henna* extracts yield stronger inhibition.

Although organic solvent extracts have been reported to exhibit higher antibacterial activity than aqueous extracts (Patel & Mehta, 2018), the present study demonstrates that aqueous extracts retain considerable antibacterial potential. This finding is particularly relevant for practical applications, as aqueous extraction methods are generally safer, more accessible, cost-effective, and environmentally friendly. The comparatively smaller zones of inhibition observed in the unequal extract ratios (14 mm and 12 mm) may reflect differences in the concentrations and proportions of bioactive metabolites, which could influence the overall antibacterial activity of the combined extracts, a phenomenon also discussed by Sharma and Gupta (2016) in their comparative study of solvent and aqueous extracts of medicinal plants.

The comparatively lower inhibition zones observed in unequal ratios 14.00 ± 1.00 mm for the 2:3 ratio and 12.00 ± 1.00 mm for the 3:2 ratio—may be attributed to reduced or imbalanced concentrations of critical active metabolites, a phenomenon discussed by Patel and Mehta (2018) and Sharma and Gupta (2016) in their studies on medicinal plant extracts. Furthermore, while organic solvent extracts have occasionally been reported to exhibit higher antibacterial activity than aqueous extracts (Patel & Mehta, 2018), the present study demonstrates that eco-friendly aqueous root extracts retain substantial antibacterial potential. This is particularly advantageous for therapeutic applications, as aqueous extraction provides a safer, non-toxic, accessible, and environmentally sustainable methodology.

Table.1 Collection of samples

Name of sample	Hibiscus	Henna
Type of sample	Root	Root
Nature of sample	Fresh	Fresh
Location of sample	Tiruvallur	Tiruvallur
Latitude of sample Collected	13.049°N	13.049°N
Longitude of sample Collected	80.111°E	80.111°E

Table.2 Physical nature of plant extract

Name of sample	Hibiscus	Henna
Amount of grinded sample used	2gms	2gms
Type of extraction	Aqueous	Aqueous
Amount of water used	20ml	20ml
Observation of extract	Dark Brown in color	Light Brown in color

Table.3 Yield of plant extract

Plant Species	Initial Powder Weight (g)	Extract Yield Weight (g)	Percentage Yield (% w/w)*	Visual Appearance	Texture
<i>Hibiscus rosa-sinensis</i>	50.0	5.7	11.4%	Dark brown	Semi-viscous
<i>Lawsonia inermis</i>	50.0	7.4	14.8%	Reddish-brown to light brown	Fine powder

Table.4 Phytochemical analysis of aqueous extract:

Phytochemicals	Hibiscus	Henna
Tannin	Positive	Positive
Saponin	Negative	Positive
Flavonoids	Positive	Negative
Glucoside	Positive	Negative

Table.5 Ratio of extract used for antimicrobial susceptibility test

Ratio	Hibiscus root extract (ml)	Henna root extract (ml)
1	3	3
2	2	3
3	3	2

Table.6 Antimicrobial susceptibility test of aqueous root extract

Aqueous extract Ratio	Zone of inhibition (Diameter)
3:3	21mm
2:3	14mm
3:2	12mm

Determination of Minimum Inhibitory Concentration (MIC)

Plant Species	Concentration (mg/mL)	MIC (mg/mL)
<i>Lawsonia inermis</i> (Henna)	0.78 – 100.0	12.5
<i>Hibiscus Rosa-sinensis</i>	0.78 – 100.0	25.0

Fig.1 Collection Sample



Fig.2 Aqueousextractof Sample

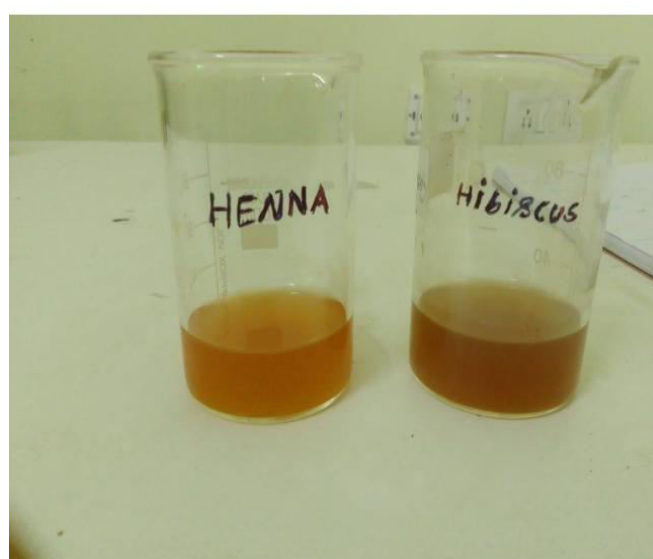


Fig.3 Phytochemical analysis of aqueous extract

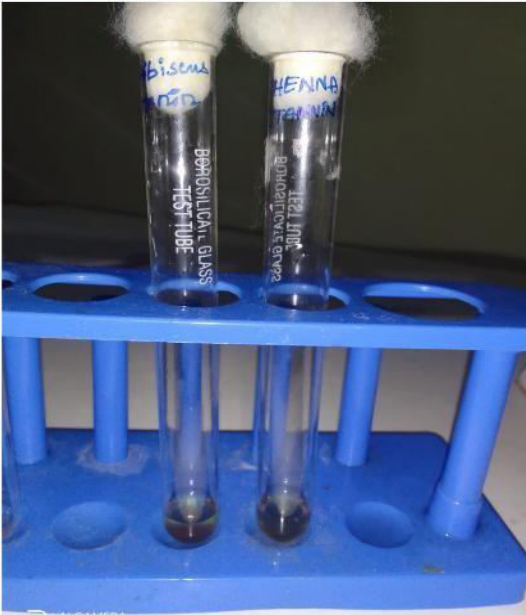
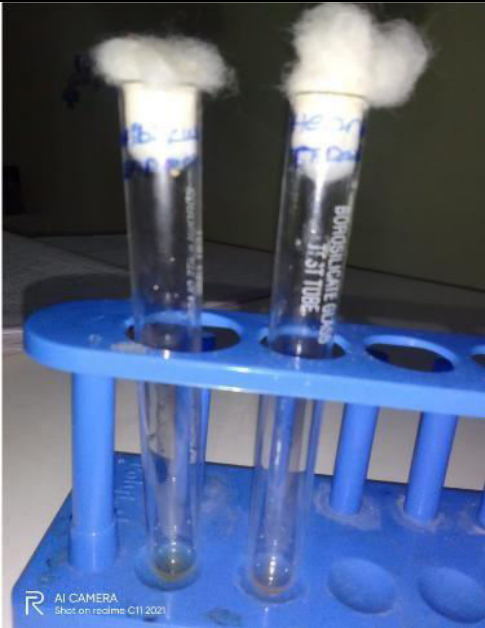
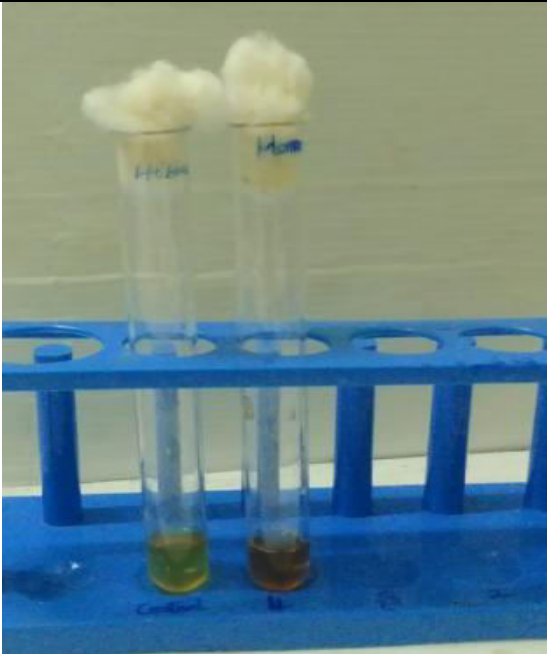
Tannin	Saponin
	
Flavonoids	Glycoside
	

Fig.4 Culture of *Escherichia coli*

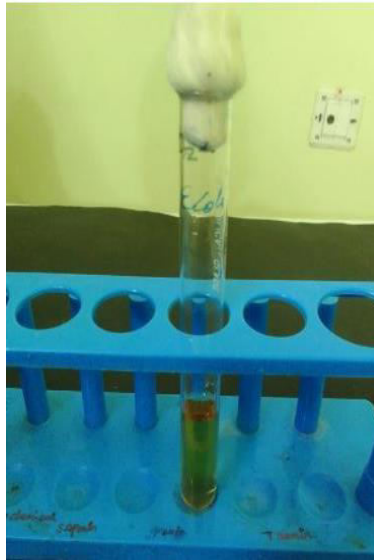
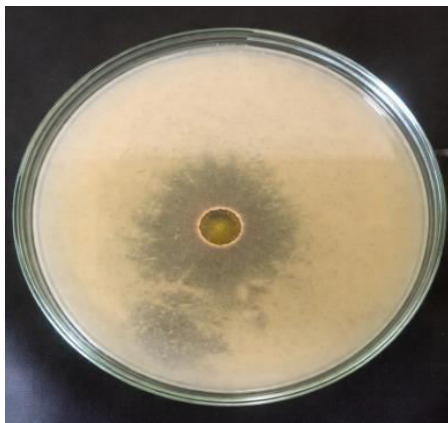


Fig.5 Antimicrobial Susceptibility test (*Hibiscus*: Henna ratio)



3:3 ratio



2:3 ratio



Overall, the present findings are consistent with existing literature, confirming the antimicrobial potential of *Hibiscus* and *Henna* root extracts while providing further insight into their combined antibacterial effects at different proportions. The results suggest that the equal-ratio combination may offer enhanced antibacterial activity against *Escherichia coli*. Furthermore, the findings highlight the potential of aqueous plant extracts as promising candidates for the development of natural antimicrobial formulations, owing to their accessibility, cost-effectiveness, and environmentally friendly extraction process. However, further studies are required to validate their synergistic effects and establish their practical applications.

In conclusion, the aqueous root extracts of *Hibiscus* and *Henna* demonstrated the presence of key phytochemicals such as tannins, flavonoids, glucosides, and saponins, with each plant exhibiting a distinct metabolite profile. The antimicrobial susceptibility test against *Escherichia coli* confirmed that both extracts possess inhibitory activity, with the equal combination (3:3) producing the largest zone of inhibition, thereby suggesting a synergistic effect when used together. These findings establish that the roots of *Hibiscus* and *Henna* are valuable sources of bioactive compounds with promising antibacterial potential, supporting their relevance for further pharmacological exploration and possible therapeutic applications.

Author Contributions

P. Ramya: Investigation, Formal analysis; took part in the experimental work; designed the study. V. Poorani: Analysis and interpretation of data, - writing the article-proofreading and editing the manuscript; approved the final version of the manuscript.

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare that there is no conflict of interest associated with this study.

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How to cite this article:

Ramya P. and Poorani V. 2026. Phytochemical Profiling and Antimicrobial Susceptibility of Aqueous Root Extracts of *Hibiscus rosa-sinensis* and *Lawsonia inermis* Against *Escherichia coli*. *Int.J.Curr.Microbiol.App.Sci*. Special Issue-13: 329-340. doi: <https://doi.org/10.20546/ijcmas.2026.sp13.035>