WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 4, 586-597. Research Article ISSN 2277–7105

AN IN VITRO ANTIBACTERIAL ASSESSMENT OF RUBIA CORDIFOLIA, OPERCULINA TURPETHUM, AND STRYCHNOS POTATORUM AGAINST ESCHERICHIA COLI AND STAPHYLOCOCCUS AUREUS

Dr. Nikita Sarkar[1]*, Prof. (Dr.) Khagen Basumatary[2]

[*1]Final Year MD Scholar, Dept. of Samhita Siddhanta, Govt. Ayurved College and Hospital, Jalukbari Guwahati, 14, Assam, India.

[2]Professor & Head P.G. Department of Samhita & Siddhant Govt. Ayurvedic College & Hospital, Jalukbari, Guwahati.

Article Received on 15 Jan. 2026,
Article Revised on 06 Feb. 2026,
Article Published on 16 Feb. 2026,

https://doi.org/10.5281/zenodo.18659819

***Corresponding Author**

Dr. Nikita Sarkar

Final Year MD Scholar, Dept. of Samhita Siddhanta, Govt. Ayurved College and Hospital, Jalukbari Guwahati, 14, Assam, India.

How to cite this Article: Dr. Nikita Sarkar[1]* Prof. (Dr.) Khagen Basumatary[2] (2026). An In Vitro Antibacterial Assessment of Rubia Cordifolia, Operculina Turpethum, and Strychnos Potatorum Against Escherichia Coli and Staphylococcus Aureus. World Journal of Pharmaceutical Research, 15(4), 586–597.

ABSTRACT

1. Introduction: In the era of escalating antimicrobial resistance, medicinal plants offer promising alternatives for novel drug discovery. In vitro studies serve as crucial tools for evaluating the antimicrobial potential of plant-based compounds. This study focuses on the antimicrobial screening of three Ayurvedic medicinal herbs—Rubia cordifolia (Manjistha), Strychnos potatorum (Katak), and Operculina turpethum (Trivrit)—traditionally recognized for their therapeutic properties. **Objectives:** To evaluate the antimicrobial activity of the selected Vishaghna herbs by integrating traditional Ayurvedic principles with modern microbiological evidence, and to comparatively assess their efficacy against both Gram-positive (Staphylococcus aureus) and Gram-negative (Escherichia coli) bacterial strains through in vitro analysis. **Methodology:** Crude extracts were prepared using methanol, aqueous, and ethyl acetate solvents. The Broth Dilution Method was employed to assess in vitro activity against Staphylococcus aureus (Gram-positive) and Escherichia coli (Gram-negative). Minimum Inhibitory Concentration (MIC) was determined using spectrophotometric absorbance at 600 nm. **Results:** Strychnos potatorum showed the strongest antimicrobial effect, especially against E. coli, followed by

Operculina turpethum. Rubia cordifolia exhibited minimal activity. **Conclusion:** The study confirms the in vitro antimicrobial efficacy of Strychnos potatorum and supports the continued exploration of ethnobotanical.

KEYWORDS: Antibacterial activity, Rubia Cordifolia, Strychnous Potatorum, Operculina Turpethum.

1. INTRODUCTION

Antimicrobial resistance (AMR) is one of the most urgent public health challenges facing the world today. Though antibiotics have revolutionized medicine over the past century, their overuse and misuse have led to the rise of multidrug-resistant bacterial strains, threatening the effectiveness of conventional treatments. With the development of new synthetic antibiotics slowing down, attention is turning back to traditional sources—particularly medicinal plants—as potential reservoirs of novel antimicrobial agents. Ayurveda, India's ancient medical tradition, has long recognized the healing power of plants. Its holistic approach to health includes numerous herbs used for blood purification, wound healing, and managing what we now identify as microbial infections. Classical texts such as the Charaka Samhita[1] and Sushruta Samhita[2] describe disease-causing entities like Krumi, Jantu, and Bhuta, which conceptually parallel modern germs and parasites. Ayurvedic practices such as fumigation, disinfection, and management of Aupsargika Roga[3] (contagious diseases) reflect an early understanding of hygiene and infection control. Among the many Ayurvedic herbs, Rubia cordifolia[4] (Manjistha), Strychnos potatorum[5] (Katak) and Operculina turpethum[6] (Trivrit) are traditionally known for their Vishaghna (detoxifying and anti-poisonous) properties. They have been widely used for conditions like skin disorders, wound infections, and gastrointestinal ailments. While modern phytochemical studies have shown that these herbs contain compounds such as anthraquinones, alkaloids, glycosides, and flavonoids—many with known antimicrobial effects—systematic evaluation using standard microbiological techniques is still limited.

This study aims to bridge that gap. It evaluates the antimicrobial efficacy of these three herbs against Staphylococcus aureus[7] and Escherichia coli,[8] two clinically relevant bacteria that represent Gram-positive and Gram-negative groups, respectively. Both are common culprits in skin infections, urinary tract infections, and hospital-acquired infections, and both show increasing resistance to conventional antibiotics. The plant materials were extracted using three solvents of varying polarity—methanol, ethyl acetate, and water—to ensure a broad

spectrum of phytochemical capture. Their antimicrobial activity was tested using the Broth Dilution Method, with Minimum Inhibitory Concentration (MIC) determined by turbidity readings and spectrophotometric absorbance at 600 nm.

The research draws from both Ayurvedic literature and contemporary scientific studies. Classical descriptions of contagious diseases and herbal detoxifiers find resonance in modern findings that plant-derived compounds can inhibit microbial growth by targeting cell walls, enzymes, or communication pathways like quorum sensing. Each herb contributes uniquely to this exploration: Rubia cordifolia offers wound-healing anthraquinones; Operculina turpethum delivers membrane-disrupting resin glycosides; and Strychnos potatorum, known for its use in water purification, contains alkaloids with potent antimicrobial action.

By combining traditional knowledge with modern analysis, this study not only reaffirms ancient practices but also highlights the potential of plant-based agents in addressing today's antibiotic crisis.

2. MATERIALS AND METHODS

2.1 Selection and Maintenance of Microorganisms

Two bacterial strains were selected to represent Gram-positive and Gram-negative classes: Staphylococcus aureus and Escherichia coli. These organisms are commonly implicated in human infections and are widely used as model organisms for antimicrobial screening. The strains were sub-cultured on Nutrient Agar slants and incubated at 37°C for 18–24 hours to obtain fresh, viable cultures. For short-term storage, the cultures were maintained at 4°C on slants and sub-cultured periodically to ensure viability throughout the study period.

2.2 Preparation of Inoculum

A loopful of each test organism was inoculated into sterile Nutrient Broth and incubated at 37°C until moderate turbidity developed, generally reaching an optical density comparable to the 0.5 McFarland standard (~10^8 CFU/mL). The bacterial suspension was then diluted to achieve an inoculum size of approximately 10^5 CFU/mL for the assay, ensuring consistency across all replicates.

2.3 Collection and Identification of Plant Materials

Three medicinal plants were selected based on traditional Ayurvedic use and previous reports of bioactivity

Rubia cordifolia Linn. (Manjistha)
Operculina turpethum (Linn.) (Trivrit)
Strychnos potatorum Linn. (Katak)

Authenticated plant materials were procured from local herbal sources and verified by a taxonomist. The plant parts used were roots for Rubia cordifolia and Operculina turpethum, and seeds for Strychnos potatorum. All samples were shade-dried and ground into fine powder using a mechanical grinder.

2.4 Extraction Procedure

Each powdered sample was subjected to sequential extraction using solvents of increasing polarity
Methanol
Ethyl acetate
Distilled water

Approximately 50 g of each powdered sample was soaked in 500 mL of the respective solvent for 72 hours with intermittent shaking. The mixtures were filtered through Whatman No. 1 filter paper, and the filtrates were concentrated under reduced pressure using a rotary evaporator at temperatures not exceeding 40°C to preserve heat-sensitive phytoconstituents. Aqueous extracts were concentrated by freeze-drying. The whole process was done in Rotary Evaporator.[9]
The dried extracts were labelled as follows
RC1: Rubia cordifolia methanol extract
RC2: Rubia cordifolia ethyl acetate extract
RC3: Rubia cordifolia aqueous extract

OT1: Operculina Turpethum methanol extract
OT2: Operculina Turpethum ethylacetate extract
OT3: Operculina Turpethum aqueous extract

SP1: Strychnous Potatorum methanol extract
SP2: Strychnous Potatorum ethylacetate extract
SP3: Strychnous Potatorum aqueous extract

All extracts were stored in airtight containers at 4°C until use.

The extraction procedure was done in INSTITUTE OF ADVANCE STUDY IN SCIENCE AND TECHNOLOGY (IASST), GUWAHATI, ASSAM.

2.5 Preparation of Stock Solutions

Each extract was dissolved in Dimethyl Sulfoxide (DMSO) to prepare stock solutions at a concentration of 1 mg/mL. Solutions were filter-sterilised through 0.22 µm syringe filters when necessary to prevent contamination.

2.6 Broth Dilution Method[10]

The antimicrobial activity was determined by the Broth Dilution Method as per CLSI guidelines with minor modifications. A sterile 96-well microtiter plate was employed. Serial two-fold dilutions of each extract were prepared in Nutrient Broth to yield final concentrations of 200, 100, 50, 25, and 12.5 µg/mL.

Each well received 100 µL of the respective extract dilution and 100 µL of the standardised bacterial inoculum. Positive controls (Ampicillin) and negative controls (broth plus bacteria without extract) were included for validation. A blank control (broth only) ensured sterility.

The microtiter plates were sealed with parafilm to prevent contamination and evaporation and incubated at 37°C for 18–24 hours.

2.7 Determination of Minimum Inhibitory Concentration (MIC)

After incubation, wells were visually examined for turbidity. Clear wells indicated inhibition of bacterial growth, while turbid wells indicated bacterial proliferation. The MIC was recorded as the lowest concentration of the extract that showed no visible growth. Observations were recorded in triplicates to ensure reproducibility.

This antimicrobial assay was done in NATIONAL INSTITUTE OF PHARMACEUTICAL AND RESEARCH, GUWAHATI (NIPER-G).

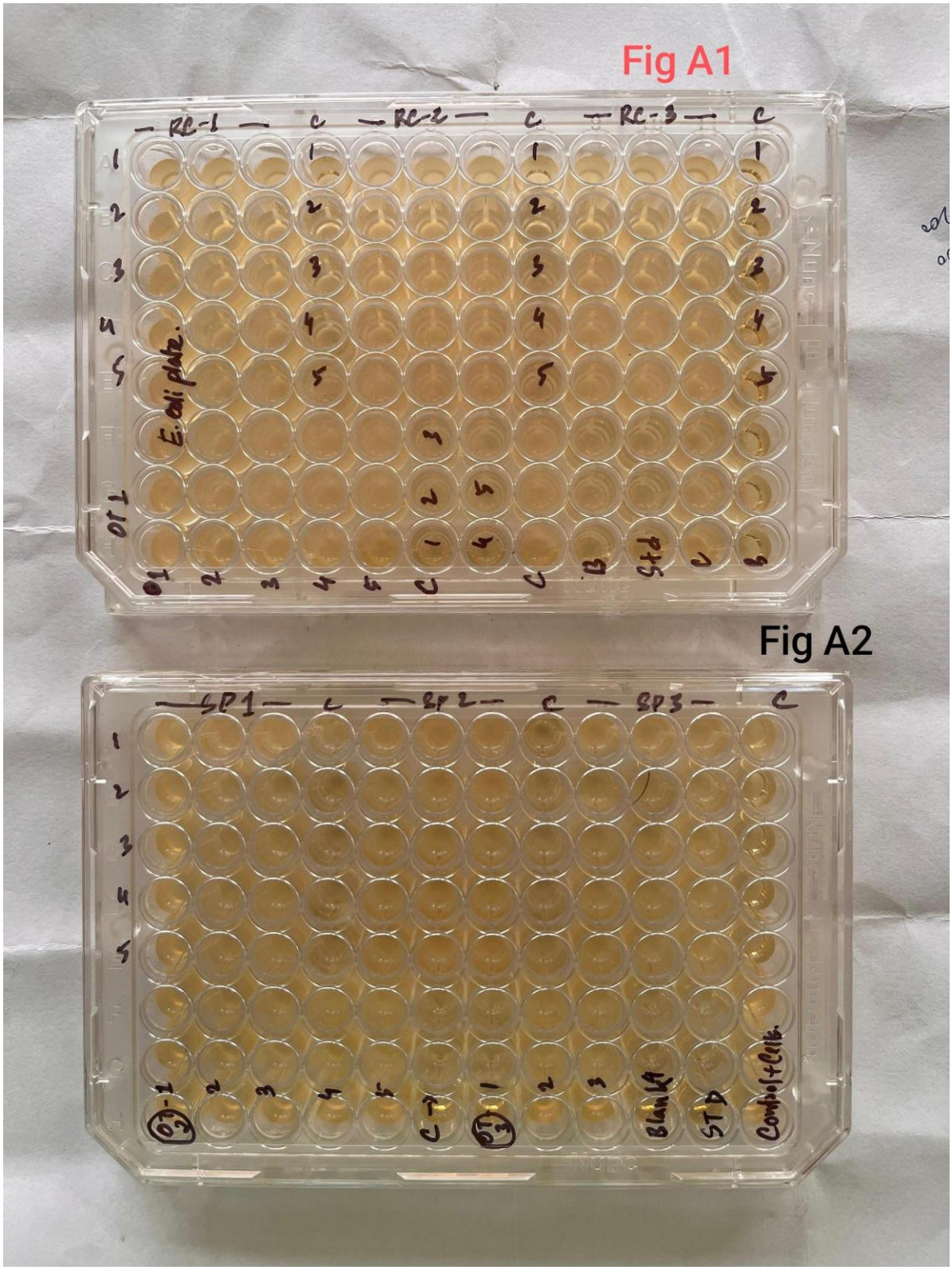
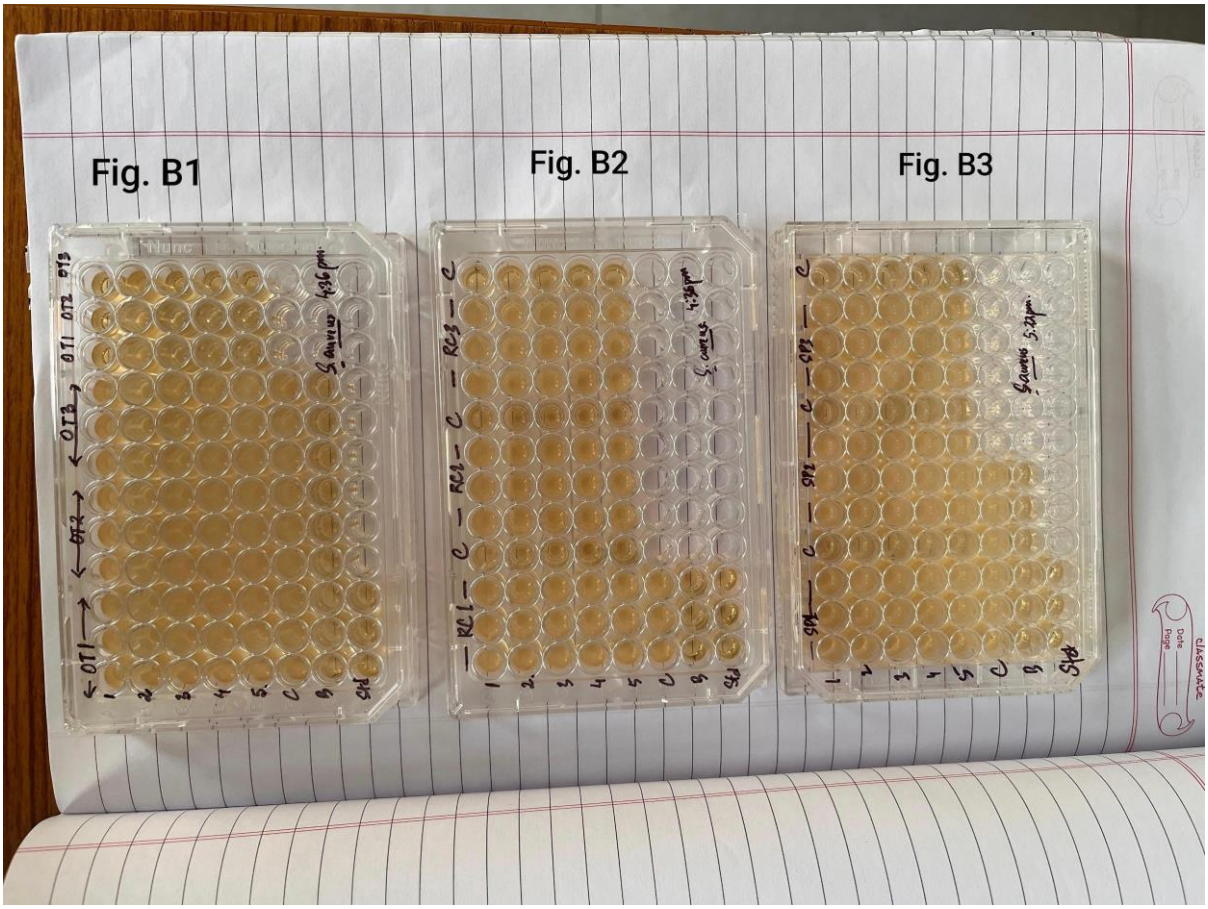


Fig A1
RC-1
RC-2
RC-3
E.coli plate.
Fig A2
SP1
SP2
SP3
Blank
STD
Control+Cells.



2.8 HERE IS THE CONCISE SUMMARY TABLE FOR BETTER UNDERSTANDING

Table No: 1

Plant	Extract (Fraction)	S. aureus Effectiveness	E. coli Effectiveness
RC	Methanol (RC1)	No inhibition	Weak to no inhibition (slight at 12.5 µg/mL
RC	Ethyl acetate (RC2)	No inhibition	Weak to no inhibition (slight at 12.5 µg/mL)
RC	Aqueous (RC3)	No inhibition	Weak to low (small spike at 12.5 µg/mL)
OT	Methanol (OT1)	Moderate to good inhibition at higher concentrations	Moderate inhibition
OT	Ethyl acetate (OT2)	Moderate inhibition at mid concentrations	Weak to moderate
OT	Aqueous (OT3)	Good to strong inhibition across higher & mid	Moderate to good

		concentrations	
SP	Methanol (SP1)	Weak to moderate	Moderate at all concentrations
SP	Ethyl acetate (SP2)	Weak to moderate	Moderate to good
SP	Aqueous (SP3)	Moderate to good	Moderate to good

3. RESULT AND ANALYSIS

3.1 Plant Authentication and Extract Preparation

All three plant materials were authenticated via macroscopic and microscopic examination to ensure botanical identity and purity. Extraction was performed using methanol, ethyl acetate, and water under standardized conditions. The yield of the extracts ranged between 2.0% to 4.76% (w/w). These extracts were subjected to antimicrobial testing using visual turbidity observation in microtiter plates and percentage inhibition graphs to quantify the antibacterial activity in broth dilution method.

3.1 Strychnos potatorum (Katak)

Among the three tested herbs, Strychnos potatorum (SP) demonstrated the most potent antibacterial activity, particularly against E. coli. Its ethyl acetate extract (SP2) and aqueous extract (SP3) showed significant, dose-dependent inhibition, with up to 35–40% inhibition at 200 µg/mL. These fractions also maintained consistent trends across lower concentrations, indicating the presence of bioactive polar and semi-polar compounds.

Against Staphylococcus aureus, the SP extracts also showed moderate activity, particularly SP2, suggesting that semi-polar phytoconstituents might be responsible for the antibacterial effect. The results align with the traditional Ayurvedic categorization of Strychnos potatorum as a Vishaghna herb, supporting its historical use in detoxification and microbial-related disorders.

3.2 Operculina turpethum (Trivrit)

Operculina turpethum (OT) displayed moderate antimicrobial activity. Its methanol (OT1) and aqueous (OT3) extracts were more effective than the ethyl acetate (OT2) extract, especially against S. aureus, where they achieved up to 20–25% inhibition at higher concentrations.

A dose-responsive trend was evident in both OT1 and OT3, particularly against E. coli. This suggests that polar solvents effectively extracted the active antimicrobial constituents of O. turpethum. These findings are consistent with Ayurvedic descriptions that emphasize Trivrit's

detoxifying and disease-modifying properties, potentially linked to its activity against microbial toxins or infections.

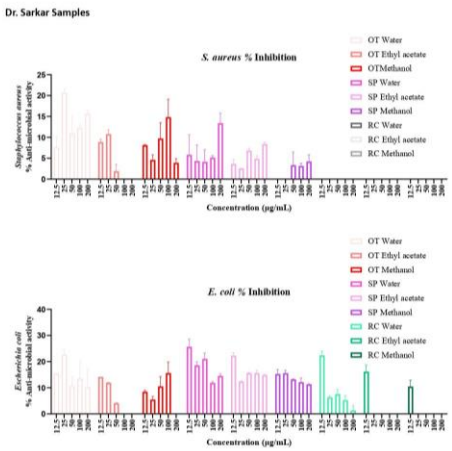
3.3 Rubia cordifolia (Manjistha)

Rubia cordifolia (RC) exhibited the least antimicrobial activity among the tested herbs. None of its extracts—methanol (RC1), ethyl acetate (RC2), or aqueous (RC3)—achieved inhibition beyond 10%, even at the highest tested concentration of 200 µg/mL. The wells remained visibly turbid across all replicates, indicating limited or absent microbial inhibition.

A minimal and inconsistent response was noted for RC3 at 12.5 µg/mL against E. coli, but the lack of reproducibility and absence of dose dependency rendered the result insignificant. This suggests that the antimicrobial efficacy of R. cordifolia may require alternative extraction methods, higher concentrations, or synergistic formulation to become bioactive.

3.4 Comparative Efficacy Between Test Organisms

Interestingly, all extracts exhibited better inhibitory activity against Escherichia coli than Staphylococcus aureus, which is contrary to the expected trend, as Gram-negative bacteria typically present more resistance due to their double membrane and efflux mechanisms. This observation may be attributed to the specific phytochemical interactions that better penetrate or disrupt the outer membrane of E. coli, or it may reflect the unique phytoconstituent profile of the selected herbs.



3.5 Graphical and visual Observations

The results were supported through

Microtiter well turbidity observations, visually confirming microbial growth inhibition.

Bar graphs that illustrated percentage inhibition across all solvent extracts and concentrations.

4. DISCUSSION

This study aimed to explore the antimicrobial efficacy of three traditionally used Ayurvedic Vishaghna herbs—Strychnos potatorum (Katak), Operculina turpethum (Trivrit), and Rubia cordifolia (Manjistha)—against Staphylococcus aureus and Escherichia coli, representing Gram-positive and Gram-negative bacteria respectively.

Among the tested herbs, Strychnos potatorum demonstrated the most promising activity, particularly in its aqueous (SP3) and ethyl acetate (SP2) extracts. The aqueous fraction showed consistent, dose-dependent inhibition of E. coli, with visible clarity in wells and significant reduction in optical density at lower concentrations. Moderate inhibition against S. aureus was also observed in SP1 and SP2, suggesting that Katak contains bioactive compounds across a range of polarities. This supports classical Ayurvedic references where Katak is listed under Vishaghna dravyas and used in conditions involving jantu (minute organisms), rakshasa (unseen harmful agents), and visha (toxins).

Operculina turpethum also displayed noteworthy antimicrobial activity, with both its methanol (OT1) and aqueous (OT3) extracts inhibiting bacterial growth, especially at higher concentrations. OT3 showed strong action against E. coli and moderate inhibition of S. aureus, reinforcing the potential of Trivrit's polar phytoconstituents. Although its ethyl acetate extract (OT2) was less effective, overall results suggest that Trivrit harbors antimicrobial components, aligning with its traditional therapeutic use in detoxification and infectious conditions.

In contrast, Rubia cordifolia showed minimal to no inhibitory effect in this study. The lack of significant inhibition across all extracts suggests that its therapeutic value may not lie in direct antibacterial activity but perhaps in other pharmacological domains such as immunomodulation, blood purification, or anti-inflammatory action, as often emphasized in Ayurvedic texts.

Comparatively, both Katak and Trivrit outperformed Manjistha in inhibiting bacterial growth. The strongest activity was seen against E. coli, which may be due to differences in bacterial cell wall composition. Gram-negative bacteria, though often more resistant, can be more susceptible to certain plant-based compounds that interact with their outer membrane. This

pattern was reflected in the dose-response curves and turbidity observations across extracts, particularly in SP3 and OT3, where clear MIC thresholds were noted.

Ampicillin, used as a standard control, exhibited 100% inhibition and validated the reliability of the assay. The observed variations among the herbal extracts also highlight the influence of extraction methods and solvent polarity on bioactive compound yield.

From an integrative perspective, the results partially validate Ayurvedic descriptions of microbial analogues under terms like visha, jantu, and rakshasa, offering a conceptual bridge between ancient wisdom and modern microbiology. The study underscores the value of re-examining traditional herbs using rigorous scientific methodology to uncover their true pharmacological potential.

In summary, Strychnos potatorum and Operculina turpethum show potential as natural antibacterial agents, particularly in their aqueous and ethyl acetate fractions. These findings support their traditional use and open avenues for further phytochemical and pharmacological investigation, whereas Rubia cordifolia may require different extraction strategies or combinatorial formulations for antimicrobial relevance.

5. CONCLUSION

The present antimicrobial screening confirmed that

5.1 Strychnos potatorum holds promising antibacterial potential, particularly its ethyl acetate and aqueous fractions, showing significant dose-dependent inhibition of E. coli and moderate activity against S. aureus.

5.2 Operculina turpethum extracts also exhibited moderate antibacterial activity, with the methanol and aqueous fractions being more effective.

5.3 Rubia cordifolia extracts were least effective against both test bacteria under the studied conditions.

In conclusion, Strychnos potatorum and Operculina turpethum extracts can be considered as potential sources for further phytochemical isolation and development of novel antimicrobial agents, especially targeting Gram-negative bacteria like E. coli. Further research, including phytochemical profiling and in vivo validation, is recommended to confirm their therapeutic relevance and to elucidate the specific bioactive constituents responsible for the observed

effects.

6. ACKNOWLEDGEMENT

Author wants to give acknowledgement to Govt. ayurved college jalukbari, Guwahati, Guwahati University, IASST guwahati, NIPER guwahati.

7. CONFLICT OF INTEREST: No conflict of interest.

8. REFERENCE

1. Agnivesha (Kashinath Pande, Ed.). Charaka Samhita, Sutra 17/27, 29, 36, 39, 2018; 1: 333). Varanasi: Chaukhambha Bharati Academy.
2. Sushruta (Shastri, Ed.). Susruta Samhita, Nidana 5/19–20 2005; 2: 250). Varanasi: Chaukhamba Sanskrit Sansthan.
3. Shastri AD, Sushruta Samhita with Ayurveda Tattva Sandipika Commentary by, Nidana Sthana, Chapter 5 Chaukhambha Sanskrit Sansthan, Varanasi, Eleventh Edition, 1997; 1.
4. Sharma PV. Dravyaguna Vijnana. 1st ed. Varanasi: Chaukhambha Bharati Academy; 2006; 2: 180–184.
5. Hegde PL, Harini A. A Textbook of Dravyaguna Vijnana. Reprint ed. Varanasi: Chaukhamba Publications; 2018; 365.
6. Sharma PV. Classical Uses of Medicinal Plants. Reprint ed. Varanasi: Chaukhamba Vishwabharati; 2018; 822.
7. Ananthanarayan R, Paniker CKJ. Ananthanarayan and Paniker's Textbook of Microbiology. 10th ed. Hyderabad: Universities Press; 2017; 1–10, 174–180, 289–295.
8. Parija, S. C. (2013). Textbook of Microbiology and Immunology (2nd ed., p. 252). New Delhi: Elsevier India.
9. Pavia, D. L., Lampman, G. M., Kriz, G. S., & Engel, R. G. (2015). Introduction to Organic Laboratory Techniques: A Microscale Approach (5th ed.). Cengage Learning.
10. Clinical and Laboratory Standards Institute (CLSI). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard—11th ed. CLSI document M07-A11. Wayne, PA: CLSI; 2018.