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Antibacterial Potential of *Rhinacanthus Nasutus* (L.) Kurz Leaf Extract against Pathogenic Bacteria

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Abstract

The *in vitro* antibacterial activity of *Rhinacanthus nasutus* leaf was investigated against various strains of bacteria *Bacilus cereus, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus* (Gram positive) and *Escherichia coli, Klebsiella pneumonia, Klebsiella oxytoca, Proteus Mirabilis, Pseudomonas aeruginosa, Serratia marcescens* (Gram Negative) by disc diffusion method. Among the various solvent extracts tested methanol leaf extract showed greater inhibitions against *Enterococcus faecalis* (9.3 mm), *Escherichia* ***coli*** (8.3 mm), and *Bacilus cereus* (8.0 mm). Petroleum ether and acetone extracts also demonstrated moderate antibacterial activities, while chloroform and ethanol extracts showed relatively weaker inhibition. The prominent inhibitory effect of methanol extract can be attributed to the presence of phytochemicals such as flavonoids, tannins, alkaloids, and naphthoquinone derivatives like Rhinacanthin. *In vivo* evaluations are essential to isolate the key active constituents and validate their efficacy against resistant pathogens.

***Keywords*:** *Rhinacanthus nasutus,* antibacterial activity, disc diffusion assay, methanol extract, Inhibition zone, Bacterial pathogen.

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INTRODUCTION

Bacterial pathogens predominantly cause infectious diseases [1]**.** Bacterial species, such as *Klebsiella pneumonia, Escherichia coli, and Staphylococcus aureus*, have developed resistance to various antimicrobial agents [2, 3]**.** Including Gram-positive and Gram-negative bacteria, increasing antibiotic resistance among bacterial pathogens is making treatment more challenging [4]**.** Due to the considerable increase in multidrug-resistant bacteria, Slow progress in developing novel antimicrobial agents and innovative approaches to combat bacterial infections is essential [5,6]**.** Drugs that may stop bacteria from growing (bacteriostatic) and eradicate harmful germs (bactericidal) are referred to as antibacterials, or antibiotics in pharmacology [7, 8]**.**The mechanisms by which antibiotics work include reducing enzyme activity, disrupting bacterial protein production, changing the permeability of bacterial membranes, and damaging bacterial cell walls [7, 9]**.** Because antibiotics are used to treat bacterial infections, they have a major impact on the worldwide decline in infectious diseases. The overuse of antibiotics, however, can reduce their effectiveness and cause bacterial resistance to spread globally [10]**.** Natural substances extracted from plants are widely recognized as important sources of bioactive compounds with medicinal potential [11,12]**.** Renewed efforts are focusing on plants as a rich source of new antimicrobials to address the global issue of growing antibiotic resistance [13]. Historically, medicinal herbs have been integral to traditional healthcare systems, aiding in the treatment of many illnesses, especially infectious diseases [14]. These sources aid in creating drugs that is less toxic to host cells and hold great promise for identifying potent therapeutic compounds [11]. *Rhinacanthus nasutus*, a species in the Acanthaceae family is native to Southeast Asia and China. It is traditionally referred to as Nagamalli, herbaceous perennial herb 1-2 cm in height [15]. It has been employed in the remedy of various health issues, including skin inflammation (eczema), respiratory tuberculosis, viral infections like herpes, liver ailments such as hepatitis, metabolic disorders like diabetes and

obesity, elevated blood pressure, malignancies, leprosy, scurvy, fungal infections like dhobi's itch, and multiple other skin-related diseases [16]. *Rhinacanthus nasutus* foliage contains a diverse range of biologically active substances, including flavonoid and alkaloid groups, phenolic acids, benzenoid compounds, anthraquinones, coumarin derivatives, naphthoquinone types, as well as saponins and tannins. The principal compound identified in the leaves is rinacanthin. It possesses antibacterial, antifungal, anti-inflammatory, and anticancer properties [17]. This study presents the antibacterial properties of *Rhinacanthus nasutus* extract against chosen Bacterial pathogens, demonstrating notable effectiveness against a range of bacterial species, especially Gram-positive ones. Significant antibacterial action is shown by the leaf extracts, particularly against Methicillin-resistant *Staphylococcus aureus* (MRSA). The extracts have been added to herbal soaps and have been shown to effectively suppress a variety of infections, including *Yersinia enterocolitica* and *Bacillus cereus* [18,19]. Its potential as a natural antimicrobial agent has also been increased by the green manufacture of silver nanoparticles from *R.nasutus* leaves, which has demonstrated encouraging antibacterial properties against strains of bacteria resistant to antibiotics [20]. Although *R.nasutus's* antibacterial activity has been the subject of several earlier investigations, our study offers new information on a variety of Gram-positive and Gram-negative bacteria.

MATERIAL AND METHODS

Experimental Microorganisms

The referred microbial strains of bacteria *Bacilus cereus, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus* (Gram positive) and *Escherichia coli, Klebsiella pneumonia, Klebsiella oxytoca, Proteus Mirabilis, Pseudomonas aeruginosa, Serratia marcescens* (Gram Negative) were obtained from Doctor Diagnostic Center, Tiruchirappalli.

Plant sample collection and extract preparation

The experimental plants with high use value were collected from their natural habitats in Different villages of Karur district, Tamilnadu, India. Identification and authentication of *Rhinacanthus nasutus* was done by The Rapinat Herbarium, St.Joesph's College, Tiruchirappalli. The plant parts were thoroughly examined, including old insect-damaged and fungus-infected leaves. The stems and roots were removed. The selected healthy plant portions were spread out and shade dried in the laboratory at room temperature for 5 - 8 days, or until they broke readily with hand. The dried plant components were pulverized into a fine powder using an electronic blender and stored in a closed container at room temperature for future use. Soxhlet extraction of dried plant materials uses solvents such as petroleum ether, chloroform, ethanol, methanol,and acetone. Fifty grams of plant materials (leaves and roots) were boiled individually with 300 ml of each solvent (acetone, methanol, ethanol, chloroform, and petroleum ether) in a soxhlet apparatus for 48 hours at various temperatures. At the end of 48 hours, each extract was filtered through whatmann paper. The filtrates from No. 1 filter paper were concentrated at room temperature. The paste-like extracts were stored at the final weight of the container. The screw cap bottles were stored in the refrigerator at 4˚C.

Antibacterial screening methods

Disc diffusion assay (Maruzella and Henry, 1958) [21]

Sterile liquid Muller Agar medium (pH 7.4±2) was poured (10-15ml) into each sterile petriplates. The growth media also seem to play an important role in the determination of the antibacterial activity. Based on the report by Lin *et al.* Muller- Hinton agar appears to best medium to study [22]**.** After solidification, 100 µl of suspension conataining 108 CFU/ ml of each test bacteria was spread over Muller Hinton Agar plates. The sterile filter paper discs (6mm in diameter) were impregnated with 10µl of the 3mg/ml extracts (30 µg/ disc) placed on the inoculated agar. Negative controls were prepared in using the same solvents employed to dissolve the plant extract. Chloramphenicol (30µg/disc) was used as positive reference control to determine the sensitivity of the plant extract on each bacterial species. The inoculataed plants were incubated at 37˚C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zones. Each assay was conducted in triplicate.

RESULT

The antibacterial properties of various organic solvents derived from the leaf extract of *Rhinacanthus nasutus* were examined against opportunistic bacterial pathogens.The outcomes of the leaf extracts were evaluated against these pathogens. The findings from the antibacterial screening of petroleum ether, chloroform, acetone, methanol, and ethanol leaf extracts of *Rhinacanthus nasutus* (30 mg/disc) are detailed in the results. All extracts

Table 01: Antibacterial Activity of *Rhinacanthus nsutus* by Disc diffusion method

Test bacteria	Petroleum ether (30 µg/disc)	Chloroform (30 µg/disc)	Acetone (30 µg/disc)	Methanol (30 µg/disc)	Ethanol (30 µg/disc)	Positive Control Chloramphenicol (30 µg/disc)
Gram positive	Experimental (30µg/disc)	Experimental (30µg/disc)	Experimental (30µg/disc)	Experimental (30µg/disc)	Experimental (30µg/disc)	Chromophenicol (30µg/disc)

B. cereus	8.3±0.5	-	7.3±0.5	8±1	5.3±4.7	28.3±1.1
E. faecalis	8.3±0.5	-	8±1	9.3±0.57	10±1	42.6±2.8
S. aureus	-	-	-	-	-	43.6±1.5
S. epidermidis	-	-	3±5.1	-	-	37.6±2.3
S. saprophyticus	-	-	-	-	-	33.3±0.5
Gram-negative						
E. coli	8±1	3±5.1	7±0	8.3±0.5	5±4.3	47±1
K. pneumoniae	5±4.35	-	4.6±4.1	6.6±5.5	-	26±3.6
K. oxytoca	-	-	-	-	-	33.6±2.5
P. mirabilis	-	-	-	-	-	17.6±2.5
P. aeruginosa	-	9.6±0.5	-	-	-	36.6±1.5
S. marcescens	-	-	-	-	-	37.3±2.0

'-' represents as 'no inhibition'

demonstrated antibacterial activity against at least two of the pathogens tested. Among the solvents tested, the methanol extract exhibited the most significant antibacterial activity against both gram-positive and gram-negative bacteria, followed by the petroleum ether and acetone extracts. In the methanol extract, a high level of antibacterial activity was noted against *Enterococcus faecalis* (9.3 mm), *Escherichia coli* (8.3 mm), and *Bacillus cereus* (8 mm). Conversely, the petroleum ether extract showed a notable degree of inhibition against *Bacillus cereus* and *Enterococcus faecalis* (8.3 mm). Similarly, the acetone extract displayed a significant level of inhibition on *Enterococcus faecalis* (8.0 mm) in (Table 01)

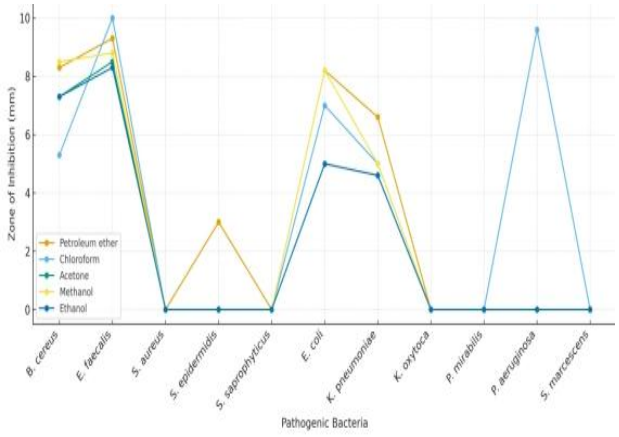

Fig 01: Antibacterial Screening of *Rhinacanthus nasutus* leaf extract

DISCUSSION

The present study results are in accordance with the work done by [23] in *Rhinacanthus nasutus* where they reported the significant antibacterial activity against gram positive bacteria such as *Bacillus cereus, Bacillus subtilis* and *Staphylococcus aureus* and gram negative bacteria such as *Proteus mirabilis, Pseudomonas aeruginosa* and *Escherichia coli*. The present study results are also supported by thework done by Senthilkumar *et al* 2016 [24] where methanol extract has shown the high degree of inhibition against all the bacteria tested. Similarly, the highest zone of inhibition was noted against *Bacillus megaterium* by methanol stem bark etracts of *Albizzia lebbeck* [25]. Similar results were obtained by Yavuz et al, [26] in their studies where they reported methanol extract of *Scutellaria salviifolia*had significant antibacterial activity against various bacterial strains with the inhibition zone ranging from 11 to 17 mm in diameters. The methanol leaf extract of *Xylocarpus granatum* exhibited 18 mm zone of inhibition around the disc. The highest zone of inhibition was observed in methanol extracts of *Ruta chalapensis* against *Staphylococcus aureus* and *Pseudomonas aeruginosa* [27]. The maximum zone of inhibition (12.02±0.14 mm) was notice in methanol leaf extract of *Tagetes erecta* [28]. Methanol extract, found in leaf extracts, exhibits significant antibacterial activity due to its ability to dissolve and extract secondary metabolites with antimicrobial properties, including

flavonoids, tannins, phenols, saponins, glycosides, and terpenoids [29].

CONCLUSION

The extract of *Rhinacanthus nasutus* demonstrated significant antimicrobial activity against various pathogenic Bacteria. The methanol extract of the leaves effectively inhibited the growth of Bacteria including *B. cereus, E. Faecalis, S.aureus, S. Epidermidis, S. Saprophyticus, E. Coli, K. pneumonia, K. oxytoca, P. Mirabilis, P. Aeruginosa, S. Marcescens.* The Greatest inhibition observed was *Enterococcus faecalis* (9.3 mm), *Escherichia* ***coli*** (8.3 mm), and ***Bacilus cereus*** (8.0 mm) indicating its potential as an antimicrobial agent. This results supports the ethnopharmacological use of *Rhinacanthus nasutus* for treating microbial infections. The presence of phytochemicals such as flavonoids, tannins, alkaloids, and naphthoquinone derivatives like Rhinacanthin in the extract may contribute to its antimicrobial properties**.**

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AUTHORS CONTRIBUTION STATEMENT

Thangaraj Francis Xavier has designed the whole study including antibacterial assay at Department of Botany, St.Joseph's College, Trichy and prepared the manuscript. Antony Sami Auxilia conducted antibacterial assay and prepared contribution part of manuscript. Kesavan Vinisha prepared the part of the manuscript. Antony kanthi Freeda Rose prepared the part of manuscript. All the authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Chandekar C. Evaluation of antibacterial activity of plant extracts on antibiotic resistant bacteria. Int J Curr Microbiol Appl Sci. 2018;7(12):1164–70.
2. Khan R, Islam B, Akram M, Shakil S, Ahmad AA, Ali SM, et al. Antimicrobial activity of five herbal extracts against multidrug resistant strains of bacteria and fungus of clinical origin. Molecules. 2009;14(2):586–97.
3. Solís-Salas LM, Sierra-Rivera CA, Cobos-Puc LE, Ascacio-Valdés JA, Silva-Belmares SY. Antibacterial potential by rupture membrane and antioxidant capacity of purified phenolic fractions of Persea americana leaf extract. Antibiotics (Basel). 2021;10(5):508.
4. Wahab MA, Li L, Li H, Abdala A. Silver nanoparticle-based nanocomposites for combating infectious pathogens: recent advances and future prospects. Nanomaterials (Basel). 2021;11(3):581.
5. Abreu AC, McBain AJ, Simões M. Plants as sources of new antimicrobials and resistance-modifying agents. Nat Prod Rep. 2012;29(9):1007–21.
6. Bhatia P, Sharma A, George AJ, Anvitha D, Kumar P, Dwivedi VP, et al. Antibacterial activity of medicinal plants against ESKAPE: an update. Heliyon. 2021;7(2):e06310.
7. Magani AK, Tallei TE, Kolondam BJ. Uji antibakteri nanopartikel kitosan terhadap pertumbuhan bakteri Staphylococcus aureus dan Escherichia coli [Antibacterial test of chitosan nanoparticles against the growth of Staphylococcus aureus and Escherichia coli]. J Bios Logos. 2020;10(1):7–12.
8. Sadikin N, Bintari S, Widiatningrum T, Dewi P. Isolasi, karakteristik, dan uji aktivitas antibakteri dari bakteri endofit daun kelor (Moringa oleifera) [Isolation, characteristics, and antibacterial activity test of the endophytic bacteria of Moringa leaves (Moringa oleifera)]. Life Sci. 2021;10(2):109–19.
9. Septiani D, Dewi NH, Wijayanti I. Aktivitas antibakteri ekstrak lamun (Cymodocea rotundata) terhadap bakteri Staphylococcus aureus dan Escherichia coli [Antibacterial activity of seagrass extract (Cymodocea rotundata) against Staphylococcus aureus and Escherichia coli]. Saintek Perikanan. 2017;13(1):1–6.
10. Rani GN, Budumuru R, Bandaru NR. Antimicrobial activity of honey with special reference to methicillin resistant Staphylococcus aureus (MRSA) and methicillin sensitive Staphylococcus aureus (MSSA). J Clin Diagn Res. 2017;11(8):DC17–20.
11. Jayapriya G, Gricilda Shoba F. Phytochemical analysis and antimicrobial efficacy of Rhinacanthus nasutus (L.) Linn. J Pharmacogn Phytochem. 2015;3:83–6.
12. Pallavali R, Avula S, Degati L, Penubala M, Damu AG, Durbaka V. Data of antibacterial activity of plant leaves crude extract on bacterial isolates of wound infections. Data Brief. 2019;24:103896.
13. Pacyga K, Pacyga P, Topola E, Viscardi S, Duda-Madej A. Bioactive compounds from plant origin as natural antimicrobial agents for the treatment of wound infections. Int J Mol Sci. 2024;25(4):2100.
14. El-Saadony MT, Saad AM, Mohammed DM, Korma SA, Alshahrani MY, Ahmed AE, et al. Medicinal plants: bioactive compounds, biological activities, combating multidrug-resistant microorganisms, and human health benefits – a comprehensive review. Front Immunol. 2025;16:1491777.
15. Brimson MJ, Prasanth MI, Malar DS, Brimson S, Tencomnao T. Rhinacanthus nasutus "tea" infusions and the medicinal benefits of the constituent phytochemicals. Nutrients. 2020;12:3776.
16. Bukke S, Raghu PS, Sailaja GR, Kedam T. The study on morphological, phytochemical and pharmacological aspects of Rhinacanthus nasutus (L.) Kurz: a review. J Pharm Sci Innov. 2011;1:1–7.

17. Gomes TA, Elias WP, Scaletsky IC, Guth BE, Rodrigues JF, Piazza RM, et al. Diarrheagenic Escherichia coli. Braz J Microbiol. 2016;47(Suppl 1):3–30.
18. Febria FA, Rahmadeni Y, Bachtiar A. Antibacterial potential ethanol extract of kayu racun leaf (Rhinacanthus nasutus) against Staphylococcus aureus and methicillin resistant Staphylococcus aureus. JPMIPA. 2021;12(2):180. doi:10.26418/JPMIPA.V12I2.46801
19. Abdul Rahman KAM, Ahmad N, Mukhtar NA, Zarkasi KZ. Antimicrobial soap development from leaf extract of Rhinacanthus nasutus. BIO Web Conf. 2024;131:02004.
20. Nagasinduja V, Shahitha S. In-vitro antimicrobial activity of green synthesised silver nanoparticles of leaf extract of Rhinacanthus nasutus against bacterial food borne pathogens. J Clin Diagn Res. 2022;16(1):DC01–DC05. doi:10.7860/JCDR/2022/56581.16713
21. Maruzzella JC, Henry PA. The antimicrobial activity of perfume oils. J Am Pharm Assoc. 1958;47(7):471–6.
22. Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jäger AK, et al. Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and antimicrobial activities. J Ethnopharmacol. 1999;68:267–74.
23. Sattar MA, Abdullah NA, Khan A, Noor AM. Evaluation of antifungal and antibacterial activity of local plant Rhinacanthus nasutus (L.). J Biol Sci. 2004;4(4):498–500.
24. Senthilkumar RP, Bhuvaneshwari V, Sathiyavimal S, Malayaman V, Amsaveni R, Kalaiselvi M. Phytochemical analysis and antibacterial activity of Rhinacanthus nasutus. Int J Biosci Nanosci. 2016;3:8–13.
25. Gawade SP, Khan ZS, Chavan-Patil VK. Phytochemical analysis and antibacterial activity of plants. J Pharm Res. 2010;1(2):163–9.
26. Yavuz C, Kiliç DD, Ayar A, Yildirim T. Antibacterial effects of methanol extracts of some plant species belonging to Lamiaceae family. Int J Secondary Metabolite. 2017;4:429–33.
27. Priya PS, Sasikumar JM, Gowsigan G. Antibacterial activity of methanol extract of Ruta chalapensis (L), Quercus infectoria (Oliver) and Canthium parviflorum (Lam). Anc Sci Life. 2009;29(2):28–31.
28. Mech P, Chaturvedi VK, Priyadarsini S. Antimicrobial activity of six different plant extracts against clinical isolates of Staphylococcus spp. from milk origin. Int J Adv Biochem Res. 2024;8(7S):102–9.
29. Nigussie D, Davey G, Legesse BA, Fekadu A, Makonnen E. Antibacterial activity of methanol extracts of the leaves of three medicinal plants against selected bacteria isolated from wounds of lymphoedema patients. BMC Complement Med Ther. 2021;21(1):2. doi:10.1186/s12906-020-03183-0