IAMJ

INTERNATIONAL AYURVEDIC MEDICAL JOURNAL

Research Article ISSN: 2320-5091 Impact Factor: 6.719

IN VITRO EVALUATION OF THE ANTIBACTERIAL ACTIVITY OF AQUEOUS EXTRACTS OF NIRGUNDI PATRA (VITEX NEGUNDO LINN) AGAINST PATHOGENS ASSOCIATED WITH DUSHTA VRANA

Tejaswini N[1], Divya P[2], Shashirekha K S[3]

[1]*Assistant Professor, Department of Roga Nidana Evam Vikriti Vijyana, Rajeev Institute of ayurvedic medical science and research centre & Hospital, Hassan*
[2]*Professor and HOD, Department of Roga Nidana Evam Vikriti Vijyana, SDM College of Ayurveda and Hospital, Hassan*
[3]*Microbiologist , Department of Roga Nidana Evam Vikriti Vijyana, SDM College of Ayurveda and Hospital , Hassan*

Corresponding Author: drtejaswininagaraju@gmail.com

https://doi.org/10.46607/iamj0514042026

(Published Online: April 2026)

Article Received: 12/03/2026 - **Peer Reviewed:** 30/03/2026 - **Accepted for Publication:** 14/04/2026.

Check for updates

ABSTRACT

Chronic ulcers are wounds that fail to heal; in general, they have a fibrotic margin and a granulation-tissue bed, which may include areas of slough (necrotic tissue). One of the main reasons for non-healing ulcers is the presence of microbes, which promotes the chronicity of these lesions, characterised by an abnormally prolonged inflammatory phase. *Ayurveda* also lists causes of *Dushtavrana*, including '*Krimi Bhakshana'*. Commonly encountered microbial communities in chronic wounds include Staphylococcus spp., Pseudomonas spp., and Escherichia coli. Use of diagnostic tools like culture and sensitivity to identify causative microorganisms, their characteristics, attributes, and culture this organism in vitro and check sensitivity against *Nirgundi,* considered to be effective against *Staphylococcus spp, Pseudomonas spp and Escherichia coli.*

Keywords: *Chronic ulcer, Dhushta vrana, Nirgundi Patra, Pus culture and sensitivity, microorganism*

INTRODUCTION

Chronic non-healing wounds constitute a significant economic burden on healthcare systems and substantially diminish the quality of life for affected patients. These wounds frequently precede severe complications, such as limb amputations or premature mortality.[1]

Currently, approximately 1% of the adult population and 3.6% of individuals over 65 years of age are affected by this condition[2]. From a contemporary medical perspective, *Dushta Vrana* in Ayurvedic texts is analogous to chronic non-healing ulcers, exhibiting similar clinical features such as *Puyasrava* (purulent discharge) and *Deergha kalanubandhi* (persistent chronicity). *Sushruta* emphasised the importance of *Vrana* (wound) management, outlining therapeutic measures within the *Shashtir Upakramas*, including *Krimighana* (antimicrobial therapy), *Dhoopana* (fumigation), and *Raksha karma* (protective interventions). These principles align with the recognition of bacterial colonisation in non-healing wounds, in which *Staphylococcus spp.*, *Streptococcus spp.*, *Pseudomonas spp.*, and *Enterobacteriaceae* are among the most frequently isolated pathogens[3].

Classics have described *Krimighna* or *Jantughna* (microbial actions) karma of some plant drugs for the *Dushta vrana* like *Vidanga, Katuki, Nimba,* etc. Hence, drugs possessing these properties need to be revalidated against specific microorganisms, thereby generating Upashaya and Anupashaya effects of the drug on microorganisms in vitro. *Nirgundi* is one such drug having properties of *Vrana Shodana*, *Ropana, Jantugna* and *Shotahara*. It is used in the treatment of various disorders such as *Krimi, Kushta, Vrana, and Visha*[4]. Phytochemical studies on *Nirgundi* (*Vitex Negundo Linn*.) revealed the presence of Volatile Oil, Triterpenes, Diterpenes, Sesquiterpenes, Ligan, Flavonoids, Flavones, Glycosides, Iridoid Glycosides and Stilbene Derivatives. In an effort to screen the spectrum of antibacterial agents from natural sources as an *Upashaya* in non-healing ulcer, *Nirgundi* (Vitex negundo) is selected for an in vitro study against Pathogenic bacteria by culture and sensitivity in Dushta vrana, with special reference to Non-healing Ulcer.

AIMS AND OBJECTIVES

To evaluate the sensitivity of aqueous extract of *Nirgundi (Vitex negundo)* against Pathogenic bacteria from pus samples of *Dushtavrana* (Nonhealing Ulcer) patients by culture and sensitivity in vitro.

MATERIALS AND METHODS

A clinical study was conducted at a tertiary Ayurvedic hospital in Hassan, including patients of either sex and all age groups presenting with non-healing ulcers of 4–6 weeks duration associated with *Puya srava* and exhibiting one or more features of *Dushta Vrana Lakshanas,* such as *Kandu*, *Amanojna gandha*, *Atisamvruta*, *Atimrudu*, *Atyavasanna*, *Rakta/Krishna/Pandu varna*, *Putimamsa avarana*, *Shotha*, *Paka*, *Unmargi vrana*, and *Dushta vrana shonita*. Patients with ulcers of less than six weeks' duration were excluded from the study. Individuals diagnosed with tuberculosis, varicose ulcer, malignant ulcer, or systemic conditions such as HIV/AIDS, HBsAg positivity, or any other disorders that could interfere with wound healing or study outcomes were also excluded.

RESEARCH DESIGN

An observational experimental study

METHODOLOGY

The extract of *Nirgundi* is prepared by the cold maceration method. Fresh *Nirgundi* leaves (100 g) were washed, sun-dried, weighed, and finely powdered. The powder was subjected to cold maceration in 250 mL of distilled water in 1000 mL conical flasks sealed with cotton plugs and tape. The mixtures were stirred for 10–15 min at 3-hour intervals during the daytime for seven days. On day 7, extracts were filtered (yield ~150 mL) and concentrated on a water bath at 60 °C to obtain 6g crude extract, which was used for antimicrobial testing.

Patients who met the diagnostic and inclusion criteria were enrolled after a detailed clinical history was obtained. Pus samples from *Dushta Vrana* cases were collected aseptically and examined microscopically and by culture. Positive samples were subcultured on

MacConkey agar and blood agar using the streak plate method. The plates were then incubated for 24–48 hours at 37°C. After incubation, cultural characteristics, such as colony morphology, were studied 6,7,8. Antimicrobial sensitivity was assessed by agar well diffusion. The workspace was disinfected with 70% ethanol and exposed to UV light for 20 min. Sterile MacConkey agar (15 mL) was poured into Petri plates, solidified, and inoculated with 24–48 h bacterial cultures to obtain lawn growth. Six wells were made using a sterile corn borer, and extract concentrations of 7000, 6000, 3000, 1500, and 750 µg/mL were added. Plates were incubated at 37 °C for 24–48 h, and zones of inhibition were measured in millimetres.

Table No.1 Organism Identified

Organism	Frequency	Percentage
Staphylococcus spp	14	46.66
Pseudomonas spp	10	33.33
E. Coli	6	20.0

Table No 2: colony characteristics of *Staphylococcus spp*

Culture characteristics	Organism identified
Size – 2.2mm Shape- Round Surface- Smooth Elevation- Raised Edge- Entire Opacity- Opaque Colour – Yellow Consistency -Buttery	*Staphylococcus spp*

Table No 3: Colony characteristics of Pseudomonas

Culture characteristics	Organism identified
Size – 2.2-3mm Shape- Oval Surface- Smooth Elevation- Raised Edge- Entire Opacity- Opaque Colour – Bluish green Consistency -Buttery	*Pseudomonas*

Table no 4: Colony characteristics of *E.coli*

Culture characteristics	Organism identified
Size – 2.2mm Shape- Round Surface- Smooth Elevation- Raised Edge- Entire Opacity- Opaque	*E. coli*

Colour – Yellow Consistency -Buttery	

Assessment criteria

Antimicrobial activity was determined by observing clear circular zones surrounding the wells, indicating inhibition of bacterial growth. The presence of a zone of inhibition signified the organism's susceptibility to the extract, whereas its absence indicated resistance. Sensitivity was interpreted based on zone diameter measurements and classified as sensitive (S) when a distinct inhibition zone was present, intermediate/moderate (I/M) when partial inhibition was observed, and resistant (R) when no inhibition zone was detected.

Observation and result

In vitro antibacterial activity of the aqueous extract of *Nirgundi* was evaluated by the agar well diffusion method, and the zone of inhibition was measured as shown in the table.

Table No. 5. Mean values of zone of inhibition at different concentrations of aqueous extract of *Nirgundi* against

Different concentrations of aqueous extract of Nirgundi	**7000** µg/ml	**6000** µg/ml	**3000** µg/ml	**1500** µg/ml	**750** µg/ml
Total number of patients (N)	30	30	30	30	30
Mean zone of inhibition in mm	20.06	16.62	13.65	11.34	7.646

The aqueous extract of *Nirgundi* demonstrated antimicrobial activity against *Staphylococcus* spp., *Pseudomonas* spp., and *E. coli*, with zones of inhibition ranging from 24 mm to 4 mm across concentrations of 7000–750 µg/mL. The maximum inhibition of 24 mm was observed at 7000 µg/mL, while the minimum of 4 mm occurred at 750 µg/mL. Sensitivity interpretation showed 7000 µg/mL as sensitive, 6000 µg/mL as moderately sensitive, and 3000, 1500, and 750 µg/mL as resistant. Mean zones of inhibition for concentrations 7000, 6000, 3000, 1500, and 750 µg/mL were 20.06 mm, 16.62 mm, 13.65 mm, 11.34 mm, and 7.64 mm, respectively.

DISCUSSION

The present study demonstrates that pathogenic bacteria such as Staphylococcus, Pseudomonas, and E. coli are susceptible to the aqueous extract of Vitex negundo (*Nirgundi*), with zones of inhibition ranging from 18 to 22 mm, indicating sensitivity. Zones measuring 17–11 mm indicate moderate sensitivity, while values below 10 mm suggest resistance. At a concentration of 7000 µg/ml, the extract produced a mean zone of inhibition of 20.06 mm against *Staphylococcus* spp. and *Pseudomonas*, indicating sensitivity, whereas *E. coli* exhibited moderate sensitivity. At 6000 µg/ml, the mean zone of inhibition was 16.62 mm, and all three organisms (*Staphylococcus* spp., *E. coli*, and *Pseudomonas*) showed moderate sensitivity. In contrast, at lower concentrations (3000, 1500, and 750 µg/ml), these microorganisms were resistant.

The observed antimicrobial activity of *Nirgundi* may be attributed to its bioactive phytochemicals, which can disrupt microbial cell membranes and interfere with protein, nucleic acid, and enzyme synthesis, thereby inhibiting microbial growth. In addition, its antioxidant properties may reduce oxidative stress, inhibit bacterial adhesion and biofilm formation, and enhance bacterial susceptibility to host immune defenses and antimicrobial agents 9. However, at lower concentrations (1500–750 µg/ml), the extract becomes less effective against resistant strains. Pathogens such as *Staphylococcus aureus*, *Pseudomonas*

aeruginosa, and *E. coli* can form biofilms that protect them from immune responses and antimicrobial therapy. *S. aureus* produces toxins and enzymes that damage host tissues, and resistant strains such as MRSA further complicate treatment. *P. aeruginosa* secretes Exotoxin A and other degradative factors and possesses efflux pumps that contribute to antimicrobial resistance. *E. coli* utilizes adhesion factors to colonies tissues, form biofilms, and release toxins that promote inflammation and delay healing. Such antibiotic-resistant organism's complicate management and prolong infections 10.

The variation in zones of inhibition at different concentrations may also reflect differences in diffusion rates of active constituents. Intrinsic characteristics, including cell wall structure and membrane permeability, influence microbial susceptibility. Certain phytochemicals may fail to exert antimicrobial effects due to organisms' cytological features and variations in membrane porosity, which limit penetration 11. Higher concentrations (7000 and 6000 g/ml) of aqueous or alcoholic extracts exhibit greater antimicrobial activity due to higher levels of active compounds. As concentration decreases, the reduced availability of these molecules may be insufficient to disrupt bacterial capsules or membranes, leading to apparent resistance. Differences in virulence among strains, along with cellular components that facilitate attachment, invasion, and immune evasion, also contribute to variability in response. The phytochemicals present in the extracts may interact with membrane enzymes and proteins, disrupt proton gradients, inhibit amino-acid biosynthesis, and ultimately cause microbial cell death. Their hydrophobic properties may further compromise microbial cell structure and permeability by altering surface tension and interacting with extracellular proteins and nucleic acids. As the concentration of *Nirgundi* extract increased, the zone of inhibition increased. Hence, it was concluded that as the concentration increases, antibacterial activity against Staphylococcus aureus, Pseudomonas, and E. coli increases.

REFERENCES

1. Jarbrink K, Ni G,Sonnergrenn H, Schmidtchen A, Pang C, Bajpai R,et al.The Humanistic and Economic Burden of Chronic Wounds: A Protocol for a Systematic Review. Syst Rev.2017 Jan 24;6:15.
2. Agale S V. Chronic Leg Ulcers: Epidemiology, Aetiopathogenesis, and Management. Hindawi Publishing Corporation Ulcers.2013;2013(413604):9 http://dx.doiorg/10.1155/2013/413604
3. Panda P, Tripathy Sarvodaya – prevalence of microbial agents associated with chronic non-healing ulcer: A cross–sectional study. Vol 13(5).2020.447-453
4. Sharma P V, Dravyaguna Vijnan (Vegetable drugs)-Vol II, 4th ed. : Varanasi: Chaukhamba Sanskrit Sansthan; 1978, p.67.
5. Jothik S O, Borse N V – A case study of Nirgundi (Vitex Nigundo Linn.) Taila in the management of dushta vrana.Vol.3(3)Jul-Sept2020
6. B S Nagoba, Asha Pichare, Medical Microbiology and Parasitology, 4th ed. Haryana, Elsevier RELX India Pvt. Ltd; 2020 P.3
7. B S Nagoba, Asha Pichare, Medical Microbiology and Parasitology, 4th ed. Haryana, Elsevier RELX India Pvt. Ltd; 2020 P.3
8. B S Nagoba, Asha Pichare, Medical Microbiology and Parasitology, 4th ed. Haryana, Elsevier RELX India Pvt. Ltd; 2020 P.3
9. Nagoba, B. S., & Pichare, A. (2020). Medical Microbiology and Parasitology PMFU (4th ed.). Elsevier India. ISBN: 9788131261194.
10. Nagoba, B. S., & Pichare, A. (2020). Medical Microbiology and Parasitology PMFU (4th ed.). Elsevier India. ISBN: 9788131261194.
11. Nagoba, B. S., & Pichare, A. (2020). Medical Microbiology and Parasitology PMFU (4th ed.). Elsevier India. ISBN: 9788131261194

Source of Support: Nil
Conflict of Interest: None Declared

How to cite this URL: Tejaswini N et al: In Vitro Evaluation of the Antibacterial Activity of Aqueous Extracts of Nirgundi Patra (Vitex negundo Linn) Against Pathogens Associated with Dushta Vrana.. International Ayurvedic Medical Journal {online} 2026 {cited April 2026}