WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
Coden USA: WJPRAP Impact Factor 8.63
Volume 15, Issue 21, 1450-1454. Research Article ISSN 2277–7105

# A STUDY ON ANTIMICROBIAL ACTIVITY OF *NIMBATRIVRITADI LEPA*

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Article Received on 05 Oct. 2026,
Article Revised on 25 Oct. 2026,
Article Published on 01 Nov. 2026,

https://doi.org/10.5281/zenodo.23910605

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**How to cite this Article:** Dr. Maria Thomas[1]*, Dr. Radhika Ranjan Geethesh P.[2]. (2026). A Study on Antimicrobial activity of Nimbatrivritadi Lepa. World Journal of Pharmaceutical Research, 15(21), 1450–1454.
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## ABSTRACT

Lepa Kalpana is a classical dosage form in the category of external management of various conditions. As being part of *Bahyaparimarjana chikitsa* it delivers its actions by bypassing the oral route. *Dusta Vrana* is condition with its wide range of presentations and complexity of prognosis with time. Chronic ulcer may be considered under the roof of Dusta vrana. An important aspect in the context of Chronic ulcer is the presence of microbes which worsen the condition. Various formulas have been mentioned in classics with either less or a greater number of ingredients for its properties like *Vrana Shodhana* properties. The present work was conducted to understand the antimicrobial potential of *Nimbatrivritadi Lepa*, by emphasising on its HPTLC and in-vitro parameters on selected microorganisms. This study analyses the concept of *Vrana Shodana* property of *Nimbratrivritadi Lepa* by evaluating the antimicrobial activity. The understanding of *Vrana Shodana* must not only be done with the antimicrobial activity but all such factors or process so as to prepare the *Vrana Sthana* suitable for *Ropana*.

**KEY WORDS:** *Nimbatrivritadi lepa, Vrana Shodana.*

## INTRODUCTION

Nimbatrivritadi Lepa is mentioned in Vangasena samhita under Dustavrana rogadhikara. This classical Ayurvedic formulation containing drugs such as *Nimba patra, Trivrit, Danti, Tila, Makshika, Saindava lavana.*[1] Most of the drugs in this combination individually are known for their, Vrana Shodana (wound cleansing), and Vrana Ropana (wound healing) properties. During the preparation of *Lepa, Mardana*; grinding may help in molecular bonding and breaking between phytochemicals, resulting in synergistic complexes. Understanding the antimicrobial potential of this combination is important to know its clinical potential. When a drug is mentioned individually for *Vranashodan*a, multifold understanding may be done for its antimicrobial or anti-inflammatory or combined. Antimicrobial study will help to know it's inhibitive potential against selected strains of bacterial and fungal pathogens.

## MATERIALS AND METHODS

### Preparation of *Nimbatrivritadi lepa*

SDM Ayurveda pharmacy Udupi is the source of herbal and mineral raw materials. Honey was bought from Dabur company. All the drugs except honey was obtained in dry form. These drugs except honey was taken 20gms each All the drugs were separately pounded in *Khalwa yantra* and made into course powder later added to the pulveriser to reduce the particle size. The individual drugs were sieved and kept in separate Ziplock cover. Since Tila is a oil seed it was dried and made into a paste and measured 20gms. Saindava lavana was mixed and not pulverized.

### Analytical studies

Physico- chemical analysis was carried out by considering both classical and modern parameters.

**Table 1: Organoleptic Characters.**

| Sl. No. | Parameters | Results |
|---|---|---|
| 1 | Sparsha | Gritty |
| 2 | Rasa | Sweet-bitter |
| 3 | Gandha | Pleasant |
| 4 | Rupa | Blackish brown |

### HPTLC[2]

The underlying principle of HPTLC is adsorption. When a mixture containing one or more components is spotted on a thin layer of adsorbent coated on a chromatographic plate and

introduced into a development tank containing the mobile phase, the mobile phase flows by capillary action. 1gm of *Nimbatrivritadi lepa* sample was dissolved in 10.0ml of ethyl alcohol warmed on a water bath and filtered. 4, 8, 12µl of the sample was applied on a precoated silica F254 on aluminum plates to a bandwidth of 8mm using Linomat 5 T.L.C. applicator. The plate was developed in Toluene-Acetone: Formic acid (9.0:6.0:1.0), and the developed plates were visualized under Short UV, Long UV, and scanned under UV 254nm and 366nm. Rf, the colour of the spots, and densitometric scan were recorded.

The steps used are: 1. Selection of chromatographic layer- Stationary phase 2. Sample and standard preparation 3. Activation of precoated plates 4. Application of sample and standard 5. Selection of mobile phase 6. Chromatographic development 7. Detection and visualization of spots 8. Scanning and quantification 9. Documentation.

**Concept of Synergism**

The interaction of two or more agents agents results in combined effects that greater than the sum of the individual parts.[3] Although a difficult one to prove this is seen within the single herb and between the herbs in a compound formulation.[4]

**Antimicrobial study[5]**

Microorganisms provide the models for the molecular study of organisms.

Invitro antimicrobial study of *Nimbatrivritadi lepa* was evaluated by Agar well diffusion method on • Staphylococcus aureus • Pseudomonas aeruginosa • Candida albicans.

**Antimicrobial activity of *Nimbatrivritadi lepa* on Staphylococcus aureus**

Method used is categorised into following steps:

Preparation of casein soya bean agar medium

Preparation of inoculum

Agar Well diffusion

The media was cooled to around 45-55°C, around 20ml each was poured into sterile Petri plates. Later 1 ml of the inoculum was immediately added to the plate, swirled for uniform distribution. It should be kept for 1 hour. Wells were bored using a sterile borer. The samples and the tibiotic were dispensed into the wells. Plates were incubated overnight at 37°C and served after 24 hours.

**Table 2: In Vitro-Antibacterial Activity Test for Nimbatrivritadi Lepa against STAPHYLOCOCCUS Aureus.**

| Sample | Volume | Zone of inhibition – (Radius in mm) |
|---|---|---|
| Nimbatrivritadi lepa (10mg/ml) | 25µl | 0 |
| Nimbatrivritadi lepa (10mg/ml) | 50µl | 0 |
| Nimbatrivritadi lepa (10mg/ml) | 75 µl | 0 |
| Nimbatrivritadi lepa (10mg/ml) | 100µl | 1 |
| Internal control ( Honey) | 50 µl | 22 |
| Standard( Ampicillin) (5mg/500µl) | 25µl | 16 |

**Table 3: In Vitro-Antibacterial Activity Test for *Nimbatrivritadi Lepa* against *Pseudomonas Aeruginosa.***

| Sample | Volume | Zone of inhibition – (Radius in mm) |
|---|---|---|
| Nimbatrivritadi lepa (10mg/ml) | 25µl | 0 |
| Nimbatrivritadi lepa (10mg/ml) | 50µl | 0 |
| Nimbatrivritadi lepa (10mg/ml) | 75 µl | 0 |
| Nimbatrivritadi lepa (10mg/ml) | 100µl | 15 |
| Internal control ( Honey) | 50 µl | 0 |
| Standard( Gentamycin) (240µg) | 25µl | 20 |

**Table 4: In Vitro-Antibacterial Activity Test for *Nimbatrivritadi Lepa* against *Candida Albicans.***

| Sample | Volume | Zone of inhibition – (Radius in mm) |
|---|---|---|
| Nimbatrivritadi lepa (10mg/ml) | 25µl | 0 |
| Nimbatrivritadi lepa (10mg/ml) | 50µl | 0 |
| Nimbatrivritadi lepa (10mg/ml) | 75 µl | 0 |
| Nimbatrivritadi lepa (10mg/ml) | 100µl | 0 |
| Internal control ( Honey) | 50 µl | 0 |
| Standard( Clotrimazole) | 25µl | 12 |

## RESULTS AND DISCUSSION

Drugs involving *Nimba, Trivrit, Danti, Tila*, Honey, and *Lavana*, this specific solvent system toluene-ethyl acetate targets non polar to moderately polar compounds. The primary types of components eluted include: Triterpenoids & Steroids: Compounds like azadirachtin, nimbin (from Nimba), and lupeol, beta-sitosterol (from Nimba and Tila) readily migrate with this solvent systems. Glycosides including steroidal glycosides and bitter principles like triterpenoid glycosides often found in Nimba bark but more in leaves. Phenolic Acids & Flavonoids: When a small amount of a polar acid (such as formic acid) is added to the toluene-ethyl acetate mixture, it successfully resolves bioactive flavonoids and phenolic acids originating from, *Nimba and Trivrit*. To clearly view and distinguish these components after elution, plates are typically derivatized using reagents like vanillin-sulphuric acid or

Liebermann-Burchard, which highlight the presence of steroids and terpenes. Azadirachtin & Nimbin from *Nimba*, disrupt bacterial cell wall structures and prevent cell division. They are notably effective against Gram-positive bacteria like Staphylococcus aurues. But the percentage of both decreases due to drying in temperature above 55 degree Celsius. Lupeol & Sitosterol present in *Nimba* and *Tila* alter cytoplasmic membrane permeability. This causes the internal contents of the bacteria to leak out, neutralizing the pathogen. The natural acids within honey lower environmental pH, creating a hostile environment that prevents bacterial proliferation. Turpentines from *Trivrit and Danti* function as natural defence mechanisms for the plant. To what extent the activity is yet to be quantified. When we see the zone of inhibition in of plates of Staphylococcus we see that zone of inhibition is nearly nil for the formulation while the vehicle has more than that of standard. While in plate were Pseudomonas were present the inhibition was near to that of standard for the formulation in higher concentration while zero in case of vehicle. This shows individual properties of a ingredient may change in a compound formula for varied environment.

## CONCLUSION

With the light of preliminary studies, the antimicrobial activity cannot be completely justified. *Vrana shodhana lepa* is the external application and may exhibit its action through various ways for say destruction of Biofilm which is one of the main cause for antimicrobial resistance and hinders the healing. It can also be It helps to know the probable nature of the combination. Further studies must be conducted for validation. The phytoconstituents vary greatly with quantitatively with factors like heat and drying. Mobile phase used in the HPTLC also is a factor for separation of compounds.

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