



COMPARATIVE IN-VITRO STUDY TO EVALUATE THE *Krimighna Karma* (Antimicrobial activity) of *Nirgundi Patra* (*Vitex negundo* L.) AGAINST *Staphylococcus aureus* BACTERIA WITH SPECIAL REFERENCE TO *Desha*

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ABSTRACT

Comparative In Vitro Study to Evaluate the Krimighna Karma (Antimicrobial Activity) of Nirgundi Patra (Vitex negundo L.) Against Staphylococcus aureus with Special Reference to Desha

Nirgundi (*Vitex negundo* L.), belonging to the family of Lamiaceae, is widely distributed throughout India and possesses several therapeutic properties, including Krimighna, Vishaghna, Shothahara, and Shulahara. Desha plays an important role in the collection of medicinal plants, as geographical and environmental factors may influence their potency. Ayurvedic classics mention that drugs collected from Jangala and Sadharana Desha possess greater potency. Hence, the present study was undertaken to evaluate and compare the Krimighna Karma of Nirgundi Patra collected from Jangala Desha (Koppal), Sadharana Desha (Hassan), and Anupa Desha (Udupi) against *Staphylococcus aureus* bacteria.

The collected samples were subjected for aqueous extraction of leaves further an in-vitro antimicrobial study was performed to compare their activity against *S. aureus*. The findings indicate that Desha has influence on the antimicrobial activity of Nirgundi Patra. However, the results did not substantiate the assumption that Jangala and Sadharana Desha samples possess greater potency. The Anupa Desha sample exhibited comparatively greater antimicrobial activity.

KEYWORDS: Nirgundi, Krimighna, Desha, Dravya Sangrahana, *Staphylococcus aureus*.

INTRODUCTION

In Ayurveda, *Dravya Sangraha Vidhi* is done by considering specific criteria like 1.Desha Sampath (Drug collected from suitable place), 2.Kaala Sampath (Drug collected in suitable season), 3.Guna Sampath (When drug gets enriched with medicinal qualities), 4.Baajana Sampath (Suitable method adopted to preserve drug)^[1], plays an important role to get ideal drug. So to obtain drug having high potency with best quality, it becomes necessary to collect drug from suitable Desha and drug with high potency become capable to cure diseases effectively and rapidly. There are many classical reference in Ayurveda specially deals with collection of medicinal plants in a specific place to obtain maximum therapeutic efficacy. Among them, Jangala and Sadharana Desha are considered as Prashasta^[2].

Nirgundi (*Vitex negundo* L.) is a shrub or small tree belonging to family Lamiaceae. It is commonly found throughout India^[3]. It possesses Karmas like Vishagna, Krimighna, Shothahara, Shulahara, Kushtagna, Kandugna, Kasahara, Keshya, Netrya, and Jwaragna^[4]. It contains phytochemicals like Vanillic acid, Luetolin, glycine, leucine and phenolic compounds such as flavonoids which are responsible for its antimicrobial activity^[5]. It is an important ingredient of Krimihara kashaya^[6], Nirgundi Rajanyadi Taila, Nirgundyadi Ghrita, Nirgundyadi Kashaya^[7]. Owing to its wide clinical importance, this drug has been selected for the study.

Staphylococcus aureus is one of the most common bacterial infections in human, which causes diseases like *Pneumonia*, *Furuncles*, *Carbuncles*, *Cellulitis*, *Impetigo*, and *Abscess*^[8]. A systematic review and a meta-analysis reported a 37% overall prevalence of MRSA (Methicillin-resistant *Staphylococcus aureus*) (2015 to 2019) in India. State-by-state stratified results of MRSA prevalence varied from 55% in Jammu & Kashmir to 21% in Maharashtra.^[9]

As Nirgundi(*Vitex negundo* L.) is mentioned in *Krimighna Mahakashaya* in Ayurveda classics^[10], it becomes relevant to revalidate the concept of Prashasta Beshaja with the help of performing Krimighna Karma (Antimicrobial activity) of Nirgundi Patra collected from 3 different deshas Jangala (Koppal), Sadharana (Hassan), Anupa (Udupi), against (*Staphylococcus aureus*) bacteria, with special reference to Desha as said by Acharya Charaka^[11].



MATERIAL AND METHODS

1. Source Of Data

- **Primary Source:** Drug collection was done from three *Deshas*- Koppal, Udipi and Hassan.
- **Secondary Source:** Literary Information was collected from *Ayurvedic* classical literatures, relevant contemporary literatures, electronic database with the pertinent past and present research works.

2. **Authentication of Drug:** was done from Department Of Dravyaguna, Shri Dharmasthala Manjunatheshwara College Of Ayurveda And Hospital Hassan

3. Preparation of Aqueous Extract of *Nirgundi Patra of Jangala, Sadharana and Anupa Desha*

➤ Materials required:

- Coarse powder of *Nirgundi Patra* collected from *Jangala, Sadharana* and *Anupa Desha*.
- Distilled water
- Conical flask
- Measuring cylinder
- Digital balance
- China dish
- Spinex orbital shaker
- Water bath

➤ Procedure:

- The coarse powder of *Nirgundi Patra of Jangala, Sadharana* and *Anupa Desha* taken separately and weighed for 5g.
- 50ml of distilled water is measured separately and taken for 3 samples separately.
- 3 conical flasks are taken and 5g of coarse powder of *Nirgundi Patra of Jangala, Sadharana* and *Anupa Desha* is added to each conical flask along with labeling *Jangala, Sadharana* and *Anupa Desha* for respective conical flask.
- Then each conical flask is added with 50ml of distilled water.
- This Conical flask containing solution is kept for rotation in spinex orbital shaker with 16rpm for 24hrs.
- After 24 hrs, these solutions were filtered using filter paper.
- The filtrates are collected in a pre weighed in china dish.
- The china dish containing filtrates were kept in water bath at 105°C till the solvent is completely dried.
- Once it get properly dried, the china dish containing these extracts is weighed once again using digital balance.
- Then the extract is collected and stored in 3 different vials by labeling it as aqueous extract of *Jangala, Sadharana* and *Anupa Desha*, Respectively.

4. Anti-Bacterial Study

Method: Agar well diffusion

Material required:

- Test strain- *Staphylococcus aureus*
- Distilled water
- Normal saline
- Test tubes
- Incubator
- Laminar air flow
- Growth media- Nutrient agar.

Procedure

Preparation of Nutrient agar media

Beef extract (1 g), Yeast extract (2 g), Peptone (5 g) and Sodium Chloride (5 g) were dissolved in 900 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally 15 g agar was added to the media and autoclaved at 121°C for 15 minutes.

Preparation of the inoculum

Staphylococcus aureus strain was procured from Microbial Type Culture Collection and Gene Bank (MTCC 3160), Chandigarh. Loopful of 24 h old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

Well Diffusion Method

The media was cooled to around 45-55°C, around 20ml each was poured into sterile petriplates. One ml of the inoculum was immediately added to the plate, swirled for uniform distribution. Wells were bored using a sterile borer. The samples and the antibiotic were dispensed into the wells. Plates were incubated overnight at 37°C and observed after 24 h.

Assessment Criteria

The sensitivity test was performed using the well diffusion method. If the drug is effective, a clear circular zone of inhibition appears around the well, indicating the absence of bacteria and demonstrating that *Nirgundi* is effective against the specific bacteria.

Interpretation and measurement of zone of inhibition in mm as follows:

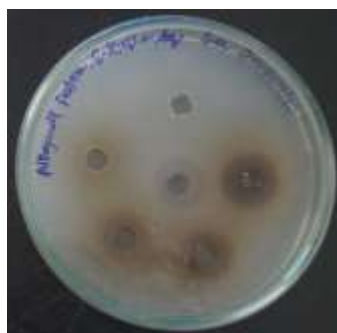
Three zones appear

1. Sensitive zone
2. Intermediate zone
3. Resistant zone.

RESULTS AND DISCUSSION



A.



B.



C.

Fig.1 Showing the antimicrobial Culture plates of test drug (*Nirgundi Patra*); A: *Jangala Desha*, B: *Sadharana Desha*, C: *Anupa Desha* against *Staphylococcus aureus* bacteria.

Table1: samples and antibiotic taken for aqueous extract of *Jangala, Sadharana* and *Anupa Desha Nirgundi Patra* against *Staphylococcus aureus* bacteria.

Samples	Concentration (in μL)	Zone of inhibition (Radius in mm)		Average
Aqueous extract of <i>Nirgundi patra</i> of (<i>Jangala Desha</i>)	25	0	0	0
	50	0	0	0
	75	6	6	6
	100	10	9	9.5
Aqueous extract of <i>Nirgundi patra</i> of (<i>Sadharana Desha</i>)	25	0	0	0
	50	0	0	0
	75	8	7	7.5
	100	11	9	10
Aqueous extract of <i>Nirgundi patra</i> of (<i>Anupa Desha</i>)	25	0	0	0
	50	0	0	0
	75	9	8	8.5
	100	11	9	10
Control-Distill Water	50	0	0	0
Standard (Ampicillin) 5mg/500 μL	25	9	9	9



- The aqueous extract of *Nirgundi Patra* from *Anupa Desha* showed zones of inhibition of 8.5 mm at 75 µL and 10 mm at 100 µL, with no activity at 25 µL and 50 µL. The activity was higher than samples from *Jangala* and *Sadharana Desha*, contrary to the expected result that these regions would show greater inhibition.
- This observation may be attributed to the fact that plants growing in *Anupa Desha* are continuously exposed to higher microbial load and potential pathogens. Such exposure creates biotic stress, compelling the plant to activate defense mechanisms for survival. As a result, specific biochemical pathways are stimulated, leading to enhanced production of secondary metabolites such as flavonoids and phenolic compounds^[12] which are responsible for showing more antimicrobial effect in comparison with the *Nirgundi Patra* collected from *Jangala* and *Sadharana Desha*.

CONCLUSION

- ❖ Among the three habitats, *Nirgundi* collected from *Anupa Desha* exhibited comparatively higher antimicrobial efficacy, while *Jangala* and *Sadharana Desha* samples showed relatively lesser activity.
- ❖ Hence through this study it is proven that the *Desha* has influence on the therapeutic efficacy of drug. But through this antimicrobial study it cannot be substantiated that drug collected from *Jangala Desha* has superior efficacy, in comparison with drug collected from *Sadharana* and *Anupa Desha*.

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