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Analytical Study And Invitro Antimicrobial Effect Of Nirgundi Malahara

DR. HARI R [1] DR. MANJUNATHA BHAT[2]*

[1] P.G. Scholar Department Of Shalyatantra Alvas Ayurveda Medical College

[2]* Professor, H.O.D Department of Shalyatantra Alvas Ayurveda Medical College

Corresponding Author: **Dr Manjunatha Bhat**

Professor, HOD, Department of Shalya Tantra, Alvas Ayurveda Medical College, Moodbidri

ABSTRACT

Nirgundi Taila, a classical Ayurvedic preparation mentioned in the Yogaratnakara, is known for its efficacy in healing *Dusta Vrana* (non-healing ulcers). To enhance patient compliance with its topical application, the formulation was modified into a *Malahara*. This study aimed to evaluate the primary analytical properties and antimicrobial effects of the modified *Nirgundi Malahara,* ensuring its safety and efficacy in promoting ulcer healing. Analytical evaluations confirmed compliance with standard parameters, with pH at 3.88, low total ash content (0.03), absence of acid-insoluble ash, and minimal loss on drying (0.03). In vitro antimicrobial assays revealed that the *Malahara* exhibited significant activity against Escherichia coli (NCIM 2065) with inhibition zones of 15 mm and 13 mm at 80% and 50% concentrations, respectively, and against Staphylococcus aureus (NCIM 2127) with an inhibition zone of 11 mm at 80% concentration. These findings suggest that the modified *Malahara* may retain the therapeutic potential of the original *Taila* and offer a more effective and convenient formulation for managing *Dusta Vrana* .

Keywords: *Malahara, Nirgundi Malahara*, Analytical study, antimicrobial study

I. INTRODUCTION

Nirgundi Taila, a classical Ayurvedic preparation mentioned in *Yogaratnakara,* is well-known for its effectiveness in managing *Dusta Vrana* (non-healing ulcers) (1). *Nirgundi* poses *Katu-Tikta Rasa* and properties such as *Kleda Shoshana, Krimighna, Shodhana, Shothahara* and *Soolahara*, all of which contribute to its wound-healing properties (2). The healing of *Dusta vrana* poses significant challenges in wound management, often requiring innovative therapeutic interventions. Ayurveda offers a rich repository of time-tested formulations for such conditions, with *Nirgundi taila* being one of the effective remedies. However, the oil-based nature of *Nirgundi Taila* can make topical application and storage difficult. To enhance patient compliance with its topical application and storage, the formulation was modified into a *Malahara* form, while preserving its therapeutic properties.

Malahara preparations, as described in *Rasatarangini,* are characterized by their semi-solid consistency, making them more convenient for localized applications. Their ease of use and reduced risk of leakage make them a better alternative for topical application for managing *Dusta vrana*, especially in challenging anatomical region

Thus, this study aimed to develop *Nirgundi Malahara* following the classical *Malahara Kalpana* preparation method and to evaluate its analytical parameters and antimicrobial efficacy against clinically significant pathogens such as Escherichia coli and Staphylococcus aureus to provide insights into its potential therapeutic applications

II. AIMS AND OBJECTIVES

To prepare *Nirgundi Malahara* by adopting classical *Malahara* preparation and to evaluate the analytical parameters and invitro antimicrobial efficacy

III. MATERIALS AND METHODS

To prepare *Nirgundi Malahara* in the classical *Malahara Kalpana* method, these steps were followed:

Collection of raw drugs: The fresh plant of *Nirgundi* (*Vitex negundo* Linn) was sourced from the local vendors in Mudbidiri, Karnataka. *Tila Taila* and *Siktha* (bee wax) were procured from Alva's Ayurvedic Pharmacy, Mijar, Moodbidri, Karnataka. The identity, purity, and strength of all ingredients were ensured following the parameters outlined in Ayurveda Pharmacopoeia of India

Pharmaceutical preparation

The entire process of preparation of *Nirgundi Taila* and *Nirgundi Malahara* was carried out under the supervision of the experts in the Rasasastra and Bhaishajya Kalpana Lab, Alva's Ayurveda Medical College, Moodbidri, Karnataka, India ensuring adherence to traditional methods.

Preparation of *Nirgundi Taila*

Nirgundi Taila was prepared following the guidelines outlined in the Yogaratnakara(1). Equal amounts of freshly prepared *Nirgundi swarasa* (juice) and *Tila Taila* (sesame oil) were combined. The *Taila* preparation process was continued until the *Kalka* (herbal paste) reached the *Madhyama Paka* stage. Heating was then halted, and the mixture was filtered through a double-layered cotton cloth into a clean, dry stainless-steel vessel.

Table 1: Ingredients of *Nirgundi Taila*

Sl. No	Ingredients	Botanical name	Family	Part used	Proportion
1	Nirgundi	*Vitex negundo* Linn	Verbenaceae	Whole plant	1
2	Tila Taila	sesame oil			1

Preparation of *Nirgundi Malahara*

Malaharas are typically made by adding bases like *Siktha* (bee wax), *Sarjarasa*, etc., to either *Pakwa* (cooked) or *Apakwa* (uncooked) *Sneha,* and then thoroughly mixing until the desired consistency, softness, and smoothness are achieved (2). Rasatarangini provides guidelines for preparing *Siktha Taila* according to the season. During the *Greeshma Rithu* (hot season), *Siktha* and *Taila* are recommended to be taken in the ratio of 1:5. In contrast, during the *Sheeta Rithu* (cold season), the recommended ratio is 1:6. *Sikta* was combined with the obtained *Nirgundi Taila* at a ratio of 1:6 respectively (3).

The *Nirgundi Taila* was initially heated in a stainless-steel vessel over a gentle flame. Scraped bee wax was gradually added to the heated *Taila* and stirred continuously until the bee wax completely melted into the *Taila.* The resulting mixture was then filtered through a clean cotton cloth into a *Khalwa Yantra* (mortar) and thoroughly triturated with a pestle until it reached a butter-like consistency. After allowing the product to self-cool, it was packaged into ointment tubes each with a capacity of 20g and stored in a clean, dry place.

Table 2: Ingredients of Nirgundi Malahara

Sl. No	Ingredients	Proportion	Quantity taken
1	*Nirgundi Taila*	6 parts	600 g
2	*Siktha*	1 part	100 g

Analytical Parameters Evaluation: Analytical evaluations were conducted at CARe KERALAM Ltd. KINFRA Park, Koratty, Thrissur, Kerala. Organoleptic properties were assessed. Parameters such as pH (10% aqueous solution) and loss on drying were determined following Ayurveda Pharmacopoeia of India (API) methods (4),(6). Total Ash and Acid insoluble Ash were determined following Indian pharmacopoeia (IP) methods.

Antimicrobial activity: The antimicrobial efficacy of *Nirgundi Malahara* was conducted at CARe KERALAM Ltd. KINFRA Park, Koratty, Thrissur, Kerala through in vitro assays against clinically relevant microorganisms in controlled laboratory conditions using the agar well diffusion method.

Test organisms used: Escherichia coli and Staphylococcus aureus

Procedure: The procedure includes inoculum preparation, preparation of Mueller-Hinton Agar (MHA) plates, inoculation of bacterial cultures, and assessment of the zone of inhibition after incubation.

Inoculum preparation: Transfer a loopful of bacterial culture from working stock slants to 5ml of Mueller-Hinton broth (MHB). Incubate the culture at 35°C until a visible turbidity equivalent to 0.5 MacFarland unit was obtained.

Preparation of MHA Plates: Prepare MHA plates in sterile Petri dishes according to standard protocols and then allow the MHA plates to solidify.

Inoculation of Bacterial Cultures: A sterile swab was taken and dipped into the broth culture. Squeeze the swab gently against the inner wall of the tube to remove excess fluid. A lawn of the growth of the test organism was then made by swabbing on the surface of the MHA plate. Then, allow the plate to dry for 5 minutes.

Preparation of Wells: Using a sterile Cork Borer of 8mm diameter, wells were prepared on the swabbed agar plates.

Sample Application: 100µl of sample concentrations was added to the wells using a micropipette. The solvent and standard drug controls were also included alongside the samples.

Incubation: MHA plates were then placed in a biosafety cabinet until complete diffusion of the sample occurred. MHA plates were then incubated at 35°C for 24 hours.

Assessment of Zones of Inhibition: After incubation, the MHA plates were observed for the presence of zones of inhibition around the wells. The diameter of the zones of inhibition was measured and recorded. The results were then compared with standard interpretations to determine antimicrobial susceptibility

IV. OBSERVATION AND RESULTS

Nirgundi Taila

Table 3: Organoleptic evaluation of *Nirgundi Taila*

Sl. No	Parameters	Results
1	Colour	Green
2	Odour	Characteristic odour
3	Consistency	Viscous

Nirgundi Malahara

Table 4: Organoleptic evaluation of *Nirgundi Malahara*

Sl. No	Parameters	Results
1	Colour	Greenish yellow
2	Odour	Characteristic odour
3	Consistency	Thick viscous

The analytical evaluation was done to ascertain the quality of the sample. The results are given in Table 5. The results comply with the standards of Ayurveda Pharmacopeia of India.

Table 5: Analytical evaluation of *Nirgundi Malahara*

Sl. No	Parameters	Results
1	pH	3.88
2	Total Ash	0.03
3	Acid insoluble Ash	Nil
4	Loss on Drying	0.03

Evaluation of the antimicrobial activity of *Nirgundi Malahara* by agar well diffusion method

The inhibitory effect of the sample against Escherichia coli and Staphylococcus aureus is given in the Table 6

The results of the antimicrobial activity tests for *Nirgundi Malahara* against Escherichia coli (NCIM 2065) and Staphylococcus aureus (NCIM 2127) can be summarized as follows:

1. Escherichia coli (NCIM 2065):

Nirgundi Malahara demonstrates antimicrobial activity against Escherichia coli, especially at concentrations of 80% and 50%.

2. Staphylococcus aureus (NCIM 2127):

Nirgundi Malahara exhibits antimicrobial activity against Staphylococcus aureus at a concentration of 80%.

Table 6: Antimicrobial activity of *Nirgundi Malahara*

Sl. No	Test organisms	Standard drug (positive control)	Test sample			Solvent control (Hexane)	Figure reference
			800000 ppm (80%)	500000 ppm (50%)	250000 ppm (25%)		
1	Escherichia coli (NCIM 2065)	Streptomycin 1000ppm – 23,23	15, 15	13, 13	NZ, NZ	NZ, NZ	Figure 1
2	Staphylococcus aureus (NCIM 2127)	Streptomycin 1000ppm – 27, 27	11, 11	NZ, NZ	NZ, NZ	NZ, NZ	Figure 2

NZ: No Zone of inhibition

Figure 1: Inhibitory activity of test sample against Escherichia coli

Figure 2: Inhibitory activity of test sample against Staphylococcus aureus

V. DISCUSSION

The analytical parameters of the prepared *Nirgundi Malahara* were determined and compared against established standards.

The pH value of 3.88 for the prepared *Nirgundi Malahara* has several implications for its use in *Dushta* and *Nadi Vrana*. It is slightly acidic and hence can promote wound healing by facilitating the breakdown of dead tissues and promoting the activities of certain enzymes involved in the healing process (7).

It also can inhibit the growth of certain microorganisms, potentially reducing the risk of infection which is crucial for preventing complications associated with anal fissures and such other infections (8). Furthermore, the acidic pH contributes to the stability of the formulation by inhibiting microbial growth, ensuring a longer shelf life.

The total ash content of a formulation indicates the amount of inorganic material present after burning. A low ash content like 0.03 suggests a relatively pure product with minimal impurities, which could imply better quality and potentially higher effectiveness in medicinal or other applications (9).

The acid-insoluble ash content in herbal products indicates the presence of impurities such as silica, sand, and other inert materials that are not soluble in acid. Higher levels of acid-insoluble ash may suggest poor quality or contamination, affecting the purity and efficacy of the product. The absence of acid-insoluble ash in *Nirgundi Malahara* signifies high purity and quality (9).

The loss on drying (LOD) refers to the amount of moisture lost when the sample is dried under specific conditions. Understanding the LOD is crucial because it can impact the stability of the product, shelf life, and dosage accuracy. A higher LOD could indicate inadequate drying or potential moisture absorption, affecting the quality and efficacy. LOD of 0.03 in *Nirgundi Malahara* suggests that the product has minimal moisture content.

This low moisture content can imply better stability, longer shelf life, and higher efficacy since excessive moisture can degrade herbal products and lead to microbial growth. Therefore, a low LOD is generally considered favourable for the quality of the product (10).

Nirgundi Malahara demonstrated antimicrobial activity against Escherichia coli and Staphylococcus aureus. The vitro antimicrobial assays revealed that the *Malahara* exhibited significant activity against Escherichia coli (NCIM 2065) with inhibition zones of 15 mm and 13 mm at 80% and 50% concentrations, respectively, and against Staphylococcus aureus (NCIM 2127) with an inhibition zone of 11 mm at 80% concentration. *Nirgundi possesses Kadu-thikta Rasa,* along with *Laghu Guna.* It exhibits properties like *Krimihara* (anti-parasitic*), Kustahara* (anti-dermatosis), and *Jantuhara* (anti-infective) (2). These characteristics substantiate the antimicrobial activity and the utility of *Nirgundi Malahara* in treating non-Healing ulcers like anal fissures and others.

VI. CONCLUSION

Nirgundi Malahara is developed based on the Malahara kalpana described in Rasatarangini. *Malahara kalpana* offers several advantages over *Taila kalpana* for local application, including more patient acceptance, ease of application, better presentation, better storage and reduced risk of leakage, especially when applied to areas like the anal region. Preliminary analytical studies conducted on *Nirgundi Malahara* show that the parameters are within standard limits, ensuring the quality and safety of the formulation. Further, the antimicrobial studies demonstrate that *Nirgundi Malahara* exhibits activity against Escherichia coli and Staphylococcus aureus. Thus, the present studies serve as a baseline, offering valuable insights and providing a foundation for future research to explore the therapeutic potential of *Nirgundi Malahara* through clinical studies.

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