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# PHARMACEUTICO–ANALYTICAL AND IN VITRO ANTIMICROBIAL ACTIVITY OF HANSPADYADI TAILA AGAINST STAPHYLOCOCCUS AUREUS

**[1]*Dr. Kalpraj Anilkumar Kothari, [2]Dr. Renuka M. Suryawanshi**

[1]PG 3rd Year Department of Rasshastra and Bhaishajyakalpana Pravara Ayurved College Shevgaon, Ahilyanagar.

[2]Guide, M.D. Ph.D, H.O.D and Professor, Department of Rasashastra and Bhaishajya Kalpana, Pravara Medical Trust's Ayurved College, Shevgaon, Ahilyanagar.

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***Corresponding Author**

**Dr. Kalpraj Anilkumar Kothari**

PG 3rd Year Department of Rasshastra and Bhaishajyakalpana Pravara Ayurved College Shevgaon, Ahilyanagar.

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## ABSTRACT

Hanspadyadi Taila is a classical Ayurvedic formulation used in Nadivrana and infected wounds. This study prepared Hanspadyadi Taila using Murchhita and Amurchhita Tila Taila and evaluated their pharmaceutical, physicochemical, microbiological, and in vitro antibacterial properties. Both formulations showed acceptable quality and no detectable bacterial contamination. Against *Staphylococcus aureus*, Murchhita and Amurchhita Hanspadyadi Taila produced inhibition zones of 19 mm and 15 mm, respectively, compared with 26 mm for streptomycin. The findings demonstrate the antibacterial potential of Hanspadyadi Taila, with comparatively greater activity in the Murchhita formulation.

**KEYWORDS:** Hanspadyadi Taila, Murchhita Tila Taila, Nadivrana, *Staphylococcus aureus*, antimicrobial activity.

## INTRODUCTION

Ayurveda is an ancient system of medicine that emphasizes the preservation of health and the prevention and treatment of disease by maintaining the equilibrium of Dosha, Dhatu, Mala, and Agni. In addition to internal medication, Ayurveda gives considerable importance to external therapies and wound management. Various Ayurvedic formulations possess Vrana

Shodhana (wound-cleansing), Vrana Ropana (wound-healing), Krimighna (antimicrobial), Shothahara (anti-inflammatory), and Vedanasthapana (analgesic) properties.[1]

Nadivrana is described in Ayurveda as a deep-seated tract or sinus associated with persistent discharge, pain, inflammation, recurrent infection, and delayed healing. It may develop due to the improper management of wounds, retained foreign bodies, trauma, deep tissue suppuration, or recurrent infection.[2] In modern clinical practice, Nadivrana may be correlated with chronic sinus wounds, pilonidal sinus, fistulous tracts, and non-healing infected ulcers. These conditions are often difficult to manage because persistent microbial contamination and inflammation interfere with normal tissue repair.[3]

Among the microorganisms responsible for skin, soft-tissue, and wound infections, *Staphylococcus aureus* is one of the most important human pathogens. It is commonly associated with abscesses, boils, cellulitis, postoperative wound infections, chronic ulcers, and infected sinus tracts.[4] Its ability to produce toxins, enzymes, and biofilms contributes to persistent infection and a reduced response to treatment. Furthermore, the emergence of antibiotic-resistant strains of *S. aureus* has become a major challenge in clinical practice.[5] Although antibiotics remain the principal treatment for bacterial infections, their prolonged or inappropriate use may contribute to antimicrobial resistance, hypersensitivity reactions, adverse effects, and recurrence of infection.

Sneha Kalpana is an important pharmaceutical process described in Ayurveda for the preparation of medicated oils and ghee. In this process, Sneha Dravya is processed with Kalka Dravya and a prescribed liquid medium under controlled heating. This method facilitates the incorporation of both lipid-soluble and water-soluble constituents into the final formulation.[6] Tila Taila, obtained from *Sesamum indicum*, is widely used as a base for Ayurvedic medicated oils because of its Sukshma, Vyavayi, and Yogavahi properties. Its penetrating and carrier properties are believed to facilitate the delivery of herbal constituents into deeper tissues.

Taila Murchhana is a preliminary pharmaceutical procedure performed before the preparation of medicated oil. It is traditionally believed to improve the colour, odour, stability, acceptability, and therapeutic quality of the oil while reducing unpleasant smell and susceptibility to rancidity. The process may also influence the physicochemical properties of the oil and the extraction or retention of active phytoconstituents during subsequent

pharmaceutical processing.[7] Therefore, comparison of formulations prepared with Murchhita and Amurchhita Tila Taila is important for understanding the pharmaceutical relevance of Murchhana.

Hanspadyadi Taila is a classical Ayurvedic formulation mentioned in *Bhaishajya Ratnavali* for the management of Nadivrana. It contains Hanspadi (*Adiantum lunulatum*), Nimba Patra (*Azadirachta indica*), and Jati Patra (*Jasminum grandiflorum*), processed in Tila Taila (*Sesamum indicum*).[8] Hanspadi is traditionally used in wounds and inflammatory conditions and has demonstrated antimicrobial potential attributed to flavonoids, tannins, and phenolic compounds. Nimba is recognized for its Krimighna, Kandughna, and Vranashodhaka properties and has reported antibacterial, antifungal, anti-inflammatory, and immunomodulatory activities. Jati Patra is traditionally used for ulcers, wounds, and skin disorders and has also demonstrated antimicrobial and anti-inflammatory properties.

Scientific standardization is essential for establishing the identity, quality, purity, stability, and reproducibility of Ayurvedic formulations. Organoleptic assessment, physicochemical analysis, chromatographic profiling, and microbiological evaluation provide measurable parameters for determining pharmaceutical quality.[9] In vitro antimicrobial testing provides preliminary evidence regarding the inhibitory activity of a formulation against selected pathogenic microorganisms. The agar well diffusion method is commonly employed for this purpose, in which antimicrobial activity is assessed by measuring the zone of inhibition around the test sample.[10]

Although the individual ingredients of Hanspadyadi Taila possess documented antimicrobial and wound-healing properties, limited scientific evidence is available regarding the complete formulation, particularly its activity against *Staphylococcus aureus*. Furthermore, the effect of Taila Murchhana on its pharmaceutical, analytical, and antimicrobial characteristics has not been adequately investigated. Therefore, the present study was undertaken to prepare Hanspadyadi Taila using Murchhita and Amurchhita Tila Taila, evaluate their pharmaceutical and analytical characteristics, assess their microbiological quality, and compare their in vitro antimicrobial activity against *Staphylococcus aureus*.

**AIM**

To prepare and comparatively evaluate Hanspadyadi Taila using Murchhita and Amurchhita Tila Taila through pharmaceutico-analytical parameters and to assess their in vitro

antimicrobial activity against *Staphylococcus aureus*.

## METHODOLOGY

Two samples of Hanspadyadi Taila were prepared using Murchhita and Amurchhita Tila Taila according to the classical reference in *Bhaishajya Ratnavali*. Both formulations were evaluated for organoleptic characteristics, refractive index, specific gravity, iodine value, saponification value, acid value, and pH. Thin-layer chromatography was performed to identify phytochemical markers, and total bacterial count was determined to assess microbiological quality. Antimicrobial activity against *Staphylococcus aureus* was evaluated using the agar well diffusion method, with streptomycin as the standard.

### Nadivrana and wound infection

Nadivrana is described in Ayurveda as a chronic condition in which a narrow tract develops within the tissue and produces persistent discharge. It may arise when pus remains confined within a swelling, a wound is improperly treated, or a foreign body is retained within the tissue. If the underlying cause is not removed, the tract may become chronic and resist healing.[11] The principal management of Nadivrana includes cleansing of the tract, removal of unhealthy tissue and foreign material, control of infection, and promotion of healthy granulation and wound healing.[12]

Nadivrana may be clinically compared with chronic discharging sinuses, infected fistulous tracts, pilonidal sinus, and other non-healing wounds. Wound healing is a complex biological process consisting of haemostasis, inflammation, proliferation, and tissue remodelling. The presence of microorganisms can prolong the inflammatory phase, damage newly formed tissue, and delay wound closure. Other factors such as poor blood supply, repeated trauma, systemic disease, and impaired immunity can further interfere with healing.[13]

*Staphylococcus aureus* is a Gram-positive bacterium frequently isolated from skin, soft-tissue, postoperative, and chronic wound infections. It possesses several virulence factors, including surface proteins, toxins, coagulase, and enzymes that promote tissue invasion and immune evasion. It can also form biofilms on damaged tissues, thereby increasing its persistence and reducing susceptibility to antimicrobial treatment.[14] The emergence of methicillin-resistant and multidrug-resistant strains of *S. aureus* has increased the difficulty of treating such infections and highlights the need to investigate alternative antimicrobial agents.[15]

**Hanspadyadi Taila**

Hanspadyadi Taila is a classical Ayurvedic medicated oil indicated for Nadivrana in *Bhaishajya Ratnavali*. The formulation contains Hanspadi, Nimba Patra, and Jati Patra processed in Tila Taila. The combination is traditionally intended to cleanse the sinus tract, control microbial involvement, reduce inflammation, and promote wound healing.[16] The therapeutic action of the formulation may be attributed to the combined effects of its herbal ingredients and the penetrating and carrier properties of Tila Taila.

Hanspadi, botanically identified as *Adiantum lunulatum*, is traditionally used in inflammatory conditions, wounds, fever, and certain infectious disorders. Phytochemical investigations have reported the presence of flavonoids, tannins, phenolic compounds, terpenoids, and other secondary metabolites. These constituents may contribute to its antioxidant, anti-inflammatory, and antimicrobial effects. Extracts of *Adiantum lunulatum* have demonstrated inhibitory activity against different pathogenic microorganisms, supporting its inclusion in formulations intended for infected wounds.[17]

Nimba, botanically identified as *Azadirachta indica*, is an important medicinal plant possessing Tikta and Kashaya Rasa and is traditionally described as Krimighna, Kandughna, Shothahara, and Vranashodhaka. Neem leaves contain biologically active compounds such as nimbidin, nimbolide, quercetin, and other limonoids and flavonoids. These constituents have demonstrated antibacterial, antifungal, antioxidant, anti-inflammatory, and immunomodulatory activities.[18] The antimicrobial and wound-cleansing properties of Nimba may help reduce microbial load and local inflammation in infected wounds.

Jati Patra, obtained from *Jasminum grandiflorum*, is traditionally used in the management of ulcers, wounds, and skin diseases. The leaves contain flavonoids, phenolic compounds, tannins, glycosides, and other bioactive constituents. Experimental studies have reported antimicrobial, antioxidant, anti-inflammatory, and wound-healing activities of the plant.[19] Jati Patra may therefore support both the cleansing and healing actions of Hanspadyadi Taila.

Tila Taila, obtained from *Sesamum indicum*, serves as the lipid base of the formulation. Ayurveda considers Tila Taila suitable for medicated oil preparation because of its Sukshma, Vyavayi, and Yogavahi properties. These properties help the oil spread over tissues and act as a carrier for the active constituents of incorporated medicinal plants. Sesame oil contains sesamin, sesamolin, sesamol, tocopherols, and unsaturated fatty acids that possess nutritional

and antioxidant properties.[20] Its emollient nature may also protect the wound surface and provide a favourable environment for healing.

**Pharmaceutical importance of Taila Murchhana**

Taila Murchhana is a preliminary processing procedure carried out before the preparation of medicated oil. During this procedure, Tila Taila is processed with selected herbal drugs and water under controlled heating. Murchhana is traditionally performed to remove the undesirable odour and impurities of the oil and to improve its colour, fragrance, stability, acceptability, and therapeutic properties.[21]

The Murchhana process may influence physicochemical parameters such as refractive index, specific gravity, iodine value, saponification value, and acid value. A lower acid value generally indicates reduced liberation of free fatty acids and better resistance to hydrolytic rancidity. Changes in iodine and saponification values may reflect alterations in the degree of unsaturation and fatty-acid composition of the processed oil. Therefore, comparative analytical evaluation of Murchhita and Amurchhita formulations helps determine the pharmaceutical influence of the Murchhana procedure.[22]

**Analytical standardization**

Standardization is necessary to ensure the quality, safety, consistency, and reproducibility of herbal and Ayurvedic medicines. Organoleptic parameters such as colour, odour, appearance, texture, and consistency provide preliminary information about the identity and acceptability of a formulation. Physicochemical parameters provide objective information regarding the characteristics, purity, degradation, and stability of medicated oils.[23]

Thin-layer chromatography is a simple and useful technique for detecting and comparing phytochemical constituents in herbal formulations. Marker compounds produce characteristic spots according to their retention factor values. The identification of quercetin in Murchhita Hanspadyadi Taila and gallic acid in Amurchhita Hanspadyadi Taila indicates the presence of bioactive phenolic and flavonoid constituents. Both compounds are known to possess antioxidant, anti-inflammatory, and antimicrobial properties that may contribute to the observed biological activity of the formulations.[24]

Microbiological quality assessment is equally important because herbal preparations may become contaminated during the collection, processing, preparation, or storage of raw

materials. Determination of the total bacterial count provides information about the hygienic quality of a formulation. The absence of detectable bacterial contamination in both samples indicates satisfactory preparation and storage conditions under the conditions of the study.[25]

**Evaluation of antimicrobial activity**

The agar well diffusion method is commonly used for the preliminary evaluation of antimicrobial activity. In this method, a standardized microbial suspension is inoculated over the surface of an agar medium, and the test formulation is introduced into wells prepared in the agar. Following incubation, antimicrobial activity is assessed by measuring the diameter of the clear zone around each well. A larger zone generally indicates greater microbial growth inhibition under the specified experimental conditions.[26]

In the present study, Murchhita Hanspadyadi Taila produced a 19 mm zone of inhibition against *Staphylococcus aureus*, while Amurchhita Hanspadyadi Taila produced a 15 mm zone. Streptomycin, used as the standard antimicrobial drug, produced a 26 mm zone of inhibition, whereas the control did not demonstrate inhibitory activity. The findings indicate that both formulations possessed in vitro antibacterial activity, with comparatively greater inhibition observed for the Murchhita formulation.

The difference between the two formulations may be related to changes produced by Taila Murchhana, including the incorporation of additional phytoconstituents, improvement in oil stability, and modification of its physicochemical characteristics. However, because the study was based on descriptive observations without repeated experimental replicates, the findings should be interpreted as preliminary. Further studies should include replicate testing, determination of minimum inhibitory and bactericidal concentrations, evaluation against additional wound pathogens, advanced chromatographic analysis, toxicity assessment, and controlled clinical trials.

## PHARMACEUTICAL RESULTS

**Table 1: Pharmaceutical preparation details.**

| Preparation | Oil used | Swarasa/Water | Kalka | Time required | Initial quantity | Final quantity | Loss |
|---|---|---|---|---|---|---|---|
| Murchhita Tila Taila | Tila Taila, 250 mL | Water, 1000 mL | Murchhana Kalka, 54.6 g | 90 min | 250 mL | 230 mL | 20 mL |
| Murchhita Hanspadyadi | Murchhita Tila Taila, | Hanspadi-Nimba-Jati Swarasa, 920 | Kalka of constituent | 60 min | 230 mL | 210 mL | 20 mL |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Taila | 230 mL | mL | drugs, 60 g | | | | |
| Amurchhita Hanspadyadi Taila | Amurchhita Tila Taila, 250 mL | Hanspadi-Nimba-Jati Swarasa, 1000 mL | Kalka of constituent drugs, 60 g | 90 min | 250 mL | 220 mL | 30 mL |

**Table 2: Observations during Murchhana of Tila Taila.**

| Sr. No. | Observation parameter | Finding |
|---|---|---|
| 1 | Initial colour | Light yellow |
| 2 | Initial odour | Characteristic sesame odour |
| 3 | Frothing during heating | Present initially and gradually reduced |
| 4 | Change in odour | Pleasant aromatic odour developed |
| 5 | Change in colour | Light yellow to yellowish-brown |
| 6 | Consistency | Smooth and homogeneous |
| 7 | Moisture content | Gradually reduced during heating |
| 8 | Taila Siddhi Lakshanas | Observed at the completion of the process |

**Table 3: Observations during the preparation of Hanspadyadi Taila.**

| Sr. No. | Observation parameter | Finding |
|---|---|---|
| 1 | Initial appearance | Greenish oily mixture |
| 2 | Odour during processing | Characteristic herbal aromatic odour |
| 3 | Colour change | Greenish to brownish-green |
| 4 | Consistency | Homogeneous, smooth, and oily |
| 5 | Frothing | Gradually reduced during heating |
| 6 | Moisture content | Gradually reduced and absent at completion |
| 7 | Taila Siddhi Lakshanas | Observed in both formulations |
| 8 | Filtration and storage | Filtered while warm and stored in airtight amber-coloured glass containers |

**Table 4: Taila Siddhi Lakshanas observed.**

| Sr. No. | Taila Siddhi Lakshana | Observation |
|---|---|---|
| 1 | Phenashanti | Cessation of frothing was observed |
| 2 | Shabda Pariksha | Crackling sound was absent |
| 3 | Gandha | Characteristic pleasant herbal odour developed |
| 4 | Varna | Proper characteristic colour developed |
| 5 | Varti Pariksha | Wick-like formation from Kalka was observed |
| 6 | Moisture | Moisture was absent at the completion of heating |

**Table 5: Organoleptic characteristics of the prepared formulations.**

| Parameter | Murchhita Hanspadyadi Taila | Amurchhita Hanspadyadi Taila |
|---|---|---|
| Colour | Brownish oil with a greenish tinge | Light-brown oil with a greenish tinge |
| Odour | Characteristic aromatic odour | Characteristic odour |
| Appearance | Clear oily liquid | Clear oily liquid |
| Consistency | Smooth and unctuous | Smooth and unctuous |

Both Hanspadyadi Taila formulations were prepared successfully according to the classical Sneha Kalpana procedure. The appearance of Taila Siddhi Lakshanas indicated proper

completion of the pharmaceutical process. Murchhita Hanspadyadi Taila required less preparation time and showed a comparatively darker colour and more pleasant aromatic odour than the Amurchhita formulation.

## DISCUSSION

The present study evaluated the pharmaceutical characteristics, analytical profile, microbiological quality, and in vitro antibacterial activity of Hanspadyadi Taila prepared using Murchhita and Amurchhita Tila Taila. Both formulations were prepared successfully according to the classical Sneha Kalpana procedure. The appearance of Taila Siddhi Lakshanas, including cessation of frothing, absence of crackling sound and moisture, development of characteristic colour and odour, and wick-like formation of Kalka, indicated satisfactory completion of the pharmaceutical process.

During Murchhana, the colour of Tila Taila changed from light yellow to yellowish-brown, and its characteristic sesame odour was replaced by a pleasant aromatic odour. These changes may have occurred due to the transfer of pigments, volatile components, and lipid-soluble constituents from the Murchhana drugs into the oil. The final Murchhita Tila Taila was smooth, homogeneous, and more aromatic than the untreated oil.

The initial quantity of Tila Taila used for Murchhana was 250 mL, of which 230 mL was recovered, resulting in a loss of 20 mL. The final yields of Murchhita and Amurchhita Hanspadyadi Taila were 210 mL and 220 mL, respectively. The loss observed during preparation may have occurred because of the absorption of oil by the Kalka, adherence of the formulation to the vessel and muslin cloth, and handling during filtration.

Organoleptic evaluation showed that both formulations were clear, smooth, and unctuous oily liquids. Murchhita Hanspadyadi Taila was brownish with a greenish tinge and possessed a comparatively pleasant aromatic odour. Amurchhita Hanspadyadi Taila was light brown with a greenish tinge and had a characteristic odour. The darker colour and improved aroma of the Murchhita formulation may be attributed to the preliminary processing of Tila Taila with Murchhana drugs.

The refractive indices of Murchhita and Amurchhita Hanspadyadi Taila were 1.46 and 1.48, respectively. The minor difference may reflect variations in chemical composition and the concentration of dissolved herbal constituents. Their specific gravity values were 0.83 and

0.87, respectively. These values confirmed the oily nature of both formulations, while the variation may have resulted from the influence of Murchhana and the extraction of herbal components.

The iodine values of Murchhita and Amurchhita Hanspadyadi Taila were 60 and 70, respectively. The comparatively lower iodine value of the Murchhita formulation may indicate a difference in the degree of unsaturation following pharmaceutical processing. The saponification values were 205 for Murchhita Hanspadyadi Taila and 200 for Amurchhita Hanspadyadi Taila. The slightly higher value of the Murchhita formulation may indicate a minor alteration in fatty-acid composition after Murchhana.

The acid values of Murchhita and Amurchhita Hanspadyadi Taila were 4 and 6, respectively. The lower acid value of the Murchhita formulation indicates comparatively less liberation of free fatty acids and may suggest better resistance to hydrolytic deterioration. However, long-term and accelerated stability studies would be required to confirm its shelf life and resistance to rancidity.

Both formulations had a pH of 6.4, indicating a mildly acidic to near-neutral reaction. This pH may be compatible with external application because it is close to the normal acidic environment of the skin. Nevertheless, dermal irritation, sensitization, toxicity, and compatibility studies are necessary before clinical use.

Thin-layer chromatography showed an Rf value of 0.52 for Murchhita Hanspadyadi Taila, which was close to the quercetin standard value of 0.54. Amurchhita Hanspadyadi Taila showed an Rf value of 0.50, close to the gallic acid standard value of 0.49. These findings suggest the probable presence of quercetin and gallic acid in the respective formulations. These phytoconstituents possess antioxidant, anti-inflammatory, and antimicrobial properties and may have contributed to the observed biological activity. However, advanced chromatographic and spectroscopic studies are required for definitive identification and quantification.

The total bacterial count was 0 CFU/mL in both formulations under the study conditions. The absence of detectable bacterial contamination indicates satisfactory microbiological quality and suggests that appropriate precautions were followed during preparation, filtration, and storage. However, this finding should not be interpreted as proof of sterility because the result

was limited to the conditions and sensitivity of the method used.

Both formulations demonstrated in vitro antibacterial activity against *Staphylococcus aureus*. Murchhita Hanspadyadi Taila produced a zone of inhibition of 19 mm, while Amurchhita Hanspadyadi Taila produced a zone of inhibition of 15 mm. Streptomycin, used as the standard, produced a 26 mm zone of inhibition, whereas the control showed no antibacterial activity.

The observed antimicrobial activity may be attributed to the combined effects of Hanspadi, Nimba, and Jati. These ingredients contain flavonoids, tannins, phenolic compounds, and other biologically active constituents with reported antimicrobial, antioxidant, anti-inflammatory, and wound-healing properties. Their extraction into Tila Taila may have contributed to the inhibitory action of the formulations against *S. aureus*.

The comparatively larger inhibition zone produced by Murchhita Hanspadyadi Taila suggests that Taila Murchhana may influence the antimicrobial profile of the final formulation. This effect may be related to the addition of bioactive constituents from the Murchhana drugs, improved extraction of lipid-soluble compounds, better stability of the oil, or modification of its physicochemical characteristics. However, the difference cannot be described as statistically significant because the study reported single observational values without experimental replicates or inferential statistical analysis.

Overall, the findings provide preliminary scientific support for the traditional use of Hanspadyadi Taila in Nadivrana and infected wound conditions. Nevertheless, this study was limited to an in vitro assessment against a single microorganism. Further research should include replicate experiments, vehicle controls, determination of minimum inhibitory and bactericidal concentrations, biofilm studies, evaluation against additional wound pathogens, advanced phytochemical profiling, stability testing, dermal safety assessment, and controlled clinical trials.

## CONCLUSION

Both Murchhita and Amurchhita Hanspadyadi Taila were prepared successfully and showed acceptable pharmaceutical, physicochemical, and microbiological quality. Both formulations demonstrated in vitro antibacterial activity against *Staphylococcus aureus*, with Murchhita Hanspadyadi Taila showing greater inhibition than the Amurchhita formulation. These

preliminary findings support its traditional use in Nadivrana and infected wounds; however, further experimental and clinical studies are required.

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