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Preparation, standardization, and *in vitro* antimicrobial efficacy of *Gairikadya malahara* – An Ayurvedic ointment

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ABSTRACT:

Introduction: *Gairikadya malahara* is a classical ointment formulation described in *Rasa Tarangini*. This drug is used externally to treat chronic ulcers. Despite many available ointments, the need of broad spectrum and innocuous dressing material is always obligatory.

Aim: The aim of this study was to evaluate the antimicrobial action of *Gairikadya malahara* on common microbial flora (Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus, Staphylococcus epidermidis,* and *Pseudomonas aeruginosa*) present over the granulation tissue.

Materials and Methods: The drug was prepared by the standard protocol as mentioned in the literature. The physiochemical, heavy metal, and microbiological analyses were performed to standardize the formulation. Antimicrobial study was conducted to see the *in vitro* action of the drug over bacteria and fungus.

Results: The prepared drug was found within prescribed physiochemical, heavy metal, and microbiological parameters as mentioned in the Ayurvedic Pharmacopoeia of India. It was found that a higher concentration (100 mg/mL) of *Gairikadya malahara* is effective against *Bacilus cereus* (MTCC 6840), *S. aureus* (MTCC 737), *Escherichia coli* (MTCC 1687), *P. aeruginosa* (MTCC 424), *Salmonella typhi* (MTCC 98), *S. epidermidis* (MTCC 96), *Enterococcus feacalis* (MTCC no. 439), and *Candida albicans* (MTCC 227).

Conclusion: *Gairikadya malahara* is innocuous and effective to prevent infection over the granulation surface. Drug standardization with physiochemical analysis, heavy metal analysis, and microbial testing suggests that the classical method of *Malahara* preparation is up to the mark. Further, a clinical study to evaluate the healing of chronic nonhealing ulcers may be recommended.

Keywords:

Antimicrobial activity, Chronic nonhealing ulcers, *Gairikadya malahara*

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INTRODUCTION

Ayurveda advocates the administration of various formulations to manage different pathological conditions through various routes including internal and external. *Malahara* is an Ayurvedic formulation for external application, used to provide local effects, and also to promote ulcer healing. An ulcer is a local defect or excavation of the surface of an organ or tissue that is produced by the sloughing (shedding) of inflamed necrotic tissue.[1] Charaka has defined nonhealing ulcers as foul-smelling, discolored, profuse discharging, and severe intense painful ulcers.[2] Sushruta has mentioned that after complete healing of the wound, the scar never disappears and its imprint persists lifelong and is called as *Vrana* (~wound/ulcer/sore).[3] Sushruta has described 60 measures for the

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comprehensive management of injury, which includes local as well as the systemic use of different drug and treatment modalities. He has also mentioned that the *Dushta vrana* (~chronic ulcers) are difficult to heal.[4] The healing process becomes diminished in chronic ulcers due to reduced tissue regeneration, angiogenesis, and neurological problems.[5] In spite of the advances that have been made, the management of chronic nonhealing ulcers is still a challenge for the clinician. Researches on drugs for chronic nonhealing ulcers are a developing area in modern biomedical sciences. Scientists who are trying to develop newer drugs from natural resources are looking toward Ayurveda, the Indian traditional system of medicine. It has been estimated that 70% of the wound-healing Ayurvedic drugs are of plant origin, 20% of mineral origin, and the remaining 10% consisting of animal products.[6]

Rasa Tarangini written by Sri Sadananda Sharma is one among the important referral books in the field of Ayurveda. *Gairkadya malahara*[7] mentioned in this text can be used as a healing agent in different types of ulcers. It contains *Siktataila, Swarnagairika* (~Orche), *Naga sindura,* and *Haridra* (*Curcuma longa* L.) [Table 1]. An attempt has been made in this study to prepare *Gairikadya malahara,* and preliminary analytical profiles have been developed along with the assessment of its antimicrobial efficacy.

MATERIALS AND METHODS

All the ingredients were procured from the local market, identified, and processed in the pharmacy of the Institute in February 2022. Raw drug materials and a sample of prepared drugs were deposited in the pharmacy for future reference (SL. no. 06/22, Batch no. 05/GM/2022, date: March 28, 2022).

Preperation of *malahara*

The formulation composition of *Gairikadya malahara* is placed in Table 1. Base of this ointment is *Sikta taila*, which was prepared by mixing sesame oil (*Tila Taila*) and bee's wax (*~Madhuchhishtha*) in 8:1 ratio.[8]

Table 1: Constituents of *Gairikadya malahara*

Drugs (Ayurvedic name)	Latin/English name	Proportion	Quantity (g)
Sikta Taila	-	6 parts	--
Madhuchhishtha	Bee's wax	1 part	420
Tila Taila	Sesame oil	8 parts	3360
Swarna Gairika	Red ochre	1 part	630
Haridra	*Curcuma longa* L.	1 part	630
Naga Sindura	Lead oxide	1/12th part	52
Total weight			5092
Final weight			4812
Loss during preparation			5.498%

Swarna gairika was crushed manually with the help of a mortar and pestle till a fine powder form. The cow ghee was melted in a container over the flame and powdered *Swarna gairika* was mixed slowly to saute within cow ghee properly.

Naga sindura was procured from a government-approved pharmacy at Bhopal, Madhya Pradesh. Powder of *Naga sindura* was mixed manually with lemon juice (*~Jambiri nimbu swarasa*) in mortar and pestle till it becomes completely dry for *Shodhana* purposes. This process of *Shodhana* was repeated thrice.

The sesame oil was taken in a large-size stainless steel (SS) vase and was kept over a mild flame. Bee's wax was cut into small pieces and mixed in lukewarm oil. The mixture was heated over a mild flame till the wax dissolves completely. The prepared *Sikta taila* was kept to cool itself. *Gairikadya malahara* was prepared after mixing all the contents – *Shuddha swarna gairika, Shuddha naga sindura,* and *Haridra* (turmeric) in already prepared *Sikta taila* in specified quantity over the flame. All the contents were mixed properly in a large size container. The prepared *Gairikadya malahara* was stored in sealed containers and kept at a cool and dry place.

Physiochemical analysis

Physiochemical analysis was done as per the methods mentioned in Ayurvedic Pharmacopoeia of India (API) for testing of *malahara* or ointment.[9] pH (10%w/v Aq. Solution), total solid, viscosity, rancidity, refractive index, specific gravity, spreadability, iodine value, free fatty acid, mineral oil, loss on drying, thermal stability (at 5°C, 25°C, and 45°C for 5 days), acid value, saponification value, peroxide value, and total fatty matter were evaluated.

Heavy metal analysis

Testing of lead, cadmium, mercury, and arsenic was done by atomic absorption spectrophotometer as per the methods mentioned for heavy metal detection in API.[10]

Microbiological analysis

Total bacterial and fungal counts were done by focusing on standard procedures.[11]

Antimicrobial activity

The antimicrobial activity was carried out by opting standard methods.[12]

Test organisms used

Cultures of the microorganisms such as *Bacilus cereus* MTCC 6840, *Staphylococcus aureus* MTCC 737, *Candida albicans* MTCC 227, *Escherichia coli* MTCC 1687,

Pseudomonas aeruginosa MTCC 424, *Salmonella typhi* MTCC 98, *Staphylococcus epidermidis* MTCC 96, and *Enterococcus feacalis* MTCC no. 439 were used. The viable microorganisms used in the test must not be more than five passages removed from the original MTCC culture or any other equivalent cultures.

Preparation of inoculums

First, the inoculums were prepared. The bacterial and fungal cultures were harvested using sterile peptone saline, the surface growth was washed, collected in suitable glassware, and then, sufficient sterile peptone saline was added to obtain a microbial count of about 1 × 108 colony-forming units (CFU)/ml. The number of CFU/ml in each suspension was determined using the conditions of media and microbial recovery incubation times 72 h to confirm the initial CFU/ml. This value serves to calibrate the size of the inoculums used in the test. The bacterial and yeast suspensions should be used within 24 h of harvest, but the fungal preparation may be stored under refrigeration for up to 7 days.

Preparation of media

For weighing media, use calibrated balance, the glassware and utensil were depyrogenated in the oven at 250°C for 60 min, weighed media carefully and dissolved in distilled water, shaken well, and heated on the hot plate for complete dissolve. Checked the water level of the autoclave if necessary adjusted the level with demineralized water. All prepared media was loaded and carefully the lid of the autoclave was closed, the power supply was checked, and the autoclave was run for sterilization. Temperature of 121°C was hold for 15 min and after which it was switched off and steam was released slowly. Before testing, the ultraviolet (UV) light of the biosafety cabinet, pass box, and biosafety cabinet room was kept switched on for 30 min. Now, the lid of the autoclave is opened to take all the media on SS trays and sent to the pass box. This was followed by entry in the airlock room and then changing room to change the dress and wear sterilize dress. Then, entry was taken in the biosafety cabinet room, the UV light of the biosafety cabinet was switched off and the white light with airflow was switched on. The hands and workbench were sterilized with 70% IPA.

Test procedure

In vitro antibacterial activity of formulations was carried out using the Kirby–Bauer agar well-diffusion method.[13] This classic method yields a zone of inhibition (ZOI) in mm result for the amount of antibacterial that is needed to inhibit the growth of specific microorganisms. Prepared *malahara* was used diluted in dimethyl sulfoxide (DMSO) at 50 and 100 mg/ml. *Malahara* sample was dissolved into DMSO and placed into wells (volume applied in each well - 100 µl). For the determination of ZOI, liquid suspension culture of each bacterial and fungal strain was poured in each well (diameter of well - 8 mm). Gentamycin (2.5 µg/mL) was used as a standard antibiotic, and fluconazole (5 µg/mL) was used as an antifungal agent and control DMSO for comparison of the results. Mueller–Hinton agar plates for bacteria and fungus were seeded with the liquid culture of bacterial strains and allowed to stay at 37°C for 24 h in an incubator. The zones of growth inhibition around the wells were measured after 18–24 h of incubation at 37°C for bacterial and 48–72 h for fungal at 25°C. The sensitivity of the microorganism species to formulation was determined by measuring the sizes of inhibitory zones (including the diameter of the well) on the agar surface with comparison to the standard antibiotic and antifungal zones.

RESULTS

It was observed that there was a 5.498% loss of contents during the process. The organoleptic characteristics of *Gairikadya malahara* are shown in Table 2. The results obtained in the analytical study are depicted in Tables 3-5 showing the prepared drug is innocuous as the values of physiochemical parameters, heavy metals parameters, and antimicrobial parameters are less than the standard values. Table 6 and Figure 1 are representing the antimicrobial activity against seven species of bacteria and one species of fungus at various concentrations.

Table 2: Organoleptic character of *Gairikadya malahara*

Parameter	Results
Appearance	Thick viscous ointment
Color	Yellowish Brown
Odor	Characteristic smell
Touch	Soft ++

Table 3: Physiochemical analysis of *Gairikadya malahara*

Test parameters	Results
pH	6.35
Total solid	98.79% w/w
Viscosity	6300 cP
Rancidity	Absent
Refractive index	1.484
Specific gravity	1.0533
Spreadability	5.0 s
Iodine value	75.46
Free fatty acid	0.06%
Mineral oil	Absent
Loss on drying	1.23% w/w
Thermal stability (At 5, 25, 45°C for 5 days)	Stable
Acid value	0.12 mg KOH/g
Saponification value	123.95 mg KOH/g
Peroxide value	21.20 m eq of O_2/Kg
Total fatty matter	51.58% w/w

w/w: Weight/weight

DISCUSSION

Modern antibiotics are frequently associated with adverse actions and the development of antibiotic drug resistance. Hence, alternate herbal medicines are much required**.** A lot of research work is present on topical Ayurvedic drugs used for chronic ulcer healing. However, no studies have been reported on the antimicrobial activity of *Gairikadya malahara* to the best of our knowledge.

The pH of *Malahara* was found 6.35, however, it should be quite identical with skin secretions, i.e., around 5.5. The total solid content of 98.79% is making it in thick semisolid form. Viscosity of 6300 centipoises makes the formulation pseudoplastic in nature. Rancidity was found absent in *Malahara* suggests no aerial oxidation of leading to unpleasant odor. *Malahra* was found 0.06% free fatty acid and no mineral oil. Cream base should spread easily without drag and should be free from friction while rubbing. Spreadabilty of *Malahara* is found good enough. Thermal stability at 5°C, 25°C, and 45°C for 5 days shows it is stable in nature. *Malahara* was also do not found having hazardous heavy metal content.

Malahara has also shown an effective zone of inhibition against *P. aeruginosa, C. albicans, E. Coli, E. feacalis,* and *S. aureus.* However, it was effective at higher concentrations against *S. typhi* and *S. epidermidis.*

Table 4: Heavy metal analysis of *Gairikadya malahara*

Test parameters	Results	Limits
Lead (Pb)	8.32% w/w	NMT 10
Cadmium (Cd)	BLQ (LOQ 0.01) mg/kg	NMT 0.3
Mercury (Hg)	0.11 mg/kg	NMT 1
Arsenic (As)	1.56 mg/kg	NMT 3

NMT: Not more than, BLQ: Below limit of quantification, LOQ: Limit of quantification

Table 5: Microbiological analysis of *Gairikadya malahara*

Test parameters	Results	Limits
Total bacterial count	40 cfu/g	NMT 100,000
Total fungal count	<10 cfu/g	NMT 1000

NMT: Not more than, Cfu: Colony-forming unit

Sushruta has mentioned different formulations for the management of injuries in *Shashti-Upakramah* (~sixty therapeutic measures for wounds) such as *Kalka* (~therapeutic use of paste in wound), *Varti* (~therapeutic wick for wound), *Taila* (topical use of medicated oils), *Raskriya* (~topical use of medicated thick syrup), *Avchurnan* (~topical dusting powders), *Vrandhupan* (~fumigation of wound), etc. However, most of the studies available were related to *Vrana ropana taila* and *Ghrita* (~wound healing measures).

Moreover, *Malahara* or ointments are generally easy to use, cost-effective, and provide better dressing material for the treatment of chronic nonhealing ulcers. *Gairikadya malahara* is having the base of *Sikta taila. Swarna gairika* is antihistaminic, soothes the burning sensation, and has an excellent healing effect.[14] Red ochre (*Swarna gairika*) paste is used traditionally in Siddha medicines. Areca nut powder and red ochre mixed in equal quantities are applied to treat herpes zoster in Tamil Nadu.[14] It has also been shown a powerful antimicrobial action in ointment formulations.[15]

Sindura has a property such as V*rana shodhana* (~wound cleansing), *Vranah ropana* (~wound healing), *Bhagna sandhankara* (~fracture healing), *Twachya* (~soothing), and *Bhutaghna* (~antibacterial). *Sindura* is commonly prescribed as a topical ulcer healing agent in the form of ointment in Unani medicine.[16] Lead is an active content of this *Malahara*. Still, after proper *shodhana* and processing of formulation, the lead content in the sample is 8.32% w/w (normal limit-not more than 10), which is acceptable. Other heavy metal contents are also under the prescribed limit. Total bacterial count and total fungal count were found very minute in the tested sample. *Girikadya malahara* may be a virtuous and harmless ulcer healing agent.

Turmeric contains flavonoid curcumin (diferuloylmethane) and various volatile oil, including

Table 6: Antimicrobial activity of *Gairikadya malahara*

Microbial strains	ZOI (mm)			
	Standard (positive control)	Test sample (50 mg/ml)	Test sample (100 mg/ml)	DMSO (negative control)
Staphylococcus aureus	22±1	10±0.5	13±1	8
Escherichia coli	25±1	13±0.5	14±1	8
Pseudomonas aeruginosa	22±1	12±1	15±1	8
Salmonella typhi	12±0.5	9±0.5	11±0.5	8
Staphylococcus epidermidis	16±0.5	8±0.4	11±0.5	8
Candida albicans	18±0.5	10±0.3	15±1	8
Enterococcus faecalis	14±0.5	10±0.5	14±1	8
Bacillus cereus	22±1	10±0.5	12±0.5	8

*Values are mean of triplicate; Diameter of well-8 mm; DMSO negative control volume applied in each well-100 μl; DMSO Negative control-Blank for all study; Gentamycin (2.5 μg/mL) as Antibacterial and Fluconazole (5 μg/mL) as antifungal. ZOI: Zone of inhibition, DMSO: Dimethyl sulfoxide

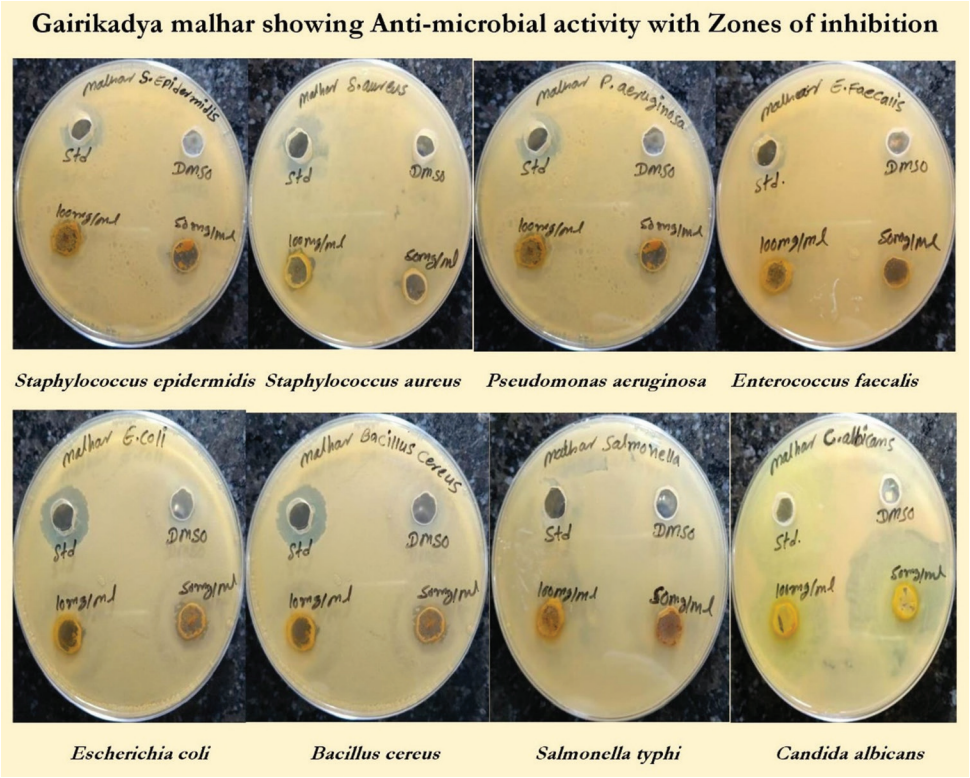

Figure 1: *Gairikadya malahara* showing anti-microbial activity with ZOI. ZOI: Zone of inhibition

turmerone, atlantone, and zingerone. It has excellent antioxidant, anti-inflammatory, and also anti-cancerous activity.[17]

CONCLUSION

The results obtained in this study suggest that the selected Ayurvedic ointment *Gairikadya malahara* showed substantial antimicrobial activity and has prodigious efficacy against Gram-positive and Gram-negative bacteria as well as anti-fungal against fungal colonies present over the biofilm of the granulating surface of chronic ulcers (~*Dushta vrana*). Thus, its active constituents may be helpful in the therapeutic treatment by local application over chronic skin ulcers. The obtained results validate the classical guidelines of the preparation of ointment. The physicochemical analysis shows that the values of different parameters are within the limit and heavy metal contents such as lead, cadmium, mercury, and arsenic are also within the prescribed limit, it can be concluded that this formulation is safe. The present study offers leads for future studies to establish its therapeutic role through pharmacological and clinical studies.

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Conflicts of interest

There are no conflicts of interest.

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