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Antimicrobial activity of *Gandhakadya Malahara* in vitro study

Nighil Gigi[1], Chaitra L.V[2], Vidhya Rani M[3].

[1]*Post Graduate Scholar, Dept. of Rasa Shastra and Bhaishajya Kalpana, Ramakrishna Ayurvedic Medical College, Hospital and Research Centre, Bengaluru, Karnataka, India.*

[2]*Associate Professor, Dept. of Rasa Shastra and Bhaishajya Kalpana, Ramakrishna Ayurvedic Medical College, Hospital and Research Centre, Bengaluru, Karnataka, India.*

[3]*HOD & Professor, Dept. of Rasa Shastra and Bhaishajya Kalpana, Ramakrishna Ayurvedic Medical College, Hospital and Research Centre, Bengaluru, Karnataka, India.*

ABSTRACT

Bhaishajya Kalpana Vigyana is the pharmacological branch of Ayurveda which deals with the preparation of herbal and herbo-mineral formulations. *Malahara* is a lately introduced dosage form to Ayurvedic system of medicine. Microorganisms such as bacteria, viruses, fungi and parasites are present everywhere in the soil, water, and air are responsible for large number of infectious diseases in human beings. To avoid such problems our *Acharyas* has mentioned many *Malaharayogas. Gandhakadya Malahara* is one among them which is mentioned in *Rasatarangini Gandhaka Vinjaniya Taranga.*

Key words: *Gandhakadya Malahara, Malaharayogas, Microorganisms, Rasatarangini Gandhaka Vinjaniya Taranga.*

INTRODUCTION

Malahara Kalpana comes under *Bahya Kalpana* (external application). The word '*Malahara*' was adapted by *Yogaratnakara*[1] from the word *Malaham* or *Marham* which is originated from Unani system of medicine.[2] The word *Malahara* means that it removes *Mala* from *Vrana, Vidradi, Twak Vikara* etc. It is a quite widely used ointment preparation with many advantages. Base materials are required for preparation of *Malahara* and *Sikta Taila* is one among them which is commonly used. Base which is the chief ingredient of *Malahara Kalpana* should be smooth, soft, should not produce irritation and sensitization of skin.

Gandhakadya Malahara is one among them which is mentioned in *Rasatarangini, Gandhaka Vinjaniya Taranga*[3] and it is a herbo-mineral formulation intended for external application in various dermatological problems, using natural ingredients *Sikta Taila* as base for preparation. Hence, this study is undertaken to evaluate pharmaceutico-analytical study of *Gandhakadya Malahara* and its antimicrobial activity.

Address for correspondence:
Dr. Nighil Gigi
Post Graduate Scholar, Dept. of Rasa Shastra and Bhaishajya Kalpana, Ramakrishna Ayurvedic Medical College, Hospital and Research Centre, Bengaluru, Karnataka, India.
E-mail: nighilgigi1993@gmail.com

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AIM OF THE STUDY

To evaluate the antimicrobial activity of *Gandhakadya Malahara*.

MATERIALS AND METHODS

Test drug

The test drug was prepared from *Rasa Shastra* and *Bhaishajya Kalpana* Department *Rasashala* of

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Ramakrishna Ayurvedic Medical College, Yelahanka, Bangalore.

Source of Chemical and Reagents

All the chemical reagents and other requirements of experimental study used from stock of Skanda Life Sciences Private Limited, R & D Centre, Sri Shaila Bramara Complex, Sy.No 47, No.10-12, Chandana layout, Srigandadkaval, Nagarabhavi, Bengaluru.

Test organisms: *Staphylococcus aureus, Proteus mirabilis, Staphylococcus epidermidis, Propioni bacterium acnes.*

Test compound as standard

Ciprofloxacin (0.1mg/ml)

Inoculum

S. aureus, P. mirabilis, S. epidermidis and *P. acnes* cell suspension were prepared and grown on Peptone broth and cultures were incubated for 24hrs at 37°C. The cell suspensions of all the cultures were adjusted to 1-2x 10^6cells/ml.

Sample details

Gandhakadya Malahara (20µl)

Test compound

Sample (100mg/ml)

Standard: Ciprofloxacin(0.1mg/ml)

Control: water

Sample preparation

100mg of sample were dissolved in chloroform and samples were used for the study.

Procedure

Determination of Antimicrobial activity

S. aureus, P. mirabilis, S. epidermidis and *P. Acnes* were inoculated on Soyabean Casein Digested agar plates. (90mm)

Test compounds: Sample 1 (10µl), Sample 2 (20µl), Standard Ciprofloxacin (20µl) for *S. aureus, P. mirabilis, S. epidermidis* and *P. Acnes* were added to the 5mm well on agar plates.

The treated plates were incubated in aerobic chamber at 37°C for 24hrs. The treated plates were observed for zone of inhibition around the wells.

Table 1: *Gandhakadya Malahara* requires following ingredients.

Ingredients	Quantity
Sikta Taila	72 gms
Shuddha Gandhaka	6 gms
Suddha Girisindhoora	6 gms
Suddha Tankana	2 gms
Karpura	2 gms

The above-mentioned quantity of *Sikta Taila* is taken in a vessel and subjected to mild heat. The other ingredients are powdered finely and kept separately. When the *Sikta Taila* melts it is taken and continuously stirred with a spoon. Now the ingredients i.e., *Gandhaka, Girisindhoora, Tankana* and *Karpura* are mixed together and added to the *Sikta Taila* with continuous stirring. After adding all the contents, the stirring is continued so that the contents get mixed homogenously and a fine red colour paste is obtained. This prepared paste is *Gandhakadya Malahara*. After that it is stored in a wide mouthed jar.

Table 2: Properties of the *Gandakadya Malahara* ingredients

Drug	*Rasa*	*Guna*	*Virya*	*Dosha Karma*	Pharmacological action
Shuddha*[4] *Gandhaka	*Katu, Tikta Kashaya*	*Sara*	*Ushna*	*Pitta Vardhaka Kapha-Vatahara*	Antifungal Antimicrobial
***Giri Sindoora*[5]**	*Katu, Tikta*	*Ushna*	*Ushna*	*Tridosha Shamaka*	Antifungal Antimicrobial

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Tankana [6]	*Kshariya*	*Ruksha Tikshna Guru*	*Ushna*	*Pittakara Vatahara Kaphanissaraka*	Expectorant Antidote
Karpura [7]	*Tikta, Katu, Madhura*	*Laghu, Rooksha*	*Sheeta*	Balances *Kapha* and *Pitta Dosha.*	bactericidal
Sikta Taila[8]	*Tikta, Katu, Madhura*	*Laghu, Rooksha*	*Sheeta*	*Kaphavatahara*	Act as stabilizer

Emulsion[9]

An emulsion is a liquid in liquid dispersion. An emulsion droplet interface has at any point the same interfacial tension and sometimes emulsions are subdivided arbitrarily regarding the droplet size (macro, mini and micro-emulsions) and hence general aspects might be lost.

Stabilizer[10]

The purposes for using stabilizers in a media is to produce smoothness in body and texture; retarder reduce crystal growth during storage, especially during period soft temperature fluctuation; provide uniformity to the product; and provide some degree of shape retention. They also contribute to mix viscosity, stabilize the protein in the mix, help in suspension of flavouring particles, slow down moisture migration from the product to the package or the air, and assist in preventing shrinkage of the product volume during storage. Some of the commonly used stabilizers are gelatine, guargum, alginate, agar, cellulose and cellulose derivatives.

Ointments

Ointments are the soft semisolid preparations meant for external application to the skin or mucous membrane. They usually contain a medicament or medicaments dissolved, suspended or emulsified in the base. Ointments are used for their emollient and protective action to the skin. They may also be used as vehicles or bases for the topical applications of medicinal substances. The absorption of medicaments by the tissues from the ointments or other semisolid preparations applied to the skin depends upon a number of factors.

Antimicrobial Study

The study was done at Skanda Life Sciences Private Limited, R & D Centre, Sri Shaila Bramara Complex, Sy. No 47, No.10-12, Chandana layout, Srigandadkaval, Nagarabhavi, Bengaluru.

Observation and Results

Evaluation of antimicrobial activity by Well Diffusion Method

The inhibitory effect of the sample against S.aureus, P. mirabilis, S. epidermidis and P. acnes is as shown in the table below

Table 3: Inhibitory activity of *Gandhakadya Malahara* against test organisms

Test Organisms	Test Compounds	Conc. per Well (µg/ml)	Zone of inhibition (mm)	Figure reference number
S.aureus	Control	-	-	Figure 1
	Ciprofloxacin (Standard)	2.0	23	
	Conc 1	2000	8	
	Conc 2	1000	0.0	
P.mirabilis	Control	-	-	Figure 2
	Ciprofloxacin (Standard)	2.0	23	
	Conc 1	2000	12	
	Conc 2	1000	6	
S.epidermidis	Control	-	-	Figure 3
	Ciprofloxacin (Standard)	2.0	23	

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	Conc 1	2000	8	
	Conc 2	1000	6	
P.acnes	Control	-	-	Figure 4
	Ciprofloxacin (Standard)	2.0	23	
	Conc 1	2000	11	
	Conc 2	1000	7	

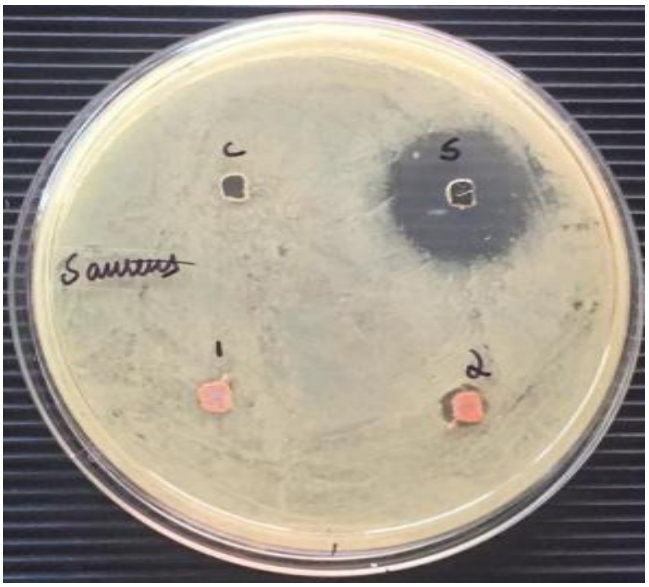

Figure 1

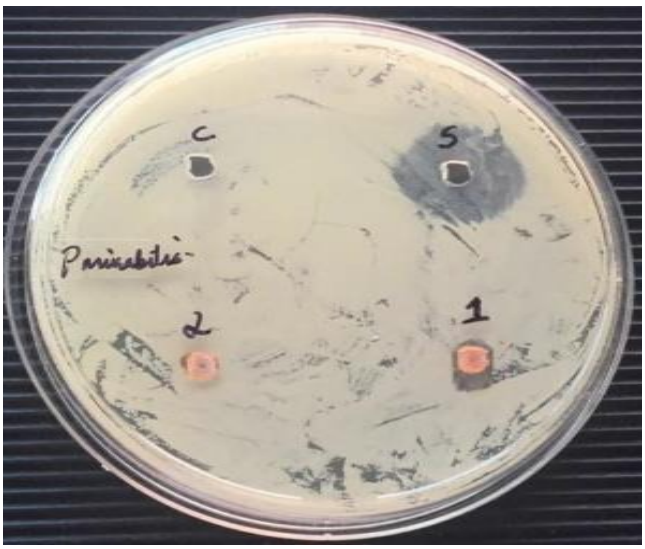

Figure 2

Figure 3

Figure 4

Figure 1-4: Inhibitory activity of test samples against *S.aureus, P.mirabilis, S.epidermis* and *P.acne* respectively S-standard (Ciprofloxacin); C -Control (water); Conc 1- 2000µg/ml, Conc 2- 1000µg/ml.

In the present study organism *S.aureus, P.mirabilis, S. epidermidis* and *P.acnes* species used for antimicrobial activity of *Gandhakadya Malahara* and its action compared with that of Ciprofloxacin.

For antimicrobial activity tested drug was used in concentration 1000µg/ml, 2000µg/ml and

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antimicrobial activity of Ciprofloxacin was used in the concentration of 2 µg/ml.

The minimum inhibition concentration was obtained by each concentration of tested drug observed. The accurate zone of inhibition for both standard and tested drug were calculated.

RESULT

The study shows that *Gandhakadya Malahara* got better results as Antimicrobial agent with high doses in the species of *P. Mirabilis* and *P acne.*

DISCUSSION

The method of preparation of *Gandhakadya Malahara* is similar to that of modern pharmaceutical preparation of ointments. The ingredients as per the literature were brought and subjected to *Shodhana* to avoid toxic effects and to get the desired therapeutic effects. The consistency of the *Malahara* was neither too hard nor too soft. It is mainly based on wax i.e., *Madhuchista*, used in the preparation and it is possible to achieve the desirable consistency by adjusting the amount of wax.

The content of *Malahara* possess *Snigdha, Tikshna, Ruksha, Sara, Ushna, Tridoshahara* properties. All the ingredients of *Malahara* have pharmacologically antifungal, antimicrobial, antimicrobial, antidote, antioxidant action, hence can effectively reduce the infection and prevent its recurrence by improving the immunity of skin by its antioxidant property.

Shudha Gandhaka is Antifungal, Antimicrobial. Vital role in immune system helps in detoxification. It helps in tissue repair and referred to as 'Nature's beauty mineral'.

Shudha Tankana is Antifungal, antibacterial. *Girisindhoora is Tridosha Shamaka* and indicated in *Kandu, Pama, Vicharchika, Sidma, Visarpa, Visha, Vrana Shodhana Ropana.*

Assessment of the influence of the test drug against the organism *S.aureus, P.mirabilis, S.epidermidis* and P.acnes species.

In the present study the significant action was noted in Ciprofloxacin group. Hence by the support of analytical and experimental evidence *Gandhakadya Malahara* double dose was proven effective in Antimicrobial activity against *P.mirabilis* and *P.acnes* species.

CONCLUSION

Gandhakadya Malahara is mentioned in *Rasatarangini,* which combination of five ingredients. Identification, collection of the drugs and preparation of *Gandhakadya Malahara* was done as per the classical reference. Analytical study results showed as followed: P-Anisidine value - 8.497 mEq/kg, Peroxide value -5.55 mEq/kg, Viscosity Index = 1.18, pH is 6.45, fatty matter - 19.6. In the antimicrobial study that was carried out to evaluate the antimicrobial activity of *Gandhakadya Malahara* was found effective in P. mirabilis and *P. acne.* By the virtue of the property of the ingredient as *Twak Vikaras (Kushta, Pama, Sidma, Kandu* etc.) *Gandhakadya Malahara* is known to act as Antimicrobial.

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