

# Comparative Evaluation of Shodhana Media on The Antimicrobial Efficacy of Gandhaka

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#### **ABSTRACT**

Gandhaka (sulphur) is a pivotal component in Ayurvedic pharmaceutics, especially in Rasashastra, used in numerous formulations for its Rasayana and detoxifying properties. However, crude Gandhaka (Ashuddha Gandhaka) often contains physical and chemical impurities such as clay, stones, *Haratala*, and *Manahshila*, necessitating its purification (Shodhana) before therapeutic application. Classical texts like Rasatarangini describe several Shodhana methods using various media. This study evaluates and compares three traditional media for Gandhaka Shodhana — Godugdha (cow milk), Bhringraj Swarasa (Eclipta alba juice), and *Churnodaka* (lime water) — representing animal, plant, and mineral sources respectively. Each method was implemented according to classical procedures, and the purified Gandhaka was analyzed for yield, physical changes, and antimicrobial activity using the Kirby-Bauer agar well diffusion method against Staphylococcus aureus, E. coli, Staphylococcus epidermidis, and Candida albicans. The antimicrobial efficacy was assessed by measuring the zone of inhibition and comparing the results with raw Gandhaka and standard antibiotics (Gentamycin and Fluconazole). Results showed that all three purification methods improved the antimicrobial profile of Gandhaka. Among them, Bhringraj Swarasa Shodhita Gandhaka exhibited the strongest effect on S. epidermidis, while Godugdha Shodhita Gandhaka was most effective against C. albicans. Churnodaka Shodhita Gandhaka, although easier to prepare, demonstrated the least antimicrobial activity and is therefore considered less effective as a Shodhana medium. The study highlights the importance of Shodhana media in enhancing the therapeutic efficacy of Gandhaka. Both Godugdha and Bhringraj Swarasa proved superior to Churnodaka in terms of yield and antimicrobial performance. Further clinical studies are recommended to substantiate these findings and explore the pharmacological implications in patient care.

**Keywords:** Gandhaka, Shodhana, Uprasa, Murcchana, Jarana, Godugdha, Rastarangini, Churnodaka, Antimicrobial Activity.

#### 1. INTRODUCTION

Gandhaka (sulphur) is an important drug used in numerous Ayurvedic pharmaceutical formulations. However, raw Gandhaka (Ashuddha Gandhaka) contains various physical and chemical impurities such as clay, sand, small stones, Haratala, and Manahshila [1] which must be removed through a purification process known as Shodhana. There are various methods for Gandhaka Shodhana detailed in classical Ayurvedic texts like Rasashastra. Yet, pharmaceutical practitioners and Vaidyas often face confusion in selecting the most suitable method for purifying Gandhaka before using it in therapeutic preparations. This necessitates the need for identifying the most effective media for the purification process.

This study aims to investigate the *Gandhaka* Shodhana methods described in the classical text *Rasatarangini* from Rasa Shastra. The text outlines various methods, and for this study, three media have been chosen: *Go-Dugdha* (cow's milk), Bhringraj Swarasa (juice of Eclipta alba), and *Churnodaka* (a mixture of lime powder and water). These media come from three different origins—animal, plant, and mineral—and each plays a critical role in the *Shodhana* process. The media's effects are crucial as they impact the purity and therapeutic properties of the final product, particularly during the *Marana* (incineration) stage, where *Gandhaka* is processed into Bhasma (incinerated medicine).

In Rasashastra, substances used in *Bhasma* preparation are categorized into four types: *Uttama* (superior), *Madhyama* (medium), *Kanishtha* (inferior), and *Durgunapradam* (antagonistic). This classification emphasizes the importance of selecting the correct medium during *Gandhaka Shodhana*, as it influences the therapeutic value of the final product. The

study is focused on evaluating the comparative efficacy and cost-effectiveness of the three selected media. By understanding how each medium affects the purification process, the study aims to identify which method produces the most effective, safe, and cost-efficient *Gandhaka* for therapeutic use.

The findings of this study will help guide pharmaceutical practitioners in selecting the best purification method for *Gandhaka*, ensuring that the resulting product is of high quality and suitable for use in Ayurvedic treatments. By applying the classical knowledge from *Rastarangini* in a modern context with the help of antimicrobial study this research will contribute to improving the standards of *Gandhaka* purification and its application in Ayurvedic practice.

### 2. MATERIALS AND METHODS

# **Drug Review-**

Gandhaka is described in Samhitas, also showing knowledge of its medicinal value from a very early period. It is the next most important drug of Rasashastra after Parada. It is described in Uparasa Varga of Ras dravyas. It is used for neutralizing the toxicity of Parada and enhancing its therapeutic effect. It is used for the preparation of Kajjali, Rasa Parpati, Rasa Sindura etc. It is being given prime importance in Rasashastra because of its Rasayana properties. It is also considered as an essential agent for the various processes of Parada such as Murcchana & Jarana etc. It is believed to impart many desirable properties to Parada and reduce its toxic effects. Probably because of this, Parada is mostly administered internally in association with Gandhaka, as Parada preparations without Gandhaka are more toxic. In addition to its value for making the Parada therapeutically useful, it is also used for Bandhana.

# Pharmaceutical Study-

Pharmaceutical study includes mainly purification of Gandhaka, and its process of standardization in which drug ratio, *dravya* quantity, and duration etc. The whole practical study of purification of *Gandhaka* with different medias held in departmental laboratory of Rasa Shastra and Bhaishajya Kalpana, Govt. Ayurvedic College and Hospital, Patna, Bihar.

1. Gandhaka Shodhana with Godugdha [2]

Sodhana of Gandhaka was carried out according to the method given in *Rastarangini* (8/7-12). Initially 250 gm Raw Gandhaka was taken and *69 gm* of Shuddha Gandhaka was obtained.

2. Bhringaraja Swarasa Nirmana [3].

There is no reference of Bhringaraja Swarasa nirmana described in Rasatarangni. Hence general method of Swarasa nirmana described in Sarangdhara Samhita, Madhyama Khanda, Prathama adhyaya is used to prepare of Bhringaraja Swarasa. Initially 2.5 kg of Bhringaraja Plant was taken and **1.150 lit** of Swarasa was obtained.

3. Gandhaka Shodhana With Bhringaraja Swarasa [4].

Sodhana of Gandhaka was carried out according to the method given in Rastarangini (8/21-22). Initially 250 gm Raw Gandhaka was taken and **71 gm** of Shuddha Gandhaka was obtained.

4. Churnodaka Nirmana [5].

Churnodaka nirmana was carried out according to the method given in Rastarangini (8/26) Initially Churna (Quick lime) 750 gm 2.25 lit of water was taken and **1.4 lit** of Churnodaka was obtained.

5. Gandhaka Shodhana with Churnodaka [6].

When both Gandhaka and Churnodaka was strongly heated together, they did not melt as described in the reference *Rasatarangini* (8/27-28). To overcome this problem Gandhaka was melted with Goghrita and poured through Goghrita lipta muslin cloth in churnodaka. Initially 250 gm of raw *Gandhaka* was taken and 209 gm of Suddha Gandhaka was obtained.

# **Method Used for Antimicrobial study:**

This microbial study of Gandhaka was carried out with the help of S.R LABS & RESEARCH CENTRE, Jagatpura, Jaipur, Rajasthan, which is AYUSH approved Laboratory & ISO-9001 Certified.

The antimicrobial activity of the test formulations was evaluated using the Kirby-Bauer agar well diffusion method. Samples, including Gandhaka dissolved in DMSO, were tested at concentrations of 50 mg/mL, 100 mg/mL, and as undiluted solutions (50% and 100%). Gentamycin and fluconazole were used as standard antibacterial and antifungal agents, respectively, with DMSO serving as the negative control.

Muller-Hinton agar plates were seeded with bacterial or fungal suspensions and wells of 8 mm diameter were filled with 100  $\mu$ L of each test solution. Bacterial plates were incubated at 37°C for 18–24 hours, and fungal plates at 25°C for 48–72 hours. The zone of inhibition (ZOI), including the well diameter, was measured to assess antimicrobial efficacy, and results were compared with those of the standard drugs and control.

# 3. RESULTS

Table No.1.1: OVERALL RESULTS OF ALL PRACTICAL

PRACRICAL NO.	NAME OF PRACTICAL	TAKEN	RESULT
1	Gandhaka Shodhana by Godugdha media	250 gm	115 gm
2	Gandhaka Shodhana by Bhringaraja Swarasa media	250 gm	117 gm
3	Gandhaka Shodhana by Churodaka media	250 gm	209 gm









Fig.1.1 Results- Raw Sulphur Powder

Table No.1.2: Antimicrobial Activity of Raw Gandhaka

Antimicrobial Activity	Zone of Inhibition (mm)			
(Values are mean of triplicate)	Standard	Test Sample (in DMSO)		
	Positive control		100%	DMSO Negative control
Staphylococcus aureus	26	18	22	8
E. coli	26	18	22 8	
Staphylococcus epidermidis	22	14	18	8
Candida albicans	28	20	24	8

As per standard antimicrobial sensitivity protocol of pharmacopoeia









Fig.1.2 Results- Purified Sulphur (Cow Milk)

Table No.1.3: Antimicrobial Activity of Godugdha Shodhita Gandhaka

Antimicrobial Activity	Zone of Inhibition (mm)			
(Values are mean of triplicate)	Standard	Test Sample (in DMSO)		MSO)
	Positive control	50%	100%	DMSO
	Positive control 30% II		10076	Negative control
Staphylococcus aureus	24	16	20	8
E. coli	22	12	14	8
Staphylococcus epidermidis	24	14	18	8
Candida albicans	28	24	26	8

As per standard antimicrobial sensitivity protocol of pharmacopoeia

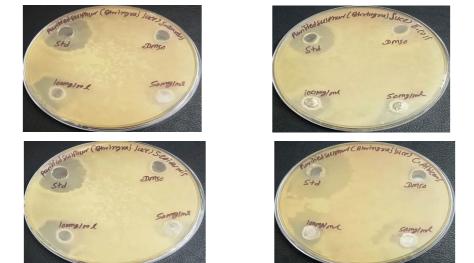


Fig.1.3 Results- Purified Sulphur (Bhringraj Juice)

Table No.1.4: Antimicrobial Activity of Bhringraja Swarasa Shodhita Gandhaka

Antimicrobial Activity	Zone of Inhibition (	Zone of Inhibition (mm)					
(Values are mean of triplicate)	Standard	Test Sa	Test Sample (in DMSO)				
	Positive control	50%	100%	DMSONegative control			
Staphylococcus aureus	24	18	22	8			
E. coli	24	16	20	8			
Staphylococcus epidermidis	24	16	22	8			
Candida albicans	28	20	24	8			

As per standard antimicrobial sensitivity protocol of pharmacopoeia

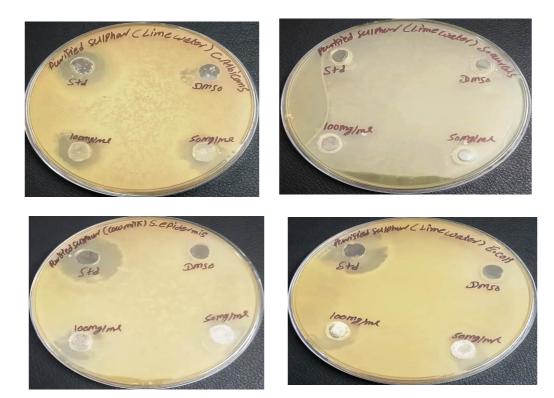


Fig.1.4 Results-Purified Sulphur (Lime Water)

Table No.1.5: Antimicrobial Activity of Churnodaka Shodhita Gandhaka

Antimicrobial Activity	Zone of Inhibition (mm)				
(Values are mean of triplicate)	Standard	Test Sample (in DMSO)			
	Positive control	50%	0% 100% DMSONegative contr		
Staphylococcus aureus	20	12	18	8	
E. coli	22	14	18 8		
Staphylococcus epidermidis	22	14	18	8	
Candida albicans	24	16	20	8	

As per standard antimicrobial sensitivity protocol of pharmacopoeia

Control is DMSO Blank for all study, Gentamycin & Fluconazole (2.5 & 5.0 µg/mL) as positive control.

Table No.1.6: Antimicrobial Activity of Samples of Gandhaka

	Zone of Inhibition (mm)							
	S. aureus		E. coli		S. epidermidis		Candida albicans	
Sample	P.C.	100%	P.C.	100%	P.C.	100%	P. C.	100%
R.G	26	22	26	22	22	18	28	24
G.G	24	22	22	14	24	18	28	26
B.G	24	22	24	20	24	22	28	24
C.G	24	18	22	18	22	18	24	20

R.G: Raw Gandhaka

G.G: Godugadha Shodhita Gandhaka

B.G: Bhringaraja Swarasa Shodhita Gandhaka

C.G: Churnodaka Shodhita Gandhaka

#### 4. DISCUSSION

Introduction: - Gandhaka is an important ingredient in Ayurvedic pharmaceuticals, but its raw form contains impurities that must be removed through Shodhana (purification). Various classical methods are described in Rasashastra, yet choosing the most suitable one remains a challenge. This study evaluates three traditional purification media—Go-Dugdha (animal origin), Bhringraj Swarasa (plant origin), and Churnodaka (mineral origin)—as mentioned in Ras-Tarangini. Since the purification medium significantly influences the quality and therapeutic value of Gandhaka and its use in Bhasma preparation, the study aims to compare these media in terms of efficacy, cost-effectiveness, and therapeutic potential using in vitro methods.

**Material:** - *Gandhaka*, an essential drug in Rasashastra, is crucial for neutralizing the toxicity of Parada and enhancing its therapeutic effects. It is used in preparing various formulations like Kajjali and Rasa Sindura and plays a key role in processes like *Murcchana* and *Jarana*. *Gandhaka* reduces Parada's toxicity, which is why the two are commonly used together, also aiding in Bandhana (binding) processes.

**Methods:** - Pharmaceutical study includes mainly purification of *Gandhaka*, and its process of standardization in which drug ratio, *dravya* quantity, and duration etc. The practical study of purification of *Gandhaka* with different medias comprised of following steps: -

## • Gandhaka Shodhana With Godugdha

Sodhana of *Gandhaka* was carried out according to the method given in *Rastarangini* (8/7-12). Initially 250 gm Raw *Gandhaka* was taken and 69 gm of Shuddha *Gandhaka* was obtained.

**Precautions**- *Gandhaka* was melted on mild heat with constant stirring, avoiding direct flame due to flammability. Filtration was done using *Goghrita*-coated muslin cloth. Residue was reprocessed to reduce loss. Overheating was avoided to maintain filterable consistency. Purified *Gandhaka* was washed with hot water (×10) to remove *Goghrita*. The process was repeated 7 times using fresh *Godugdha* and *Goghrita*, with proper cleaning of equipment after each cycle.

#### • Bhringaraja Swarasa Nirmana.

There is no reference of Bhringaraja Swarasa nirmana described in *Rasatarangni*. Hence general method of Swarasa nirmana described in *Sarangdhara Samhita*, Madhyama Khanda, Prathama adhyaya is used to prepare of Bhringaraja Swarasa. Initially 2.5 kg of Bhringraja Plant was taken and 1.150 lit of Swarasa was obtained.

### Gandhaka Shodhana With Bhringaraja Swarasa.

Sodhana of *Gandhaka* was carried out according to the method given in Rastarangini (8/21-22). Initially 250 gm Raw *Gandhaka* was taken and 71 gm of Shuddha Gandhaka was obtained.

#### • Churnodaka Nirmana.

Churnodaka nirmana was carried out according to the method given in Rastarangini (8/26) Initially Churna (Quick lime) 750 gm 2.25 lit of water was taken and 1.4 lit of Churnodaka was obtained.

### • Gandhaka Shodhana With Churnodaka.

When both *Gandhaka* and *Churnodaka* was strongly heated together, they did not melt as described in the reference *Rasatarangini* (8/27-28). To overcome this problem *Gandhaka* was melted with *Goghrita* and poured through *Goghrita* lipta muslin cloth in *churnodaka*. Initially 250 gm of raw *Gandhaka* was taken and 209 gm of Suddha *Gandhaka* was obtained.

#### • Antimicrobial Activity

Antibacterial and antifungal activities were assessed using the agar well diffusion method. Test formulations (50 mg/mL, 100 mg/mL, 50%, 100% in DMSO) were applied (100  $\mu$ L/well, 8 mm). Gentamycin and fluconazole served as standards; DMSO as control. Muller-Hinton agar plates were incubated at 37°C (18–24 h for bacteria) and 25°C (48–72 h for fungi). Zones of inhibition were measured in mm.

- After corelating all the antimicrobial study reports of Raw as well as Shodhita Gandhaka samples, it shows that –
- All four samples are result oriented. i.e. All the samples, raw as well as Shodhita *Gandhaka* has antibacterial and antifungal activity.
- All three samples (except *Churnodaka* Shodhita Gandhaka), has equally effective on *S. aureus* bacteria.
- On E. coli, Raw Gandhaka shows most effective among all the four samples.
- On S. epidermidis, Bhringraja Swarasa shodhita Gandhaka shows most effective among all the four samples.
- On Candida albicans, Godugadh Shodhita Gandhaka shows most effective among all the four samples.

### 5. CONCLUSION

Based on the antimicrobial study, it is concluded that both raw and Shodhita Gandhaka possess antibacterial and antifungal activities, with a comparatively stronger antifungal effect. Among the various purification media used, Churnodaka Shodhita Gandhaka was the least effective against all tested microbes, indicating that Churnodaka is not a suitable medium for the purification of Gandhaka. All other samples, except Churnodaka Shodhita Gandhaka, showed similar efficacy against *Staphylococcus aureus*. Raw Gandhaka was most effective against *Escherichia coli*, Bhringraja Swarasa Shodhita Gandhaka against *Staphylococcus epidermidis*, and Godugdha Shodhita Gandhaka against *Candida albicans*. These results emphasize the significant role of the purification medium (Shodhana dravya) in enhancing the antimicrobial properties of Gandhaka, a principle that may also influence the Marana process. While Bhringraja Swarasa demonstrated balanced effectiveness across all tested microbes, its limited seasonal availability may hinder its routine use. In contrast, Godugdha is easily accessible and effective, making it the most suitable medium for Gandhaka purification. Further clinical trials are recommended to substantiate these findings and explore their therapeutic relevance.

#### 6. ACKNOWLEDGEMENT

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