



## Research Article

### COMPARATIVE ANTIMICROBIAL STUDY OF RASAPUSHPA AND RASKARPUR

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#### ABSTRACT

Nirgandha kalpana is an important type of Parad Murchana. Yet, ignorance and also fear of its side effects led to its lesser and lesser usage in Ayurveda. Also, the use of Sagandha kalpana of Parad is more as compared to the former. The few widely known Nirgandha kalpanas are Mugdha rasa (grey powder) a kharaliya kalpana and Rasapushpa, Raskarpur the kupipakva kalpanas. Rasapushpa and Raskarpur both are prepared within 6 and 12 hours respectively in kupi-valukayantra by giving manda agni(100-200<sup>0</sup>C). Both are said to possess krimigna property and thus in this study an initiative was taken to confirm their antimicrobial property. Microorganisms selected were Staphylococcus aureus, Streptococcus pyogenes and Pseudomonas aeruginosa. The organisms selected were responsible for causing various skin diseases as both Rasapushpa and Raskarpur have been indicated in various skin diseases like Mandal, Kushta, Vicharchika, etc. The study started with preparation and standardisation of Rasapushpa and Raskarpur and then the Antimicrobial study was conducted using Agar Ditch method. It was found that Rasapushpa and Raskarpur showed total inhibition to streptococcus pyogenes, moderate inhibition to staphylococcus aureus and slight inhibition to pseudomonas aeruginosa. This study is just a first step towards antimicrobial study of these nirgandha kalpanas. Further elaborate studies could be conducted to acquire more details.

**Keywords:** Parad, Sagandha, Nirgandha, Murchana, Kupipakva, Rasapushpa, Raskarpur Valuka yantra, Antimicrobial.

#### INTRODUCTION

Ayurveda is an age-old science of Indian Medical system that is based on its own fundamentals. It represents totality of life and provides complete knowledge to maintain holistic balance of body and mind.

Rasashastra is an important branch of Ayurveda. It is a science which includes all life sciences. It deals with drugs prepared from metals and minerals in combination with various plants and animal products. Among all the traditional medicines that evolved from time to time Rasashastra has its own importance. It is believed that Rasashastra is an expansion of rasayan therapy of Ayurveda.

In Rasendrasarsangraha the importance of Rasaushadhis has been highlighted as those required in less dose, are tasteless hence palatable and ensures good health in short span<sup>1</sup>.

Rasayogas are basically classified into Kharaliya, Parpati, Kupipakva and Pottali kalpanas. Among these preparations kupipakva are found to be highly effective due to the extensive use of agni. It has been named so as these kalpanas are prepared in a kupi, which is a round bottom flask with a narrow neck covered with 3-7 layers of cloth and mud and the instrument used is Valuka yantra<sup>2</sup> a vessel containing sand.

Based on the presence of gandhak in the preparation, kupipakva kalpanas are again classified into sagandha and nirgandha kalpanas<sup>3</sup>. In nirgandha kupipakva kalpanas two most important preparations are Rasapushpa and Raskarpur.<sup>4</sup> Both have been indicated in acute conditions, skin diseases and epidemics yet fearing its toxicity its use gradually diminished and now only a limited group of physicians use these drugs in practice. To re-

establish the efficacy, safety and for wider acceptability of these kalpas research needs to be done.

Raskarpur has being indicated in eradicating many kinds of Krumivisha<sup>5</sup> and also used in Kushta, kandu, mandal and twak dosha vikaar. Rasapushpa too has being indicated against toxicity caused by microorganisms<sup>6</sup>. Also, it is used in wound healing. Keeping this in mind microorganisms responsible for causing multiple skin diseases were identified and selected for this study. Thus, Staphylococcus aureus which causes staphylococcal scalded skin syndrome, boils, abscess, impetigo, etc<sup>7</sup>; Streptococcus pyogenes causing impetigo, minor abrasions, infections of wounds<sup>8</sup>; and Pseudomonas aeruginosa causing lesions of wounds, bed sores, nail bed infection<sup>9</sup> were selected for this study.

#### MATERIALS AND METHODS

**Materials:** Parad(Mercury), Kasis(Ferrous sulphate) and Saindhav(Sodium chloride), Hydrochloric acid(HCl).

**Instruments:** Stone Kharal, Stainless Steel spatula, conical flask, rubber cork, "C" shaped glass tube, Spirit lamp, Kupi(7 layered), Valuka yantra, Gas-stove, Pyrometer, glass container and other analytical instruments.

#### Shodhanaa of raw materials

##### 1. Parad samanya shodhanaa

Done by HNO<sub>3</sub> method. (70% conc. HNO<sub>3</sub> =30ml, Water=70ml and Mercury =100g & asbestos chips) Soaked together for an hour.

The above method was selected from Ayurvediya Rasashastra- Siddhinandan Mishra<sup>10</sup>.

Parad before shodhana: 800 g; Parad after shodhana: 659 g (Table 1)

## 2. Parad Vishesh shodhana

Chitrak mul kwatha, Triphala kwatha, Kumari, Nagvelli patra, Adrak, Haridra swaras, Rason kalka and Saindhav (each 1/16<sup>th</sup> of Parad) were added and triturated until Parad got completely homogeneous with these drugs. Time taken 24 hours<sup>11</sup>.

The above drugs were selected as they were used in vishesh shodhana of Parad as well as since they possessed kushtagna properties. It was in the wake, if at all Rasapushpa had to be used in future for some skin disease.

Parad before shodhana: 815g. Parad after shodhana: 780 g (Table 1).

## 3. Kasis shodhana

Ashuddha Kasis was taken and heated in Bhringaraj swaras by dolayyantra method for 72 mins (3 ghatika). Kasis dissolves in the swaras whereas impurities remain in the pottali. This mixture is then dried. Ashuddha kasis 500g, after shodhana 450 g<sup>12</sup>.

## 4. Preparation of Rasapushpa mixture

Purified mercury, Purified Kasis and Saindhav are taken 150g each and triturated for 18 hours (until lustreless mixture was formed). Pista green mixture was obtained<sup>13</sup>. Mixture obtained: 420g Loss 30g.

## 5. Preparation of Rasapushpa

Rasapushpa mixture 300g was filled in a 7 layered kupi and this was then immersed in a valuka yantra and heated with constant manda agni for 6 hours on a Gas stove<sup>13</sup> (Table 2). After stopping the heat, the kupi was allowed to self cool overnight. The next morning kupi was removed from the valuka yantra, its layers scraped and broken midway to acquire flower like white coloured rasapushpa at the neck of the kupi.

Rasapushpa acquired: 95g

A red coloured powder was obtained at the bottom of the kupi which was saline in taste.

## 6. Preparation of Raskarpur

### Step I: Preparation of Mercuric Sulphate

Purified Parad(250g) and Sulphuric acid(375ml) are taken together in a conical flask. Its mouth was fixed with a rubber cork and a 'C' shaped glass tube was attached to it and on its rear end a rubber tube was fixed. The other end of the rubber tube was immersed in a glass cylinder. The mixture was heated on a kerosene stove until a white powder was formed<sup>14</sup>. Total time taken: 18 hours.

Mercuric sulphate obtained: 375g

### Step II: Preparation of Raskarpur mixture

350 g each of Mercuric sulphate and Saindhav were taken and triturated in a stone kharal until a homogeneous fine mixture is obtained<sup>14</sup>.

700g of mixture was obtained.

Time taken: 3 hours.

### Step III: Preparation of Raskarpur

Raskarpur mixture 300g was filled in a 7 layered kupi and this was then immersed in a valuka yantra and heated with constant manda and madhyam agni (up to 360<sup>o</sup>C)<sup>11</sup> for 12 hours on a gas stove<sup>14</sup>.

After stopping the heat, the kupi was allowed to self cool overnight. The next morning kupi was removed from the valuka yantra, its layers scraped and broken midway to acquire white coloured crystal like Raskarpur at the neck of the kupi.

Raskarpur acquired: 114 g. Table 3

### Confirmatory Test of Rasapushpa

A pinch of Rasapushpa was taken and dissolved in distilled water in a test tube.

Then 3 drops of ammonia was added to this solution.

Black precipitate was found at the bottom of the test tube.

This confirms that the compound formed is Mercurous Chloride (HgCl/Hg<sub>2</sub>Cl<sub>2</sub>) and Rasapushpa is known as Calomel (Mercurous chloride)<sup>15</sup>

### Confirmatory Test of Raskarpur

A pinch of Raskarpur was taken and dissolved in distilled water in a test tube.

Then 3 drops of ammonia was added to this solution.

White precipitate was found at the bottom of the test tube.

This confirms that the compound formed is Mercuric Chloride (HgCl<sub>2</sub>) and Raskarpur is also known as Mercurous chloride<sup>15</sup>.

## ANALYTICAL STUDY

### Raw material analysis

2 samples each of Kasis and Saindhav were bought from the market and their quantitative analysis was done. The one having higher concentration of the compound FeSO<sub>4</sub> and NaCl was selected for the drug preparation (Table 4)

### Namboori Phased spot test of Parad

Namboori phased spot test was performed on the crude mercury brought from the market. Also, the same test was performed post Parad shodhana with HNO<sub>3</sub> method. Before showed presence of Naag and Vanga metals and after showed the absence of them<sup>16</sup>.

For the study 10% potassium iodide paper was taken. Samples of both crude and HNO<sub>3</sub> purified mercury were prepared separately by adding 1ml of 5N HNO<sub>3</sub> to 2 g of mercury and kept still for 20 mins.

Later a drop of this solution was put on the potassium iodide paper (Whatman's paper no.1 & 10% potassium iodide solution) to see the immediate colour changes (Table 5).

### Tests performed on Rasapushpa and Raskarpur

**Organoleptic characters** such as colour, taste, odour and touch were observed (Table 6).

Ash value, Acid insoluble Ash value, estimation of mercury and chloride were done and their values were compared with the standards (Table 7).

**Namboori Phased spot test:** Two Rasapushpa and Raskarpur solutions were prepared, one by adding 0.5ml of 5N HNO<sub>3</sub> and other by adding 0.5ml of distilled water to 0.125gm of Rasapushpa and Raskarpur respectively. These samples were then heated and allowed to settle for 48hours<sup>17</sup>.

After that shake the samples well before adding 2 drops on the potassium iodide paper (Whatman's paper no.1 & 10% potassium iodide solution) and note the observations (Table 8, Table 9).

### ANTIMICROBIAL STUDY

Method: Agar Ditch Method<sup>18</sup>

Microorganisms: Staphylococcus aureus, Streptococcus pyogenes and Pseudomonas aeruginosa.

Medium: Nutrient agar

Instruments: Petridish, Nichrome wire loop, Incubator, Spirit lamp and Scalpel

#### Procedure

1. Nutrient Agar was taken, melted and then poured in two petridishes which were then allowed to cool off.
2. Then in the centre a rectangular ditch, 7cm long and 1cm deep was incised.
3. A mixture of 4 ml agar (after melting it) and 2g of drug sample was prepared by mixing them well. This way 2 mixtures of each Rasapushpa and Raskarpur were prepared.
4. These mixtures were then poured in the two incised ditches respectively and allowed to settle.
5. The incubator was adjusted on 37 °C.
6. Now the saline suspension of all 3 organisms was taken. Nichrome wire loop was dipped in each and a line drawn across the agar crossing the ditch horizontally.

7. This procedure was repeated dipping the nichrome loop in each of the 3 organisms on both the petridishes (containing both the drug groups) one below another horizontally.
8. These petridishes were then kept in the incubator for 24 hours for appropriate growth of organisms.
9. After 24 hours the dishes were removed and the growth /inhibition of growth was observed near the spirit lamp (to avoid spread of organisms).

#### Precaution

- Each time the loop is dipped in an organism, sterilize it by heating it on the spirit lamp.
- Always keep the lids of the petridishes closed to avoid spread of organisms.

### RESULTS AND DISCUSSION

Are given in the table below (Table 10).

This study initiated with preparation and standardisation of Rasapushpa and Raskarpur. Although both have been mentioned to eradicate the toxicity of microorganisms, Rasapushpa is being indicated especially in wounds caused due to Syphilis and microorganisms causing cholera. Raskarpur on the other hand is useful in eradicating microorganisms causing diarrhoea and Syphilis too. Also, it is indicated in Kushta and Kandu. And thus, the study of their Antimicrobial property needed to be done. To find the common organisms to study both the kalpa the organisms responsible in causing various skin diseases were selected. Finally using the Agar ditch method, the inhibition of the drugs to the growth of the microorganisms was observed.

Table 1: Parad Shodhana

Shodhanaa	I. Parad samanya shodhana-HNO <sub>3</sub> method			II. Parad Vishesh Shodhana		
Time required	1 hour			24 hours		
Procedure	Soaking			Trituration		
	Parad before shodhana	Parad after shodhana	Loss incurred	Parad before shodhana	Parad after shodhana	Loss incurred
	900g	839g	9%	815g	780g	9.5%

Table 2: Observations and temperature chart of Rasapushpa

Duration	Temperature		Observations
	Valuka	Kupi	
½ hour	100°C	80°C	Mixture melted
45 mins	110°C	86°C	White fumes started coming out
1 ½ hours	130°C	90°C	Fumes continue coming out
2 ½ hours	140°C	96°C	Rasapushpa starts to form at the neck of kupi
3 hours	150°C	100°C	Fumes reduce
3 ½ hours	140°C	110°C	Fumes stopped, Cork applied
6 hours	200°C	--	Heat stopped

Table 3: Observations and temperature chart of Raskarpur

Duration	Temperature		Observations
	Valuka	Kupi	
1 hour	98°C	80°C	Mixture melted
1 ½ hours	110°C	84°C	White fumes started coming out
2 ½ hours	110°C	92°C	Mixture feels like wet sand
5 hours	100°C	78°C	Fumes reduce as temp. falls down
6 hours	106°C	88°C	Mixture turns hard, fumes reduced
7 hours	130°C	110°C	White crystals start forming at the neck of kupi
9 hours	160°C	122°C	Fumes stopped coming out, Cork applied
12 hours	360°C	----	Heat stopped

**Table 4: Analytical values of Kasis and Saindhav**

Kasis sample 1	FeSO <sub>4</sub> -80%
Kasis sample 2	FeSO <sub>4</sub> -84%
Saindhav sample 1	NaCl- 96.09%
Saindhav sample 2	NaCl- 98.34%

**Table 5: NPST of Ashuddha and shuddha Parad**

Ashuddha Parad		Parad purified with HNO <sub>3</sub>	
Observations	Interpretations	Observations	Interpretations
Yellow spot in the centre surrounded by a orange band of ring. Lastly a brown ring.	Yellow spot seen at the centre signifies the presence of vanga and naag	Orange spot surrounded with brown	Absence of yellow spot signifies absence of vanga and naag.

**Table 6: Organoleptic characters of Rasapushpa and Raskarpur**

Characters	Rasapushpa	Standard	Raskarpur	Standard
Colour	White	Pink	Greyish white	White
Odour	odourless	Odourless	Odourless	Odourless
Taste	Salty	Saline	Salty	Saline
Touch	soft	---	Rough	---

**Table 7: Analytical tests of Rasapushpa and Raskarpur**

Standards	Rasapushpa	Standards*	Raskarpur	Standards*
Ash Value	4.3442	--	1.8615	--
Acid Insoluble Ash value	3.9590	--	1.2587	--
Mercury Content in %	45.26	83-85%	51.34	65-75%
Chloride content in %	16	15-20%	24.34	24-33%

**Table 8: Namboori phased spot test of Rasapushpa**

Rasapushpa Batch 1 and II			
Phase I: Immediate observation			
HNO <sub>3</sub>	Standard observation**	Distilled water	Standard observation**
Purple spot in centresurrounded by orange ring, then light orange band, cream coloured band and lastly brown ring.	Light purple central spot with brown periphery. In between both very light purple space.	Central orange with white spots in between, in periphery cream ring, white band and last greyish band.	Similar to that of HNO <sub>3</sub> sample.

**Table 9: Namboori phased spot test of Raskarpur**

Phase I: Immediate observation			
HNO <sub>3</sub>	Standard observation**	Distilled water	Standard observation**
Purple spot in centre with a light purple ring surrounding it. And lastly dark brown band forms the outermost layer.	Immediately a brown spot forms. It further turns white with moderate deep brown periphery. Before the end of the 1 <sup>st</sup> phase ½ tiny and irregular dark particles form in the centre of the spot.	Centre orange spot surrounded by broad light brown band with white circle in between.	Immediately a brown spot forms. It further turns white with moderate deep brown periphery.

\* Pharmacopoeial standards of Ayurvedic Formulations p. 295, \*\* Manual of Namburi Phased spot test. p. 39-42

**Table 10: Antimicrobial study results**

Organisms	Rasapushpa	Raskarpur
Staphylococcus aureus	++	++
Streptococcus pyogenes	+++	+++
Pseudomonas aeruginosa	+++	+

+++ : Total inhibition, ++ : Moderate inhibition, + : Slight inhibition

## CONCLUSION

The Antimicrobial study showed that Rasapushpa showed total inhibition to *Streptococcus pyogenes* and *Pseudomonas pyogenes* whereas moderate inhibition to *Staphylococcus aureus* and Raskarpur showed total inhibition to *Streptococcus pyogenes*, moderate inhibition to *Staphylococcus aureus* and slight inhibition to *Pseudomonas aeruginosa*.

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