



ORIGINAL RESEARCH PAPER

Ayurveda

A PHARMACEUTICO-ANALYTICAL STUDY OF RASAPUSHPA WITH SPECIAL REFERENCE TO ITS IN-VITRO ANTIMICROBIAL ACTIVITY

KEY WORDS: Kupipakwa preparation, Rasapushpa, physico chemical parameters, Syphilis,

Prema Kalmegh

Asst Professor, Dept. of Rasashastra & Bhaishajya Kalpana, Rajendra Gode Ayurveda College Hospital and Research Centre, Amaravati. PhD Scholar, MGACHRC, DMIMS(DU).

Bharat Rathi*

Professor Dept. of Rasashastra & Bhaishajya Kalpana, Mahatma Gandhi Ayurved College Hospital & Research Centre Salod(H) Wardha. Datta Meghe Institute of Medical Sciences (Deemed to be University) Sawangi (M) Wardha (MS) India. * Corresponding Author

Bhushan Raghuwanshi

Asst Professor, Dept. of Kayachikitsa, Rajendra Gode Ayurveda College Hospital and Research Centre, Amaravati.

ABSTRACT

Introduction: *Kupipakwa* formulations hold superior position in Ayurvedic therapeutics due to its small drug dose, rapid action, desired result, long shelf life and palatability. According to *Rasatarangini* *Rasapushpa* is an effective *Kupipakwa* preparation specifically in wounds caused due to Syphilis and microorganisms causing cholera. Hence this study was planned to assess the antimicrobial activity of the *Rasapushpa* on causative organism *Treponema Pallidum*. **Material & Methods:** *Rasapushpa* was prepared according to *Rasatarangini*. Analytical study was done to establish the basic standards for *Rasapushpa* as there is no pharmacopeia standard guideline. The formulation was tested for organoleptic parameters, physicochemical analysis and antibacterial activity against causative organism *Treponema Pallidum*. **Observation & Results:** All the Organoleptic and physico chemical parameters are within normal limits. X- ray diffraction study of *Rasapushpa* shows various peaks for crystalline structure. *Rasapushpa* has revealed good antimicrobial activity against *Treponema Pallidum*. **Conclusion:** *Rasapushpa* can be prepared with easily available drugs and can be effectively used in the management of Syphilis.

Introduction:

Since Vedic period drugs are being used after purification successfully to treat physical and mental ailments. *Rasashastra* a branch of an Ayurveda pharmaceuticals is one of the main tributary of ancient medical science includes the entire field of inorganic pharmaceutical preparation incorporating metallic and non-metallic and mercurial compounds flourished during the medieval period [1]. The *Rasa* preparation are devoid of taste, effective in less dose, quick acting having long shelf life, easy for handling etc. makes their use convenient and important in therapeutic profile[2-3].

Phiranga or Portuguese disease can be correlated with the syphilis. It is known to be sexually transmitted disease, which occurs due to sexual contact with native of *phiranga desha*. Hence it is commonly known as *phiranga*. Description of *phiranga roga* is not available in *Brihatrayee viz. Charaka Samhita, Sushruta Samhita* and *Ashtanga Hridaya*. *Acharya Bhavamishra* (16th century) was the first Ayurveda physician who documented this disease in his book *Bhavaprakasha samhita*. *Phiranga* is classified according to the manifestation. The classification of *phiranga* can be understood as different stages of syphilis[4].

According to modern science, syphilis is caused by infection of the spirochaete *Treponema pallidum*. In which main causative organism is spirochaetes. The spirochaetales include three genera that are pathogenic for humans and for variety of other animals. *Leptospira* which causes Human Leptospirosis, *Borellia* which causes relapsing fever and Lyme disease, *Treponema* which causes *Treponematoses* [5].

Here the Syphilis caused by *Treponema pallidum* which can be correlated with causative factors of *Firanga* told by *Madhavidana*[6]. After knowing the view of *Madhaw-nadana* and modern concept about the *Firanga* i.e syphilis and in the quest of updating the same, the present work was planned on one of the mercurial preparation *Rasapushpa*. According to *Rasatarangini* it is an effective preparation for the management of *Firanga*.

In *Kupipakwa* preparations, Mercury with or without Sulphur is converted in to a suitable compound, even without being reduced to ashes [7]. Through this process, the potency and efficacy of mercury enhances in proportion to the amount of Sulphur burnt in the *Jarana* process. The properties like small drug dose, rapid action, desired result, long shelf life, palatability made *Kupipakwa* formulations to hold superior position in Ayurvedic therapeutics. *Kupipakwa* medicines are very much effective in all *Vata Kapha* predominant diseases. *Rasapushpa* is indicated specifically in wounds caused due to Syphilis and microorganisms causing cholera. Few other Antimicrobial study *Rasapushpa* showed total inhibition to *Streptococcus pyrogenes* and *Pseudomonas pyrogenes* whereas moderate inhibition to *Staphylococcus aureus*. [8] Hence this study was planned to assess the antimicrobial activity of the *Rasapushpa* on causative organism *Treponema Pallidum*.

MATERIAL AND METHODS:

Material:

Raw drugs were procured from the local market and identified according to acceptable properties described in the authoritative texts of *Rasashastra* and authenticated chemically by Laboratory. All the herbal drugs were also identified and authenticated by taxonomist. Literary source of the data is obtained from the classical texts of Ayurveda.

Methods:

Shodhana (purification) of the mineral drugs was done according to the textual reference as shown in the table no1.

Table 1: Purification of ingredients of Rasapushpa

S.N.	Name of drug	Method of Purification	Reference
1.	<i>Parad Shodhana</i> [9]	Trituration in <i>Tapt Khalwa Yantra</i> with <i>Nistush Lashuna</i> and <i>Nirmalikut Saindhav Lavana</i>	Ayurveda Prakash 1/165
2.	<i>Kasis Shodhana</i> [10]	With <i>Nimbu Swaras Bhavana</i>	Brihad Rasaraj Sunder 8/163

B) Preparation of Rasapushpa

The Rasapushpa is one of the *Koopipakwa Rasayan*. In the present study the method followed for the preparation of *Rasapushpa* was as described in *Rasatarangini*.

Table No.2: Ingredients and Quantity of Raspushpa [11]

Sr. no	Ingredient	Scientific name	Raw Quantity	Obtained Quantity	Quantity taken for preparation
1.	Kasis	FeSO ₄ 7H ₂ O	500gms	276gms	150gms
2.	Parad	Mercury	500gms	392gms	150gms
3.	Saindhaw Lavana	Rock Salt	500gms	305gms	150gms

The method of preparation of Rasapushpa was divided into 3 phases as *Poorwakarma* (pre procedure), *Pradhan karma* (major procedure) and *Paschatkarma* (post procedure) as described in table 3 & 4.

Table 3: Phases of The method of preparation of Rasapushpa

S.N.	Phases	Process
1.	<i>Poorwakarma</i>	<p>Shuddha Kasis + Shuddha Parad + Nirmalikut Saindhaw Lavana (150 gm each) In <i>Khalwayantra</i></p> <p style="text-align: center;">↓</p> <p>Triturated for 18 hours (until lusterless mixture was formed)</p> <p style="text-align: center;">↓</p> <p><i>Kachkoopi</i> filled with 300 gm of mixture</p>
2.	<i>Pradhankarma</i>	<p><i>Kachkoopi</i> placed at <i>Valukayantra</i> & heated with constant mild fire</p> <p style="text-align: center;">↓</p> <p>Thermometer inserted near <i>kachakoopi</i></p> <p style="text-align: center;">↓</p> <p>Temperature recorded (half hourly)</p> <p style="text-align: center;">↓</p> <p>Vapors along with fumes expelled out</p> <p style="text-align: center;">↓</p> <p>vapors stopped completely</p> <p style="text-align: center;">↓</p> <p>Mouth of the bottle closed with cork</p> <p style="text-align: center;">↓</p> <p><i>Valukayantra</i> allowed to cool overnight</p>
3.	<i>Paschatkarma</i>	<p><i>Valukayantra</i> becomes self cool</p> <p style="text-align: center;">↓</p> <p><i>Koopi</i> was removed</p> <p style="text-align: center;">↓</p> <p>Its layers scraped and wrapped a thread soaked in kerosene and burnt</p> <p style="text-align: center;">↓</p> <p><i>Koopi</i> was broken</p> <p style="text-align: center;">↓</p> <p><i>Rasapushpa</i> was collected (at the neck of <i>koopi</i>)</p>

Table 4: Observations and temperature chart of Rasapushpa

Duration	Temperature (°C)	Observations
0 hour	25	Kajjali is in powder form
1 st hour	72	White fumes started coming out
2 nd Hour	164	Fumes continue coming out
3 rd hour	200	Rasapushpa starts to form at the neck of kupi
4 th hour	220	Fumes reduced
5 th Hour	300	Fumes stopped, Cork applied
6 th Hour	380	Heating stopped

Confirmatory Test of Rasapushpa

A small quantity of Rasapushpa was dissolved in distilled water in a test tube. Then to this solution few drops of ammonia was added. At the bottom of the test tube black precipitate was formed. This proved that the precipitate formed is Mercurous Chloride (Hg₂Cl₂) i.e. Rasapushpa [12].

C) Analytical Study

Analytical study was planned to generate the basic standards for Rasapushpa as there is no pharmacopeia standard guideline available. The prepared formulation was first tasted for organoleptic characters such as odor and color. (Table no.6) Physicochemical analysis includes pH, Loss on drying, determination of ash value, determination of acid insoluble ash and Water Soluble Extractive, Fineness of Particles and X-Ray Diffraction. Assay of Elements done to calculate the % of Mercury Iron, Sulphur, Sodium and Chlorine (Table no. 7).

Microbial specification was tasted to validate its safety for internal as well as external use. Enterobacteriaceae, Total fungus count, E-coli, Salmonella and Coliform were performed as per CCRAS parameters. (Table no. 8) Analysis of samples was conducted as per API standards in analytical lab.

D) Antimicrobial Study

Preparation of Extract of Rasapushpa:

Distilled water was used as a Solvent for extraction of *Rasapushpa* (Trial drug). The Trial drug extracts was named as Distilled water extract. Bacterias: - *Treponema pallidum*. All cultures collected are pure, authentic and obtained from standard culture collections sources.

Preparation of Nutrient Agar media

Antibacterial activity which is essential for solidification is performed by using Agar media. Module of Nutrient Agar Media (High media REF 1001) was made by Distilled water (100 ml) of pH of the media 7.4±0.2

Agar solution of required quantity was prepared according to the standard ratio with pH 7.2. The prepared media was transferred in conical flasks which were sealed with aluminium foils and sterilized in autoclave for 15 minutes. Then flasks were poured into the sterile Petri plates and incubated for 37 ± 20C for 24 hrs.

Preparation of different concentrations extracts of Rasapushpa

Concentration of extracts of *Rasapushpawas* prepared by making a suspension in distilled water, as *Rasapushpa* is not soluble in water.

Preparation of Inoculum

Bacterial culture

The sterile nutrient Agar medium was cooled to 45°C and spread with 10⁶ cells/ml of respective bacterial culture individually and 5 holes or wells about 9mm in diameter were cut in the medium with a sterile cork borer. Then Disc was prepared.

Incubation

The inoculated plate was placed on the table for 1 hour to allow the extract to diffuse into the agar. The NA plate was incubated aerobically at 37 °C for 24 hrs. Zones of inhibition produced after incubation was measured in millimetres [13].

OBSERVATIONS AND RESULTS

Table 5 : Pharmaceutical observations of Rasapushpa:-

Wt. of Ingredients (gm)	Time required (hrs)	Total weight of prepared medicine (gm)	Yield %	Loss %
300	28	49.5	16.5%	83.5%

Table 6: Organoleptic Observations of Rasapushpa

Sr. No.	Ayurved parameters	Observations
1	Shabda (Sound)	NA
2	Sparsh (Touch)	Mrudu
3	Rupa (colour)	White
4	Rasa (Test)	NA
5	Gandha (Smell)	NA

Table 7: Physico-Chemical Tests & Elemental assay

Physical Tests	
Color	White
Odour	NA
Particle Size	126.87 µm
Chemical Tests	
pH	6.6
Loss on drying at 105oC	04.32 %
Total Ash	16.38 %
Acid Insoluble Ash	01.92 %
Alcohol Soluble Extractive	03.20 %
Water Soluble Extractive	0.4 %
Assay of Elements	
Free Mercury Percentage	00.00 %
Mercury Percentage	81.93 %
Ferrous Percentage	10.72 %
Sulphur Percentage	03.75 %
Sodium Percentage	09.57 %
Chlorine Percentage	21.22 %

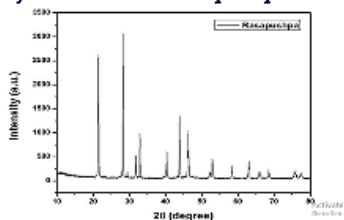
Table 8: Microbial Contamination Test of Rasapushpa

Test	Value (Cfu/g)
Total Plate Count	25 x 10
Total Fungal Count	Absent
Escherichia Coli	Absent
Salmonella	Absent
Coliform	Absent

Observations and Results of X-Ray Diffraction of Rasapushpa

In present study sample were used in powder form. In X-RD reports D- values obtained with Theta angle, intensity and its graphs. Then this graph compared with Standard JCPDF data. They are represented in graph 1.

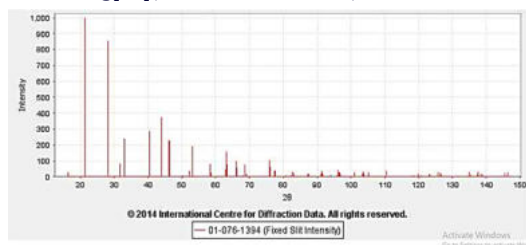
Graph 1: X-Ray Diffraction of Rasapushpa



There are two large peaks at 21.45 2 theta angle & 1820 gross intensity and 28.18 2 theta angle & 2602 gross intensity (chart 1)

Peak No.	2θ	Relative intensity	Crystalline size	Full width half maxima
1	21.45	71.3	29.29 nm	0.279 Å°
2	28.18	100	36.82 nm	0.224 Å°

Joint Committee Powder Diffraction Standards Data Match for- Hg₂Cl₂ (Mercuric Chloride)



Peak No.	2θ	Relative intensity	Crystalline size	Full width half maxima
1	21.45	71	29.29 nm	0.279 Å°
2	33.88	29	21.82 nm	0.224 Å°

Chart 1: Showing angle, d-values, Net intensity, gross intensity and relative intensity

Sex	Scan	Angle	d Value	Net Intensity	Gross Intensity	Rel. Intensity
1	rasapushpa.raw (Strip ko2) #1	21.455	4.13630	1820	1868	71.3 %
2	rasapushpa.raw (Strip ko2) #1	28.185	3.16359	2552	2602	100.0 %
3	rasapushpa.raw (Strip ko2) #1	29.419	3.03369	91.5	136	3.6 %
4	rasapushpa.raw (Strip ko2) #1	31.717	2.81891	387	432	15.1 %
5	rasapushpa.raw (Strip ko2) #1	32.883	2.72153	748	790	29.3 %
6	rasapushpa.raw (Strip ko2) #1	40.267	2.23788	487	528	19.1 %
7	rasapushpa.raw (Strip ko2) #1	43.828	2.06397	1202	1248	47.1 %
8	rasapushpa.raw (Strip ko2) #1	46.084	1.96803	437	483	17.1 %
9	rasapushpa.raw (Strip ko2) #1	46.306	1.95913	903	948	35.4 %
10	rasapushpa.raw (Strip ko2) #1	52.123	1.75332	101	143	3.9 %
11	rasapushpa.raw (Strip ko2) #1	52.898	1.72946	350	391	13.7 %
12	rasapushpa.raw (Strip ko2) #1	58.242	1.58285	237	275	9.3 %
13	rasapushpa.raw (Strip ko2) #1	62.972	1.47484	323	359	12.6 %
14	rasapushpa.raw (Strip ko2) #1	65.903	1.41618	109	147	4.3 %
15	rasapushpa.raw (Strip ko2) #1	68.482	1.36900	178	218	7.0 %
16	rasapushpa.raw (Strip ko2) #1	68.863	1.36235	47.2	86.0	1.9 %
17	rasapushpa.raw (Strip ko2) #1	75.598	1.25682	106	146	4.2 %
18	rasapushpa.raw (Strip ko2) #1	75.957	1.25177	126	166	5.0 %
19	rasapushpa.raw (Strip ko2) #1	77.295	1.23341	65.3	103	2.6 %

Observations and results of Antimicrobial study

Showing zones of inhibition in agar media in mm for Rasapushpa

Name of Organism	Rasapushpa (Zone of Inhibition in agar media in mm)
Treponema pallidum	12



DISCUSSION:

In Ayurveda herbs and the minerals are the major source of drugs for the preparation of medicines. According to the requirement, these drugs are flourished by undergoing varied modulations. Rasapushpa is one such herbomineral formulation prepared by various processes. Rasapushpa is one of the herbomineral formulation especially suggested to treat wounds caused due to Syphilis and microorganisms [14].

1) Pharmaceutical study

All the ingredients of Rasapushpa were selected strictly according to classical reference. Minerals drugs were purified before being used in the formulation. The purification of Mercury is done with garlic paste. Due to the continuous trituration in Tapta Khalwa yantra, the fineness of Parad increased and the shining property was reduced. Weight loss in Parad was observed might be due to continuous trituration each time during purification process. At the end colour changed from shiny silvery white to dull blackish white and has the fragrance of garlic.

Before purification Kasis have green colour but after purification Kasis gained faint yellow colour and became dry so that it turns in to the powder form easily. The purpose of Kasis shodhana is to discard superfluous materials from Kasisa and to increase its potency [15]. Kasis shodhana explained in all the texts by using different Medias. In the preparation of Rasapushpa, its shodhana is especially done in the lemon juice. Lemon juice is rich with vitamin C and vitamin B complex, which works as an intrinsic reason in the absorption of iron in the body due to a synergistic effect [16].

The Specific Shodhan process about Saindhav is not mentioned in any Rasa texts. So for removal of physical impurities from it, nirmalikaran process was done. Saindhav

Lavan shodhana was done by Vaporisation technique by dissolving in water.

2) Analytical Study

The prepared Rasapushpa was subjected to analytical study in laboratory for Ph, total Ash Value, Acid insoluble ash, Water soluble extractives, Alcohol Soluble extractives, Loss on drying, Colour, Odour, Assay of element as Hg, Na,S,B, Pb, Microbial contamination test, and X Ray diffraction.

- **PH:** The PH of the Rasapushpa was 6.7 and Slightly Acidic.
- **Total Ash Value:** Total Ash Value of Rasapushpa was 17.84 % and it was within the normal limit.
- **Acid Insoluble Ash:** Acid Insoluble Ash value of Rasapushpa was 2.02 % and it was within the normal limit.
- **Loss on Drying:** The Loss on Drying at 110° C of Rasapushpa was 4.28% and it was within the normal limit.
- **Particle size:** Microscopic examinations of Rasapushpa shows particle size about 136.47 µm.
- **Assay of element:** Assay of element for mercury, sulphur, sodium, ferrous, chlorine and lead for Rasapushpa was done and they were within normal limits.
- **X-Ray Diffraction:** X ray diffraction study of Rasapushpa shows various peaks for crystalline structure. Also there are small peaks for other ingredients and amorphous substances. Rasapushpa was 67.2% crystalline and 32.8% amorphous.

3) Antibacterial Study

Antimicrobial activity of Rasapushpa was studied to determine zone of inhibition in per mm against selected organisms for Treponema Pallidum. Thus Rasapushpa has revealed good antimicrobial activity for above said organism.

CONCLUSION

Rasapushpa can be easily prepared as described in the texts. Physical and chemical analysis of Rasapushpa supports in its standardisation. All physico chemical parameters are within normal limits. X-ray diffraction study shows Rasapushpa 67.2% crystalline and 32.8% amorphous. Rasapushpa has shown better zone of inhibition against *Treponema pallidum* and thus showed good antimicrobial activity.

REFERENCES:

1. Rathi B, Rathi R, Pusadkar S. Contribution of the text Rasapaddhati in the history of Indian alchemy: A review. *J Indian Sys Med* 2019;7:70-4.
2. Rathi B, Rathi R, Rajput DS. Pharmaceutical standardization of *Avalgujadi Lepaguti*. *J Indian Sys Med*. 2016;4(2):72-76
3. Rathi B, Rathi R. Pharmaceutical standardization of Bakuchi vati: a modified dosage form of Dhatriyadi Yoga. *Int. J. Res. Ayurveda Pharm*. 2017;8(1):57-61.
4. Misra Brahma Sankara, Bhavaprakash of Sribhava Misra, Part II, Chaukhambha Sanskrit Sansthan, Varanasi, 2003; p 560-561
5. Maharjan AS, Arunraj MN., Anjana .An Overview of *Phiranga* in Ayurveda. *Int J Ayu Pharm Chem* 2017;7(2):410-41
6. Upadhyaya Y. Madhava Nidana, Upadamsh Nidana, Chaukhambha Prakashana, Varanasi, 1st ed. 1999; p 171
7. Shashtri K. Rasa Tarangini, 1st Edition, Varanasi, Motilal Banarsidas, 2014; p. 103.
8. Gaonkar G. Comparative antimicrobial study of rasapushpa and raskarpur. *Int. Res. J. Pharm*. 2018;9(2):54-58
9. Ayurveda Prakasha Shri Madhava- Edited By Shri Gulraj sharma Mishra, 1/165, Varanasi, Chaukhamba Bharti Academy, 1999; p 54
10. Chaube D, Bruhat Rasa Rajasundar, Varanasi, Chaukhamba Orientalia, 3rd Edition 2000. p162
11. Kashinath shashtri; Rasa Tarangini, 6/28-31, 1st Edition, Varanasi, Motilal Banarsidas, 2014; p.108.
12. Dwivedi Vishwanath, Bhartiya Rasashastra, 1st.ed. M.P: Sharma Ayurved Mandir; 1977. p.231.
13. Kokate CK. Analytical Pharmacognosy, 5th Chap. 1st ed 1990, Nirali Prakashana. Pune.
14. Sarkar PK, Mehta NJ, Prajapati PK. Chemistry of Kupipakwa Rasayanas-A Review. *Ancient science of life*. 2008 Apr;27(4):56.
15. Sharma S . Rasa Tarangini. 11th ed. Varanasi: Motilal Banarasidas Publication, 1979; p.568.
16. Shrestha N, Shrestha S, Bhattarai A. Determination of Ascorbic Acid in Different Citrus Fruits of Kathmandu Valley. *Journal of Medical and Biological Science Research* 2016;2(1):9-14