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

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ABSTRACT

INTRODUCTION: Kupipakva 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 Kupipakva preparation specifically in wounds caused due to Syphilis and microorganisms causing choleru. 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 pharmacology 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 Brihatayee viz. Charaka Samhita, Sushruta Samhita and Ashtanga Hridaya. Acharya Bhamavamisha (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 pallidium. In which main causative organism is spirochaetes. The spirochaetes 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 Lymedisease, Treponema which causes Treponematoses [5].

Here the Syphilis caused by Treponema pallidium which can be correlated with causative factors of Firanga told by Madhavindana[6]. After knowing the view of Madhav-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 Kupipakva 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 Kupipakva formulations to hold superior position in Ayurvedic therapeutics. Kupipakva medicines are very much effective in all Vata Kapha predominant diseases. Rasapushpa is indicated specifically in wounds caused due to Syphilis and microorganisms causing choleru. 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

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Name of drug</th>
<th>Method of Purification</th>
<th>Reference</th>
</tr>
</thead>
</table>
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 Rasapushpa [11]

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Ingredient</th>
<th>Scientific name</th>
<th>Raw Quantity</th>
<th>Obtained Quantity</th>
<th>Quantity taken for preparation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Kasis</td>
<td>FeSO₄.7H₂O</td>
<td>800gms</td>
<td>276gms</td>
<td>150gms</td>
</tr>
<tr>
<td>2.</td>
<td>Parad</td>
<td>Mercury</td>
<td>500gms</td>
<td>382gms</td>
<td>180gms</td>
</tr>
<tr>
<td>3.</td>
<td>Saindhaw Lavana</td>
<td>Rock Salt</td>
<td>800gms</td>
<td>305gms</td>
<td>180gms</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Phases</th>
<th>Process</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Poorwakarma</td>
<td>Shuddha Kasis + Shuddha Parad + Nirmalikrut Saindhaw Lavana(150 gm each) In Khalwayantra</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Triturated for 18 hours (until lusterless mixture was formed)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kachkoopi filled with 300 gm of mixture</td>
</tr>
<tr>
<td>2.</td>
<td>Pradhan karma</td>
<td>Kachkoopi placed at Valukayantra&amp; heated with constant mild fire</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Thermometer inserted near kachkoopi</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Temperature recorded (half hourly)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vapors along with fumes expelled out</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Vapors stopped completely</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mouth of the bottle closed with cork</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Valukayantra allowed to cool overnight</td>
</tr>
<tr>
<td>3.</td>
<td>Paschatkarma</td>
<td>Valukayantra becomes self cool</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Koopi was removed</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Its layers scraped and wrapped a thread soaked in kerosene and burnt</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Koopi was broken</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rasapushpa was collected (at the neck of koopi)</td>
</tr>
</tbody>
</table>

Table 4: Observations and temperature chart of Rasapushpa

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

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 Mercurgus Chloride (Hg.Cl) 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, Finess of Particles and X-Ray Diffraction. Assay of Elements done to calculate the % of Mercury Ion, 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 par CCRAS parameters. (Table no. 8) Analysis of samples was conducted as par 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. Bacteria: - Treponema pallidium. 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 foil and sterilized in autoclave for 15 minutes. Then flasks were poured into the sterile Petri plates and incubated for 37 ± 20°C 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 [15].

OBSERVATIONS AND RESULTS

Table 5: Pharmaceutical observations of Rasapushpa-

<table>
<thead>
<tr>
<th>Wt. of Ingredients (gm)</th>
<th>Time required (hrs)</th>
<th>Total weight of prepared medicine (gm)</th>
<th>Yield %</th>
<th>Loss %</th>
</tr>
</thead>
<tbody>
<tr>
<td>300</td>
<td>28</td>
<td>49.5</td>
<td>16.5%</td>
<td>83.5%</td>
</tr>
</tbody>
</table>
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)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Shabda (Sound)</th>
<th>Sparsh (Touch)</th>
<th>Rupa (colour)</th>
<th>Rasa (Test)</th>
<th>Gandha (Smell)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NA</td>
<td>Mruda</td>
<td>White</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>NA</td>
<td>Mruda</td>
<td>White</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**Table 6: Organoleptic Observations of Rasapushpa**

**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 105°C: 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: 81.83 %
- Ferrous Percentage: 10.72 %
- Sulphur Percentage: 03.75 %
- Sodium Percentage: 21.22 %
- Chlorine Percentage: 09.57 %

**Test**
- Value (CFU/g)
- Total Plate Count: 25 × 10
- Total Fungal Count: Absent
- Escherichia Coli: Absent
- Salmonella: Absent
- Coliform: Absent

**Table 8: Microbial Contamination Test of Rasapushpa**

**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 fluorished 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 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 superficial 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, sulphar, 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: