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RESEARCH ARTICLE

EVALUATION OF PHYSICO-CHEMICAL ANALYSIS OF LAGHU SUTASHEKHARA RASA

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ABSTRACT

Introduction: Laghu sutashekhara rasa was prepared as per Rasatantrssara avam Sidha prayoga sangraha and efforts have been made to lay down analytical standards for laghu sutashekhara, which were not found reported till date. The parameter for standardization of laghu sutashekhara rasa are ,pH 7.6 , and disintegration time 22 min and dissolation time is 40 min, whereas hardness was 4 kg. Loss on drying was found to be 9.3% w/w, acid insoluble ash was 62.57 %w/w, water soluble extract was 42.95 % w/w and percentage of solubility is 18. **Material & method:** The aim of the present work is to standardization of laghu sutashekhara rasa and attempts to evaluate the Organoleptic characters, phytochemical study, phamacognostic study and physicochemical parameters like pH, Loss on drying at 105°C, Water soluble extract, Alcohol soluble extract, Total Ash, Acid insoluble ash etc. **Result** Results of organoleptic charcter, physical constant, elemental analysis (XRD), phytochemical constituents & HPTLC contributes to establish standards for standardisation of laghu sutashekhara rasa. **Conclusion:** The phytochemical constituents & analysis of HPTLC reveals that the Laghu sutashekhara rasa contains most of the ingredients of all the raw materials. Results of organoleptic character, elemental analysis (XRD), phytochemical constituents & HPTLC contributes to establish standards for standardisation of laghu sutashekhara rasa.

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INTRODUCTION

Herbo mineral formulation are at great demand globally for primary healthcare due to their safety margins and cost effectiveness. When herbal medicines are being manufactured on large scale then manufacturers face many problems such as low-quality & lack of authentication of raw material, non-availability of standards [Sagar Bhanu, 2005; Jagriti, 1997], lack of proper standard methodologies of single drugs and formulations and lack of quality control parameters [Dixit Renu and Reddy Vijay, ?]. So it is a prime need to standardize Ayurvedic preparations to guarantee their purity, safety, potency and efficacy. Therefore, in case of herbomineral drugs the standardization should encompass entire field of study from cultivation of medicinal plant to its clinical application [3]. WHO involves in standardization and quality control of herbal crude drugs to monitor the physicochemical evaluation of crude drugs covering the aspects of selection and handling of crude material, safety, efficacy, stability assessment of finished product [Vanishri, 1996], documentation of safety and risk, provision of product information to consumer and product promotion. Laghu sutashekhara rasa is used in the treatment of Amalpitta, Parinamshool, udarshool, anaha and atopa etc[12] . By analyzing the properties of its ingredients (gairika, shunti and nagvalli swaras (bhawna dravya) it seems that its use may be justified in these clinical conditions. Till date, no standards are available for laghu sutashekhara rasa. Hence, the present study has been carried out with aims and objectives to develop analytical profile of laghu sutashekhara rasa.

Aim and Objective

- To know the organoleptic, physical constant, phytochemical constituents of laghu sutashekhara rasa.
- To know the organic and inorganic contents of laghu sutashekhara rasa.
- To establish standards for standardization of laghu sutashekhara rasa.
- Screening of anti ulcer activity of laghu sutashekhar rasa.

MATERIALS AND METHODS [5-11,13]

Materials

Drugs

- **Laghu sutashekhara rasa:** Vati (for pharmaceutical standardization)
- **Laghu sutashekhara rasa:** Powder(for organoleptic, physical constants,
- phytochemical, XRD& HPTLC)

Methods

Physico-chemical analysis

Organoleptic characters of laghu sutashekhara rasa were tested with sense organs

Physical constants

- **Solubility test:**A pinch of laghusutashekhara rasa powder was taken in a dry test tube with one ml of solvent and shaked for one minute. Then observed for solubility, non-solubility and sparingly solubility. Solubility test was conducted with the following solvents.Distilled water, Xylene, Benzene, Toluene, Acetone, Hydrochloride acid, Ethylealchol, Methanol, Chloroform, Carbon Tetrachloride

- **Percentage of Solubility:** 100 ml of distilled water + 5gm of laghusutashekhara rasa powder was stirred well, kept for 24 hours and filtered through Whatman's filter paper no. 42. It was kept for drying and weighed the filter paper with residue after complete drying. Then % of solubility was calculated as per standard formula.
- **Determination of Moisture content:**A clean and dry petridish was weighed. 5gm of dried laghusutashekhara rasa powder was taken in the petridish and weighed. Petridish was kept in hot air oven for an hour at 105^0C. After one hour the petridish containing laghusutashekhararasa was taken out of hot air oven and weighed. Again the petridish was kept in hot air oven for one more hour at 105^0C. The procedure was repeated till the constant weight of petridish containing powder was obtained.

$$\text{Percentage of Moisture content} = \frac{\text{Difference in weight}}{\text{Weight of sample}} \times 100$$

4.Determination of Specific Gravity: Empty picnometer was weighed (w_1) and picnometer was filled with distilled water weighed ($w_{2)}$. Afterwards took 1% solution prepared by taking one gram of laghusutashekhara rasa dissolved in 100 ml of distilled water. Filled this solution in and empty picnometer weighed ($w_{3)}$. All these three weights noted.

$$\frac{W_3 - W_1}{W_2 - W_1}$$

Determination of pH Value: Digital handy pH meter was caliberated by using standard buffers solution of known pH 4.0 and 9.2 at 30^0C. The reference electrode was thoroughly washed with distilled water every time and water was drained by using filter paper.

1% solution of laghusutashekhara rasa by taking 1 gm of laghusutashekhara rasa in 100 ml of distilled water was prepared then the tip of electrode was completely immersed in the solution of laghusutashekhara rasa and pH value was recorded.

pH study of laghusutashekhara rasa in different media: Different pH solutions having 1.2, 7.1 and 9.2 pH were prepared. 1 gm of laghu sutashekhara rasa was dissolved in 100 ml of each of three pH media and the change in pH of the solution was monitored for an interval of one hour from the time of addition of laghu sutashekhara rasa for 12 hours by using digital caliberated pH meter. The results are tabulated in

Ash value: A clean and dry Silica crucible was weighed. 5gm of sample of L.S.S.R was weighed accurately and transferred to the Silica crucible. The Silica crucible along with sample was weighed. It was subjected to incineration in a electric muffle furnace till carbon free ash was obtained maintaining 450.C temp. At the end Silica crucible was taken out from the muffle furnace allowed to cool and weighed.

$$\text{Percentage of Ash} = \frac{\text{Difference in weight}}{\text{Weight of sample}} \times 100$$

Determination of water insoluble ash : Total ash obtained by the above procedure boiled with 25 ml of distilled water for five minutes and cooled. It was filtered through Whatman filter paper no. 40 and insoluble matter was collected. Dried and ignited the filter paper along with insoluble matter cooled and weighed. The percentage of water insoluble ash was calculated as follows

weight of filtrpaperwith ash - weight of filter paper / Wt of ash X 100

Determination of Acid Insoluble Ash: Total ash obtained by ash value procedure boiled with 25 ml of dilute HCl. Insoluble matter was collected on ashless filter paper (Whatman's Filter Paper no. 42). The residue was repeatedly washed with hot water, dried well and ignited in electric burner cooled and weighed. Percentage of acid insoluble ash was calculated as follows:

weight of filtrpaperwith ash - weight of filter paper / Wt of ash X 100

Phytochemical constituents of laghu sutashekhara rasa: Laghu sutashekhara rasa was subjected for extraction with distilled water. Then the concentrated extract was used for phytochemical screening to observe the phytochemical constituents.

Method of Extraction: About 50gms of powder was subjected for extraction with distilled water from soxhlet extractor. The extracts was concentrated by distilling the distilled water at low temperature and then dried on a water bath.

Aqueous extract was subjected for phytochemical analysis:

Detection of Alkaloids: Sol. of Extract + Wagner's reagent (K.I. Soln.) Reddish brown ppt indicates the presence of Alkaloids. Reddish brown colouredppt was observed in the aqueous extract of laghu sutashekhara rasa That indicates the presence of alkaloids.

Detection of Carbohydrates: Benedict's test (Test for reducing sugars) :5ml Benedict's reagent was heated in a test tube, 8-10 drops of test sol. Was added. Then brick red ppt indicates presence of carbohydrates. Brick red ppt was observed in the aqueous extract of L.S.S.R .That indicates the presence of carbohydrates.

Determination of Tannins: Sol of Extract + $FeCl_3$ (Ferric chloride) Blue colourppt was not observed in the aqueous extract of laghu sutashekhara rasa that indicates the absence of tannins.

Determination of steroids: Libermann Burchard sterol reaction :Sol of Extract + conc. H_2SO_4 + Chloroform. Blood red ppt was not observed in the aqueous extract of laghu sutashekhara rasa that indicates the absence of steroids.

Determination of Triterpenoids: Salkowski's test:Sol of Extract + conc. H_2SO_4 + Chloroform (10 ml chloroform + 90 ml distilled water i.e. 1:9) Yellow ppt was observed in the aqueous extract that indicates the presence of Triterpenoids.

Determination of Flavanoids: Sodium hydroxide solution test:Sol of Extract + 10% lead acetate sol. Yellow ppt was not observed in the aqueous extract of laghu sutashekhara rasa that indicates the absence of Flavanoids.

Detection of proteins: Millions Biuret test:Sol of Extract + 2ml 10% NaOH sol. + 2 ml 10% $CuSO_4$. Blue colourppt was not observed in the aqueous extract of L.S.S.R that indicates the absence of Proteins.

Detection of Saponins: Foam index test:Sol of Extract + 20ml D/W shaked for 15 minutes. Foam was observed in the aqueous extract of laghu sutashekhara rasa that indicates the presence of saponins.

Detection of Carotenoids: Sol of Extract + 85% H_2SO_4. Blue colour at the junction of two layers was not observed that indicates the absence of Carotenoids.

Pharmaceutical standardization of laghu sutashekhara rasa vati:

Uniformity of weight test: 10 pills of laghu sutashekhara rasa were taken randomly and weighed individually. The average weight was calculated. The weight of the individual pill was compared with the average weight.

Diameter Test: 10 pills were taken randomly and then calculated separately the diameter of these pills with the help of vernier caliper. The mean diameter was calculated. Then the diameter of each pill was compared with the mean diameter of the pills.

Hardness test: 10 randomly selected pills and Monsanto hardness tester were used to conduct hardness test. The pill to be tested was held vertically in between the jaws of hardness tester. The force applied to the edge of the pill was gradually increased by pressing the jaws with the help of hand, until pill breaks. The reading was noted from the scale, which indicates the pressure required to break the pill. Same procedure was repeated for another nine pills and mean was calculated.

Friability test:10 randomly selected pills of laghu sutashekhara rasa were weighed and placed in the tubling chamber of the friabilator. The friabilator was revolved 100 revolutions at the rate of 25 revolution /minute rate. During each revolution the pills fall from a distance of 6" to undergo shock. After 100 revolutions, the pills were again weighed. The loss in weight was noted and from which the percentage of friability was calculated by the formula.

$$\%\text{age of friability} = \frac{A-B}{A} \times 100$$

Where

A= Total weight of 10 pills before the friability test
B= Total weight of 10 pills after the friability test

Disintegration test of laghu sutashekhara rasa: One-one pill was placed in each of six tubes of the basket of disintegration apparatus and the assembly is suspended once in distilled water, pH of 3.2 acidic media and once in pH of 9.2 alkaline media, temperature was maintained at 37^0 C±2 ^{0}C. On every pill, discs were placed and apparatus was operated and recorded the time required to break down into pieces and should pass through shieve& collect in container with the use of stopwatch. This procedure was repeated with other pH solutions at the end of disintegration of 6^{th} pill the watch was stopped and the time was recorded (n=6) result is shown in

Dissolution test: A suitable volume of dissolution medium like acidic media (pH3.2), Distilled water (pH7.1), alkaline media (pH 9.2) was filled in glass vessel, which was submerged in water bath maintained at 37^0 c. The pills were introduced in basket and fitted in position. The motor was started and its revolutions adjusted to 100 rpm. The test was carried out till pills got disintegrated and form a homogeneous mixture. Time was recorded.

Observations and Results

The observation of HPTLC shows that it contains many spots.These spots are of different hydrocarbon groups from dry ginger (shunthi) and betel leaves (nagwalli).The gairika (red oxide of iron) does not appear in the HPTLC.

Table 1. Showing Organoleptic Characters of laghu sutashekhara rasa

Sr. No.	Organoleptic character	Description.
1	Colour	Light brick red
2	Odour	Pungent odour
3	Taste	Pungent
4	Touch	Smoot/soft
5	Appearance	Amorphous powder

Table No. 2 Showing Solubility of laghu sutashekhara rasa

Sr.No.	Solvent	Solubility
1	Distilled Water	SS
2	Methanol	NS
3	Ethyl alcohol	NS
4	Petroleum ether	NS
5	Acetone	NS
6	Benzene	NS
7	Toluene	NS
8	Chloroform	NS
9	Xylene	NS
10	Carbon tetrachloride	SS

NS = Not soluble, SS = Sparingly soluble,

Table No.3. Showing Physico-chemical standards of laghu sutashekhara rasa

Sl. No.	Physical constants	Results
1	pH value	7.6
2	Specific gravity	1.7
3	Moisture content	1.8%
4	Ash value	32.6%
5	Water insoluble ash	57.05%
6	Water soluble ash	42.95%
7	Acid insoluble ash	62.57%
8.	% of solubility	18%

Table No.4 Showing phyto-chemical constituents of laghu sutashekhara rasa

Sl. No.	Organic Constituents	Results
1	Alkaloids	+ve
2	Carbohydrates	+ve
3	Tannins	-ve
4	Steroids	-ve
5	Triterpenoids	+ve
6	Flavanoids	-ve
7	Proteins	-ve
8	Saponins	+ve
9	Carotenoids	-ve

Table No. 5Showing Acidic, Alkaline, Neutralizing activity of laghu sutashekhara rasa

Time	Different media		
	pH 1.2	pH 7.1	pH 9.2
0 Hour	1.7	8.6	7.5
1 Hours	1.7	8.5	7.4
2 Hours	1.7	8.3	7.5
3 Hours	1.8	8.3	7.6
4 Hours	1.8	8.4	7.6
5 Hours	2.0	8.5	7.7
6 Hours	2.0	8.3	7.8
7 Hours	2.0	8.4	7.5
8 Hours	2.1	8.3	7.8
9 Hours	2.0	8.3	7.6
10 Hours	2.0	8.3	7.7
11 Hours	2.1	8.4	7.8
12 Hours	2.1	8.4	7.8

Table No.6 Showing Pharmaceutical standard tests of laghu sutashekhara rasa.

Pharmaceutical standards	**Results**
Uniformity of weight	125.9mg
Uniformity in size	4.340 mm
Hardness	4 kg
Friability	0.39%
Disintegration of laghu sutashekhara rasa pills in acidic media 1.2 PH	25 min
Disintegration of laghu sutashekhara rasa pills in D/W	22minutes
Disintegration of laghu sutashekhara rasa pills in alkaline media 9.2 PH	27 min
Dissolution of laghu sutashekhara rasa pills in acidic media 1.2 PH	1 hrs &30min
Dissolution of laghu sutashekhara rasa pills in D/W	40 min
Dissolution of laghu sutashekhara rasa pills in alkaline media 9.2 PH	60 min

DISCUSSION

Laghu sutashekhara rasa was prepared as per the reference of the ayurvedic formulary of india part-2 page no.-282 (Rasa tantra sara avam sidha prayoga sangraha); kharliya rasayana-330 and ausadhi gundharma shastra & was subjected to organoleptic, physico-chemical & phytochemical analysis.

The sample shown organoleptic characters like brick red in colour, pungent in odour, slight alkaline in test, smooth to touch and amorphous powder. The sample was subjected to physical constants analysis. pH of sample was 7.6. Acid & alkaline neutralizing activities in different media shown in acidic media (1.2 pH) initially pH 1.7 after one hour 1.7 and at the end of 12 hrs it was 2.1. In alkaline media (9.2) initially pH 7.5 after one hour 7.4 and at the end of 12 hrs it was 7.8. In neutral media (7.1pH) initially Ph 8.6 after one hour 8.5 and at the end of 12 hrs it was 8.4. The sample was sparingly soluble in distilled water and carbon tetra chloride. Moisture content of sample was 1.8% & specific gravity 1.7. Ash value was 32.6%, water insoluble ash 57.05%, water soluble ash 42.95% and acid insoluble ash 62.5% were noted. The phytochemical constituents like alkaloids, carbohydrate, triterpenoids & saponin were noted. Elements like Fe,Si, Ni, Ca, P, Co, V & Al were noted.

The purified gairika has been estimated gravimetrically and found to contain fe_2O_3 as major component. Analysis also shows that Fe is present as ferrous ion to the extent of 8.82%. In addition to Fe there are other elements such as silicon (Si) about 12.68%, phosphorus and manganese in minute amount about 1105 ppm and 1570 ppm respectively. These elements do not appear in the HPTLC. Ginger (shunti) which contains essential oils, olive oil, resinous matter, gingiberaceae, gingerol etc. which are organic hydrocarbons and appears in the HPTLC. The mixture of gairika&shunti on trituration with the extract of betel leaves which contains carbohydrate 0.5%, protein 3%, minerals 2.3% fat 0.4% tannin 0 .1 to 1.3 % and some alkaloids also get associates with the mixture and appeared in the HPTLC. The pharmaceutical standard of laghu sutashekhara rasa vati was carried out, uniformity of weight 125.9 mg, uniformity in size 4.340mm, hardness 4 kg, friability 0.39% were observed. Disintegration of pills in acidic media 1.2 pH was 25 minute, in distilled water 22 minute, in alkaline media 9.2 pH 27 minute was noted. Dissolution of vati in acidic media (1.2 pH) was 1 hrs & 30 minute, in distilled water 40 minute and in alkaline media (9.2 pH) 60 minute was observed. Results of organoleptic charcter, physical constant, elemental analysis(XRD), phytochemical constituents & HPTLC contributes to establish standards for standardisation of laghu sutashekhara rasa.

Conclusion

The phytochemical constituents like alkaloids, carbohydrate, triterpenoids & saponin were present. The Monitoring & analysis of HPTLC reveals that the Laghu sutashekhara rasa contains most of the ingredients of all the raw materials. Results of organoleptic character, physical constant, elemental analysis (XRD), phytochemical constituents & HPTLC contributes to establish standards for standardisation of laghu sutashekhara rasa.

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