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Research Article

COMPARATIVE STUDY OF DIFFERENT *ANAND-BHAIRAV RASA* AND ITS STANDARIZATION (W.S.R. TO BHAISHAJYA RATNAVALI)

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ABSTRACT

Introduction: Standardization of Ayurvedic drug is an important criteria for selection, processing, efficacy & safety wise, to meet the WHO guidelines for the world wide acceptability of Ayurvedic formulations. Therefore different preparations of *Anandbhairav rasa* from classic Bhaishajya Ratnavali has been selected and studied for standariztion.

Materials & Methods: Three samples of *Anand Bhairav Rasa* were prepared according to *Jwaratisar prakaran* of Bhaishajya Ratnavali and three samples according to *Atisar Prakaran* of Bhaishajya Ratnavali were made and subjected to various physico-chemical analyses so that their physical as well as chemical changes can be analyzed.

Conclusion: Both formulations showed the difference in pharmaceutically, organoleptic examination as well as in chemical analysis. However, the results obtained from physico chemical analysis of all the three samples are very close together and within fixed physico-chemical norms as described in pharmacopeial standards for Ayurvedic formulations.

KEYWORDS: *Anandbhairav rasa*, Ayurvedic drugs, Standardization.

INTRODUCTION

Ayurveda is one of the oldest medical science of the world has been practicing since its existence. Ayurveda advocates a complete promotive, preventive and curative system of medicine. Hence Ayurvedic texts describe a comprehensive schedule of health regimen for prevention of health popularly known as *Svasthavrtta* and *Sadavratta*. If body is diseased, the objective of curative treatment supervenes and for this our Acharyas (Scholars) emphasized on the use of *Aushaddha* (Drugs). Various studies and continuous experimentations by our disciples of Ayurveda has put forward the uniqueness and integrity of these drugs.

As the time advanced, a specific branch known as Rasa -Shastra came into existence, which was in rudimentary form in Brihatrayees. Rasa - Shastra gave the fast acting metallic/mineral preparations incorporated with herbal/mineral products to cure the diseases. Even today it is seen that a classical Ayurvedic approach needs a lot of time, skill and many herbs are not easy available. Different Rasa preparations got position over the classical herbal system because of its longer life, low dose, quicker action etc. Also efficiency of these Rasa preparations depend upon its purity and quality, this can be ensured by its standardization.

The concept of standardization and quality control of drugs can be found even in the ancient Ayurvedic texts. In those days, the physician himself would check the raw drugs by their typical taste, color, smell, shape and texture and prepare the medicines. But in the modern time these tests are not sufficient to give scientific explanation and quality control. The W.H.O. also has been encouraging and promoting the traditional herbal medicines in health care programmes. Hence the standardization of the raw drugs, processing, finished products, verification of the claims, mechanism of action and purity from metallic and microbial contamination are some of the major issues which have to be taken into consideration for increasing the world wide acceptability of herbal products and also to achieve clinical success and maximum therapeutic effect.

Purpose of Study: Aims and Objectives of the present research is to.

a) Examine two different formulations of *Anand Bhairava Rasa* pharmaceutically and physico-

chemically so that physical as well as chemical changes can be analyzed.

b) And to standardize different samples of *Anand Bhairava Rasa*.

Place of Study: The study was conducted as a part of post graduate thesis research work in Post Graduate Department of Rasa Shastra and Bhaishjya Kalpana, Rishikul State Ayurvedic College, Haridwar, Uttarakhand (UK) in the year 2008.

MATERIAL AND METHODS: the present study has been divided into following parts :

A. Collection and identification of raw drugs

B. Pharmaceutical preparations of *Anand Bhairav Rasa*

C. Standardization of *Anand Bhairav Rasa*

Collection and identification of raw drugs

The authentic ingredients of *Anand Bhairav Rasa* were procured from the local markets of Haridwar and Dehradun and were thoroughly checked and botanically identified by the experts in the Dept. of Dravya-Guna & Rasa Shastra, State Ayurvedic college, Rishikul Haridwar, UK. Moreover, the mineral ingredients of the formulations were identified and tested for their authenticity by IIT., Roorkii and Divya Pharmacy, Haridwar, UK through various analytical procedures.

a) Identification and Test for *Hingul* – 0.4 gm of sample was taken in kjeldal standard flask of 300 ml capacity and added equal part of water and HNO_3. It was heated gently until the liquid formed colorless. Then 150 ml of water was added. 1% of $KMnO_4$ solution was added to remove excess of permanganate. The solution was titrated with N/10 ammonium Thiocyanate using ferric alum as indicator till brick red color appears. Volume of N/10 Ammonium Thiocynate was noted (V ml) and 86.1 % of Mercury was found in the raw sample of *Hingul*.

b) Identification and Test for Sulphur – when it was heated, it burns with a blue flame emitting sulphur dioxide which changed colour of moistened blue litmus paper to red.

1 gm of sample was taken in 100 ml beaker and 25 ml of carbon di sulphide was added to dissolve it and suspension was heated on water bath. The solution was filtered through previously cleaned & weighted sintered crucible. Residue was washed three times with carbon disulphide and dried at 105 ^{0} C in electric oven for 1 hour. Crucible was cooled in desiccators for 1 hrs and weighted. 98.16 % sulphur was found in sample of *Gandhaka*.

c) Identification and Test for Borax- first, Borax solution is made by dissolving its 4 gm in carbon dioxide free water and diluted it to 100 ml with same solvent, then 1 ml of this solution was mixed with sulphuric acid abd methanol, then it was ignited, the flame has a green border. pH of solution was come to 9.7

Pharmaceutical study: this portion includes (a) purification of the ingredients and then (b) preparation of the *Anand Bhairav Rasa*. Three samples of *Anand Bhairav Rasa* were prepared according to *Jwaratisar prakaran* of Bhaishajya Ratnavali[1]and three samples according to *Atisar* Prakaran of Bhaishajya Ratnavali.[2].To prepare *Anand Bhairav Rasa*, first all ingredients i.e., *Hingul*, *Gandhaka, Tankana* and *Vatsanabh* were purified as per the instructions given in the texts.

Purification of *Hingul* was done first by its *Swedan* in *Dola Yantra* with *Jambiri rasa* and then triturating it 7 times with Goat's urine. After washing three times with hot water it became purified[3]. *Gandhaka* purification was done by melting it in cow's ghee and then pouring it into cow's milk with the help of fine cloth. Then it was thoroughly washed with warm water to remove impurities and oiliness[4]. *Tankana* purification was done by being steeped it in *Kanjika* for a night and then in Human's urine, Cow urine, and *Jambiri rasa* for twelve hours respectively. Then after mixing it with *Maricha*, it was washed thoroughly with water[5]. *Vatasnabh* was purified by dipping gram like pieces of it into Cow's urine in sunlight and then pilling its outer layer[6], it was used.

1. In one formulation (A) *Shuddha Hingula, Shuddha Gandhaka, Shuddha Tankana*, *Shuddha vatsnabh, Trikatu* were taken in equal parts and were titurated with *Jambiri Nimbu rasa* for 6 hours to prepare *Anand Bhairava Rasa* and tablets equal to *Gunja* were prepared.
2. In second formulation (B) *Shuddha Hingula, Shuddha Gandhaka, Shuddha Tankana, Shuddha vatsnabh, Maricha* & *Pippali* were taken in equal parts and were titurated with water to prepare *Anand Bhairava* Rasa and tablets equal to *Gunja* was prepared.

Table 1: Showing ingredients (A+B) of *Anand Bhairava Rasa*

Formulation A (According to *Jwaratisara Prakaran of Bhaisajya Ratnavali*)				**Formulation B (According to *Atisara Prakaran of Bhaisajya Ratnavali*)**			
S.N.	**Name of drug**	**Botanical name**	**Weight (gm)**	**S.N.**	**Name of drug**	**Botanical name**	**Weight (gm)**
1.	*Hingula*	Cinnabar red mercury(II) sulfide (HgS)	60	1.	*Hingula*	Cinnabar Red mercury(II) sulfide (HgS)	60
2.	*Gandhaka*	Sulphur Persian(S)	60	2.	*Vatsanabh*	*Aconitum ferox* Wall.	60
3.	*Tankan*	Borax $Na_2B_4O_7.10H_2O$	60	3.	*Tankan*	Borax $Na_2B_4O_7.10H_2O$	60
4.	*Vatsanabh*	*Aconitum ferox* Wall.	60	4.	*Maricha*	*Piper nigrum* Linn.	60
5.	*Shunthi*	*Zingiber officinale*	20	5.	*Pippali*	*Piper longum* Linn.	60
6.	*Maricha*	*Piper nigrum* Linn.	20	6.			
7.	*Pippali*	*Piper longum* Linn.	20	7.			
8.	*Jambiri*	*Citrus jambhiri* Lush.	100ml	8.			

These preparations were done at P.G. Department of Rasa Shastra and Bhaishjya Kalpana, Rishikul State Ayurvedic College, Haridwar U.K. and prepared tablets were studied for standardization through organoleptic characters and physico-chemical properties viz. colour, odour, taste, texture,pH value, loss on drying at 110^0c, total ash, acid insoluble ash, water soluble, alcohol soluble and ether soluble extraction, Thin layer chromatography (TLC). All these tests were conducted at I.I.T., Roorkee & Divya Pharmacy, Haridwar. The details of observation and results of the study is as follows.

OBSERVATION AND RESULTS

Table 2: Showing pharmaceutical processing (A+B) of *Anand Bhairava Rasa*

Sample No.	**date**	**Time of Commencement**	**Time of Completion**	**Amount of *Jambiri rasa***	**Original amount (gms)**	**amount after process (gms)**	**% gain**
Sample 1A	17.10.07	11.00 a.m.	3.00 p.m.	75 ml	300	310	3.3
	18.10.07	11.00 a.m.	1.00 p.m.	25 ml			
Sample 2A	30.11.07	11.00 a.m.	3.00 p.m.	75 ml	300	312	4
	1.12.07	11.00 a.m.	1.00 p.m.	25 ml			
Sample 3A	11.01.08	11.00 a.m.	3.00 p.m.	75 ml	300	313	4.3
Sample 1B	19.10.07	11.00 a.m.	3.00 p.m.	75 ml	300	302	0.66
	20.10.07	11.00 a.m.	1.00 p.m.	25 ml			
Sample 2B	03.12.07	11.00 a.m.	3.00 p.m.	75 ml	300	302	0.66
	04.12.07	11.00 a.m.	1.00 p.m.	25 ml			
Sample 3A	14.01.08	11.00 a.m.	3.00 p.m.	75 ml	300	301	0.33
	15.01.08	11.00 a.m.	1.00 p.m.	25 ml			

Table 3: Showing organoleptic characters (A+B) of *Anand Bhairava Rasa*

Sample code	**Appearance**	**Touch**	**Odour**	**Taste**
A.B.1A	Reddish brown, fine powder	Smooth	Pleasant	Astringent, pungent in taste
A.B.2A	Reddish brown, fine powder	Smooth	Pleasant	Astringent, pungent in taste

A.B.3A	Reddish brown, fine powder	Smooth	Pleasant	Astringent, pungent in taste
A.B.1B	Brick red, fine powder	Smooth	Faint	Astringent, slight pungent in taste
A.B.2B	Brick red, fine powder	Smooth	Faint	Astringent, slight pungent in taste
A.B.3B	Brick red, fine powder	Smooth	Faint	Astringent, slight pungent in taste

Determination of % Loss on Drying[7]

It is the amount of volatile matter of any kind that can be driven off under condition specified. First of all accurately weighted sample of A.B. Rasa was taken in a previously washed and dried weighing bottle. This bottle was then kept in an electric oven whose temperature was maintained at 110^{0}C. after 3rd, 4th, 5th hrs. the bottle was allowed to cool in a dessicator and weighed. The process was continued till constant weight. The same process was carried out for the rest of samples of Rasa in duplicate sets. The values of % loss on drying for different samples are shown in table no.5

Ash Value[8]: this parameter is helpful in knowing the organic and inorganic matter present in the drug. First of all accurately weighted sample of A.B. Rasa was taken in a previously washed and weighing silica crucible which was heated on mild heat very gently so as to avoid burning of Rasa. When all the fumes exhaust, samples was transferred in the muffle furnance maintained at approx. 450 – 550^{0}C for 3 hrs. until free from carbon. Then crucible was withdrawn from furnance kept in dessicator, cool and weighed. The same process was carried out for rest of the drug samples taking two sets of each. The values of % of ash contents for different samples are shown in table no.5

Acid Insoluble Ash[9]: This parameter is helpful in knowing the silicates (sand) present in a sample. Silicates remain insoluble in acid. For this 2 gm of ash sample was taken and dissolved it in ratio of 1: 1 HCL and boil the content for about 5-10 min, filter through a sinctered glass crucible. It was then kept in an electric oven whose temperature wad maintained at 110 C. after 3rd, 4th, 5th hrs. it was withdrawn from oven and allowed to cool in a desiccators and weighed. The process was continued till constant weight. The loss in weight represented the acid insoluble ash. The same process was carried out for rest of the drug samples taking two sets of each. The values of % of acid insoluble ash for different samples are shown in table no.5

Water Soluble Extractive[10]: it is the parameter to determine polar molecules responsible for therapeutics of drug. 5 gm of sample was macerated with 100 ml alcohol chloroform water in a closed flask for 24 hrs, shaking frequently during 6 hrs and allowed to stand for 18 hrs. 25 ml of filtrate was taken for evaporation in a previously weighted beaker. It was then kept in an electric oven whose temperature was maintained at 110 C. After 3rd, 4th, 5th hrs. it was allowed to cool in a dessicator and weighed. The process was continued till constant weight. The same process was carried out for rest of the drug samples. The values of water soluble extractive for different samples are shown in table no.5

Alcohol Soluble Extractive[11]: It is the parameter to determine non polar molecules responsible for therapeutics of the drug. 5 gm of sample was macerated with 100 ml alcohol in a closed flask for 24 hrs, shaking frequently during 6 hrs and allowed to stand for 18 hrs. 25 ml of filtrate was taken for evaporation in a previously weighted beaker. It was then kept in an electric oven whose temperature was maintained at 110 C. After 3rd, 4th, 5th hrs. it was allowed to cool in a dessicator and weighed. The process was continued till constant weight. The same process was carried out for rest of the drug samples. The values of alcohol soluble extractive for different samples are shown in table no.5

Thin Layer Chromatography (TLC)[12]: it is a technique in which a solute undergoes distribution between two phases, a stationary phase acting through adsorption and a mobile phase in form of a liquid. Seven samples were taken for TLC examination. *Pippali, Maricha* and *Vatsanabh* were extracted in methanol by keeping at room temperature overnight. *Shunthi* was extracted in chloroform by keeping at room temperature. The TLC examination was done by comparison of the raw drugs with *Anand Bhairava Rasa*. For comparison the TLC plates were developed in different mobile phases. Benzene: Ethyl acetate (2:1:10), Benzene: Ethyl acetate (2:1), Chloroform : Methyl Alcohol (9 :1), n Hexane : Ether (40 : 60).

After complete development of TLC plates, comparison was done by detection with 5 % sulphuric acid or Iodine vapours or Dragandroff reagent and heated in oven at 110 C for upto 5 min.

the detail results of TLC studies are shown in table no.7

Table 4: Showing Physico chemical analysis (A+B) of various ingredients of *Anand Bhairava Rasa*

S.N.	Sample	Loss on drying (w/w)	Ash value (w/w)	Acid insoluble ash	Water soluble extractive (w/w)	Alcohol soluble extractive (w/w)
1.	*Shunthi*	12.90	4.39	1.25	1.52	2.98
2.	*Maricha*	12.61	3.53	0.50	6.4	5.6
3.	*Pippali*	11.86	4.85	0.30	12.8	12.8
4.	*Vatsanabh*	10.98	5.13	1.65	25.12	8.6

Table 5: Showing Physico-chemical analysis (A+B) of different samples of *Anand Bhairava Rasa*

S.N.	Sample code	pH value	Loss on drying (w/w)	Ash value (w/w)	Acid insoluble ash	Water soluble extractive (w/w)	Alcohol soluble extractive (w/w)	% of 'Hg'	% of 'S'
1	A.B.1A	7.55	13.8619	14.0143	2.0231	26.5914	5.9931	12.51	20.15
2	A.B.2A	7.45	13.3066	14.5912	1.9848	25.1213	5.5449	12.36	19.00
3	A.B.3A	7.30	12.2094	14.8739	1.9711	24.0010	5.1482	11.86	20.12
4	A.B.1B	9.14	10.7515	13.6244	1.5415	28.2941	6.0512	11.51	2.54
5	A.B.2B	9.21	10.5584	13.5690	0.7298	28.2941	5.9312	11.44	2.69
6	A.B.3B	9.09	10.1911	13.5698	1.5538	27.1032	5.9316	12.00	2.61

Table 6: Showing presence of trace metals (A+B) in different samples of *Anand Bhairava Rasa*

I.C.P.	A.B.1A	A.B.2A	A.B.3A	A.B. 1B	A.B. 2B	A.B.3B
Cr	0.00079	0.00082	0.00084	0.00099	0.00086	0.00076
Mn	0.004385	0.004292	0.004294	0.00277	0.002698	0.002754
Pb	0.0009337	0.0008321	0.0007345	0.000766	0.000715	0.000763
Cu	0.00123	0.001131	0.001113	0.001308	0.001315	0.001295
Ni	0.000341	0.0003192	0.0002928	0.000451	0.000449	0.000449
Cd	0.0000132	0.0000128	0.0000122	0.000007	0.0000121	0.0000105
Zn	0.003836	0.008812	0.006794	0.0082	0.0065	0.007
As	0.2985	0.3714	0.4910	0.5852	0.5982	0.5790

Table 7: showing TLC study of various samples

S.N.	Raw Herbal drugs/ *A.B.Rasa*	Mobile phase	Stationary phase	Visualization by spraying
1	*Piper nigrum*	Benzene : Ethyl acetate 2:1	Silica gel	One spot seen at RF=0.44 with vanillin sulphuric acid reagent
2	*Piper longum*	Benzene : Ethyl acetate : Diethy ether 2:1:10	Silica gel	One spot seen at RF=0.55 with vanillin sulphuric acid reagent
3	*Zingiber officinalis*	nHexane: Ether 40:60	Silica gel	One spot seen at RF=0.26,0.48 with vanillin sulphuric acid reagent
4	*Aconitum ferox*	$CHCl_3$: CH_3OH 9 : 1	Silica gel	Six spots seen at RF=vapours 0.10,0.20,0.39,0.56,0.74, 0.96 Detection by Iodine(a) Two spots seen at RF=0.39, 0.36 Detection by Dragandroff reagent(b)
5	*A.B.Rasa* sample A	Benzene: Ethyl acetate : 2:1	Silica gel	In this solvent system, RF =0.44 Detection by vanillin sulphuric acid

		Benzene : Ethyl acetate: Diethyl ether 2:1:10	Silica gel	In this solvent system, RF =0.55 Detection by vanillin sulphuric acid
		nHexane: Ether 40:60	Silica gel	In this system, RF =0.26, 0.48 Detection by vanillin sulphuric acid
		$CHCl_3$: CH_3OH 9 : 1	Silica gel	In this solvent system, RF obtained at 0.10,0.20,0.39,0.56,0.74, 0.96 Detection by Iodine(a)
		$CHCl_3$: CH_3OH 9 : 1	Silica gel	Two spots seen at RF=0.39, 0.96 Detection by Dragandroff reagent(b)
6	*A.B.Rasa* sample A	Benzene: Ethyl acetate: 2:1		In this solvent system, RF =0.44 Detection by vanillin sulphuric acid
		Benzene: Ethyl acetate: Diethyl ether 2:1:10	Silica gel	In this solvent system, RF =0.55 Detection by vanillin sulphuric acid
		nHexane: Ether 40:60	Silica gel	In this solvent system, RF values not obtained
		$CHCl_3$: CH_3OH 9 : 1	Silica gel	In this solvent system, RF obtained at 0.10,0.20,0.39,0.56,0.74, 0.96 Detection by Iodine(a)
		$CHCl_3$: CH_3OH 9 : 1	Silica gel	Two spots seen at RF=0.39, 0.96 Detection by Dragandroff reagent(b)

DISCUSSION AND CONCLUSIONS

The three samples of both formulations were studied for their organoleptic characters and subjected to physico-chemical analysis to obtain standard values of the final products. There are large numbers of techniques and procedures available for standardization of herbal drugs, among them few suitable techniques were adopted on the basis of their availability and cost effectiveness. Both formulations showed the difference in pharmaceutically, organoleptic examination as well as in chemical analysis. In pharmaceutical processing, there was a net gain in weight was observed in different samples of *Anand Bhairav rasa*. However more gain in weight was recorded in formulation A of *Anand Bhairav rasa*, as its contents were titurated with *Jambiri rasa* as shown in table no. 2.

In organoleptic analysis, difference in color was observed in both formulations of *Anand Bhairav rasa*, the vary in color of different samples could be because of difference in ingredients of both formulation. Brick red color is due to presence of *Hingul* and absence of *Gandhaka* in formulation B, which was present in formulation A, hence imparted Reddish brown color. Comparative organoleptic character are summarized in table no.3

In formulation A of *Anand Bhairav ras*a, free sulphur is present and it has higher percentage of sulphur as compared to formulation B of *Anand Bhairav rasa*. pH of formulation A of *Anand Bhairava Rasa* is low as compared to formulation B. All toxins (heavy metals) have a safe threshold toxicity (table no.6), below which there is no toxicity. As the results obtained from physico chemical analysis of all the three samples are very close together and within fixed physico-chemical norms as described in pharmacopeial standards for Ayurvedic formulations. Evaluation of physico- chemical parameters and standardization helps to assess the quality and identify the presence of specific ingredients in a drug and application of chromatographic techniques which aid in recognition of ingredients and also to assess the purity by comparing with standard ones. Thus can be concluded that the procedure adopted for the preparation of *Anand Bhairava Rasa* can be supposed to be a standard one and will help the practitioners in curing the disease properly.

Abbreviations:

- A.B.Rasa : Anand Bhairava Rasa
- ml. : milliliter
- gm : grams
- TLC : thin layer chromatography
- Hg : mercury
- S : sulphur
- % : percentage
- ICP : inductively coupled plasma

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