WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 10, Issue 12, 1527-1536. Research Article ISSN 2277– 7105

PHARMACEUTICO - ANALYTICAL AND ANTIMICROBIAL STUDY OF "BHAIRAV RASA"

Dr. Tanuja Bharti*[1], Dr. M. C. Patil[2], Dr. Manbi Sharma[3] and Dr. Ankush Kumar[4]

[1]Assistant Professor, Dept. of Rasa Sashtra & Bhaishajya Kalpana, Abhilashi Ayurvedic Medical College and Research Institute Chail Chowk, Mandi H. P.

[2]Professor and H.O.D., Dept. of Rasa Sashtra & Bhaishajya Kalpana, Shri S.B.S. Ayurvedic Medical College and Hospital Mundargi Distt. Gadag Karnataka.

[3]Assistant Professor, Dept. of Stri and Prasuti Tantra, Abhilashi Ayurvedic Medical College and Research Institute Chail Chowk, Mandi H. P.

[4]Assistant Professor, Dept. of Kaumarbhritya, Abhilashi Ayurvedic Medical College and Research Institute Chail Chowk, Mandi H. P.

Article Received on
29 July 2021,

Revised on 18 Aug. 2021,
Accepted on 06 Sept. 2021

DOI: 10.20959/wjpr202112-21734

***Corresponding Author**
Dr. Tanuja Bharti
Assistant Professor, Dept. of Rasa Sashtra & Bhaishajya Kalpana, Abhilashi Ayurvedic Medical College and Research Institute Chail Chowk, Mandi H. P.

ABSTRACT

Micro-organisms namely Bacteria, viruses, fungi, worms etc. are present everywhere. The fact that these Micro-organisms can cause certain diseases to human beings was known to Ayurveda even in ancient eras. They even describe the methods to destroy these Micro-organisms and prevent infection caused by them. However modern science has made tremendous progress in this area and acquired deep knowledge about Micro-organisms. But still infections caused by these, still ranks very high as a cause of death in the world. Even though there are number of Antibiotics present and new are being developed, the Micro-organisms are developing resistance towards these Antibiotics, hence there is need to find out a better medicine which can contradict all the Micro-organisms. Hence in present study Bhairav Rasa, a herbomineral preparation has been selected which fall Under Kharaleeya Rasayana. It is indicated in Karanrogas, Sannipataj jwara, Agnimandya, Grahani vikaras and Shotha.

KEYWORDS: Bhairav Rasa, Micro-organisms, Kharleeya Rasayana.

INTRODUCTION

Research is the unveiling of facts about a particular object or thing substantiated by current available scientific tools. Now, the total world is seeing at our herbo mineral drugs are to get relief from incurable ailments. Modern people are gradually budging towards Ayurvedic drugs, because of their endurable effect and relative safety compared to modern synthetic drugs. Rasashastra is a branch of medicine, which deals with metals and minerals to produce the drugs with higher efficacy in lower doses and with good palatability. Thus it became the branch, which fulfills the aims and objectives for preparation of "Shreshtha Bhaishajya" i.e. "Best Medicine Infection and immunity involve interaction between the body (host) and infecting organisms. These micro-organisms cause plenty of infectious diseases in human beings.[1] To overcome such conditions many antibiotics and antifungal are heavily prescribed by medical practitioners, these may cause many hazards to the body such as nausea, vomiting, gastric irritation, metallic taste, destruction of gastric flora and anaphylactic reactions causing even death.[2] Amongst the massive world of medicines, Antimicrobial agents are one of the widely used and misused drugs available to medical profession. Once upon a time they were called as the magic bullets for their great contribution in curing an infection, but recently these bullets also backfired in way of causing resistance, and decreasing their effectiveness.[3] In recent years no of antimicrobial agents have been discovered but unfortunately even with the advent of numerous antimicrobial agents, still most of the physicians are unable to treat these infection appropriately due to hindrance by treating the infection develop of resistance, adverse effects and patient affordability, etc. though the centre for diseases control and prevention has taken steps to curb antimicrobial resistance, still it is flaring at a rampant rate due to over prescribing of these drugs. This is driven largely by patient demand, time pressure on clinicians and diagnostic uncertainty fueling an ever increasing need for newer drugs.[4] Making antibacterial drug therapy effective safe and affordable has been the focus of interest during recent years, some of the Ayurvedic formulations may be considered as an ideal replacement for treating certain bacterial and fungal infection.[5] Hence there is a pressing need for establishment of new antimicrobial compound producing no or minimal side effects. Many krimihara formulations have been extensively described in our Ayurvedic classics. Bhairav Rasa is one among them which has been described in Karanarogadhikara in Bhaishajyaratnavali. It is a herbomineral preparation and is considered as an ideal one, because exhibits faster and greater efficacy even in minute dose. Hence in the present study an effort will be made to evaluate Pharmaceutico – Analytical and antimicrobial study of Bhairav Rasa.

Aims and Objectives of The Study

The present study has been done with the following aim and objectives:

1. Preparation of BHAIRAV RASA.
2. Physico-chemical analysis of BHAIRAV RASA.
3. To evaluate the Antimicrobial activity of BHAIRAV RASA.

MATERIAL AND METHODS

Raw materials collection and authenticity

All the raw the drugs both herbal and minerals for the preparation of Bhairav Rasa were collected from the Chaitanya Ayur Formulations to get the particulars quality drugs and those were screened for classical grahya lakshanas and those were certified by the concerned Pharmacy. The ingridients of Bhairav Rasa are listed below in table:

Ingredients of Bhairav Rasa: Bhaishajya ratnavali Karanroga Chikitsa P-983.

Sl. No.	Drugs English/Botanical Name	Quantity
1.	Shodhit Parada Mercury	1Part
2.	Shodhit Gandhak	1Part
3.	Shodhit Tankana (Sodium pyroborate)	1Part
4.	Shodhit Vatsanabh (Aconitum ferox)	1Part
5.	Marich (Piper nigrum)	1Part
6.	Varata Bhasma (Cowris)	1Part
7.	Ardraka Swaras (Zingiber officinalis)	Q.S.

Ayurveda has enlisted certain drugs, which will cause adverse effects or no therapeutic effects if used in the impure state or may lead to complication. So proper purification is necessary to counteract the probable adverse effects so before undergoing to the preparation of formulation Shodhana procedures were carried out as per classical text.

The entire pharmaceutical study design includes the following procedures

- Hingula Shodhana.
- Hingulotta Parada Nirmana
- Hingulotta Parada shodhana
- Preparation of Kajjali
- Shodhana of Gandhaka.
- Shodhana of Tankan
- Shodhana of Vatasnabh
- Preparation of Marich Churna

- Preparation of Varatika Bhasma
- Preparation of Bhairav Rasa

Preparation of Bhairav Rasa

Name of the Procedure	**Preparation of Bhairav Rasa**
Reference	Bhaishjyaratnavalikaranrogachikitsa p-983**.**
Materials	1.Higulotta Parada: 4GM 2.Shudha Gandhaka: 4GM 3.Shudha Tankan: 8GM 4.Shudha Vatasnabh: 8GM 5. Marichchurna: 8GM 6. Vratabhasm: 8GM 7.Aardarak Swarasa: 100ML.
Days taken	66 Days

PROCEDURE

- The prepared Kajjali, Shudha Tankana, Shudha Vatasanabh and fine powder of Marich, Varat Bhasma are taken in above mentioned quantity and mixed well in a Khalva yantra. Required quantity of Ardark swaras was taken.
- For the first bhavana 80 ml of Ardarak Swarasa was added to powdered mixture and triturated uniformly at a rate of 30 strokes / min for 3 hr 20 min.
- The prematerial was subjected for continual and cautious mardana till powder completely absorbs the swarasa and this completes one bhavana.
- This bhavana was repeated for another 20 times by taking fresh Aardark Swarasa each times.
- Then allowed to dry in shade.
- After completion of bhavana, Gunja Pramana 1 ratti (125mg) each vati was prepared.

Observations during preparation of Bhairav Rasa with Ardarak Swarasa bhavana as below

Bhavana No.	**Initial Weight**	**Final Weight**	**Duration**	**Weight Gain**	**Color**
1.	56 gm	56.12 gm	3hrs 10min	0.12 gm	Olive dark green
2.	56.12 gm	56.40 gm	3hrs 30min	0.2 gm	Olve dark green
3.	56.40 gm	56.52 gm	3hrs 25min	0.12 gm	Sage green
4.	56.52 gm	56.68 gm	3hrs 10min	0.16 gm	Sage green
5.	56.68 gm	56.72 gm	3hrs 5min	0.04 gm	Ash green
6.	56.72 gm	56.82 gm	3hrs 20min	0.10 gm	Dark green
7.	56.82 gm	56.95 gm	3hrs 20min	0.13 gm	Greyish black

RESULT

- 56.95 grams of Bhairav Rasa was obtained.
- Total weight gain during the procedure: 0.95gm.

13. Kajjali

14. Ashodhit tankan

15. After bharjan

16. Ashudh Vatasanabh

17. After shodhan

18. Marich

19. Marich choorna

20. Ashodhit varatika

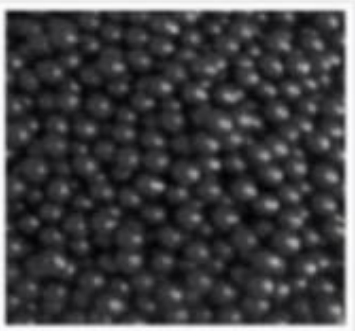
21. During shodhan in kulath kwath

22. Shodhit varatika

23. During marana procedure

24. Shudh varata bhasma

25. Mardana of all ingredients
by giving bhavana with aardark swarasa



26. Bhairav Rasa in vati form 125 mg

Analytical Study

- pH (10% solution) = 9.09
- Loss on Drying = 6.198%
- Total ash = 22.201%
- Water soluble ash = 2.611%
- Water Insoluble Ash = 12pH (10% solution) = 9.09
- Loss on Drying = 6.198%
- Total ash = 22.201%
- Water soluble ash = 2.611%
- Water Insoluble Ash = 12.653%
- Determination of disintegration time =15 minutes.
- Hardness Test (Monsanto Hardness test) = 4 kg/cm
- **XRD, SEM, AAS** tests were carried out during the study**.**

Experimental Study

- **Aim of the Experimental study**

To evaluate the Anti-microbial (Antibacterial and Antifungal) activity of Bhairav Rasa.

- **MATERIALS and METHODS**

To evaluate the antibacterial and antifungal activity of Bhairav Rasa a study was carried out by Cup plate method.

- **MATERIALS**

A) Drugs

1. Test drug: Bhairav Rasa
2. Standard antibacterial drug: Ciprofloxacin
3. Standard antifungal drug: Fluconazole

B) Micro organisms

Bacteria Fungi

a) Staphylococcus pneumoniae a) Candida albicans

b) Staphylococcus aureus b) Aspergillusniger

c) Staphylococcus albus d) Pseudomonas aeruginosa

e) Escherichia coli

OBSERVATION AND RESULT

The results reveal that the organisms have shown varied response to Bhairav Rasa compared to Standard drugs (Ciprofloxacin and Fluconazole). Test drug (Bhairav Rasa) has shown less or more response to bacterial organisms in all the concentrations. Whereas the response of Bhairav Rasa compared to Fluconazole in fungal organisms shows less response.

In bacterium Staphylococcus aureus, Ciprofloxacin solution showed 36mm of zone of inhibition, 2% solution of Bhairav Rasa showed 24mm of zone of inhibition, 5% solution of Bhairav Rasa showed 27mm of zone of inhibition, 10% solution of Bhairav Rasa showed 25mm of zone of inhibition showed 2mm of zone of inhibition. So the test drug was less and more effective compared to standard and among the 3 different concentrate solutions of test drug, 2% was more effective.

In Fungus Candida albicans, Fluconazole solution showed 34mm of zone of inhibition, 2% and 5% solution of Bhairav Rasa showed 9mm of zone of inhibition, 10% solution of Bhairav Rasa showed 0mm of zone of inhibition. So the test drug was less effective compared to standard and among the 3 different concentrate solutions of test drug, 10% was more effective. In Fungus Asphargillusniger, Fluconazole solution showed 26mm of zone of inhibition, 2% solution of Bhairav Rasa showed 5mm of zone of inhibition, 5% and 10% solution of Bhairav Rasa showed 0mm of zone of inhibition. So the test drug was less and more effective compared to standard and among the 3 different concentrate solutions of test drug, 5% and 10% were more effective.

DISCUSSION

Kharaliya Rasa is the most important type of preparation in the Ayurved, and the Bhairav Rasa is most effective Kharaliya Rasayana in the treatment of Karan Roga Sannipattaj Jwara, Agnimandya, Grahani vikaras and Shotha. In the preparation of Bhairav Rasa, Bhavana (levigation) and Mardana (trituration) are the most important factors. The act of trituration of drug with any liquid not only reduces the drug particle to a finer state but also facilitates the breakage and reunion of bonds in the material during trituration. As a result of which we find an entirely different compound formation by the end of total trituration. Hingula Shodhana was performed by Nimbu Swarasa Bhavana for 7 times. After purification these was significant increase in weight of Shodhita Hingula (1.03%). This may be due to addition of solid content of Nimbu Swarasa. The pH of Nimbu Swarasa was 2 and it is acidic media. Nimbu Swarasa might help in detoxification of Hingula due to its Amla Rasa. Nimbu

Swarasa is rich in complex of organic acids such as citric acid, mallic acid, which may react with the unwanted materials in Hingula and from a complex, which is soluble in water. The Hingula was washed with water thoroughly so that it may help in separation of water-soluble complex of impurities. Prakshalana continued till Hingula attained Ujjwala Varna and loses Amlatva of Bhavana dravya. Then shodhan of Gandhak is carried out before making the Kajjali. Kajjali has yogavahi property. After Shodhana of Tankana loss of total amount (350gm) was observed which may be due to evaporation of water content from Tankana as the chemical formula of Tankana (Na2B2O7.10H2O) contains 10 parts of water. Tankan has anti-inflamatory properties. Prior preparation Vatsanabha should be used after Shodhana. Shodhana of Vatsanabha was carried out in Go-mutra. It became soft so that the pin can be easily pierced. Then the power was prepared. After shodhan 30 gm of loss was observed. Then Varatika shodhan was done by giving swedna in Dolayantra and the liquid media was kulatha kwatha. Then Marana was done. Then by adding marich churna with all ingredients Bhavana was given with Ardarak Swarasa. And the weight increased but at the time of Adrak Swarasa Bhavana weight was increased because of starch part of Adraka (Adraka major constituent upto 50% starch (carbohydrate). Adraka having Guru Guna that was control the and balance the Laghu Guna of above drugs, and also seen Tikshna Guna that is the quality which is responsible for the quick activity of a drug or sharpness of a drug.[6] Adraka having Vit. A and gingerals (6,8,10 gingerol), gingerols are phenolic compounds. Effectiveness of Adraka as an Antioxidan antioxidant, anti-inflammatory agent, antinausea and anti-cancer, infectious disease.[7] Bhairav Rasa is Dark Greyish in colour, Characteristics odor, Tablet were prepared. Loss on drying is revealing the presence of moisture. Total ash reveals that presence of organic matter. Disintegrate within the time. In Bhairav Rasa Mercury is 5.81%**.** The trial drug Bhairav Rasa has been proved to be anti- microbial during the experimental study conducted by the cup plate method. Bhairav Rasa showed susceptible sensitivity against the Gram positive bacteria Staphylococcus aureus, Staphylococcus albus. Gram negative Escherichia coli, Pseudomonas aeruginosa when treated with Bhairav Rasa have shown less sensitivity as compared to Ciprofloxacin. The fungi candida albicans and aspargillus niger, when treated with Bhairav rasa have shown very less sensitivity as compared to Fluconazole. Bhairav Rasa has antifungal, antipyretic, antioxidant, anti-inflammatory, and effective in infectious disease gastrointestinal complaints.

CONCLUSION

Pharmaceutical standardization of formulation is an important and essential requirement to establish the safety and efficacy, and Physic-chemical parameters and standardization helps to assess the quality of the drugs or formulation. Application of XRD, SEM and AAS techniques which is identification and purity the drug by comparing with standard ones. Shodhana is a process of separation by which physical and chemical impurities get separated from the substance by different process with various drugs, which literally means purification and converting the drug fit for further procedure. Bhavana is an important Samskara with the help of which, not only the potency of a drug can be altered, but is also capable to bring about changes in characteristics of drug viz. regulation, addition of new or deletion of undesirable characteristics. Bhairav Rasa has antifungal, antipyretic, antioxidant, anti-inflammatory, and effective in infectious disease gastrointestinal complaints. Thus, can be concluded that the procedure adopted for the preparation of Bhairav Rasa all procedure can be considered ideal and will help the further study.

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