INTERNATIONAL RESEARCH JOURNAL OF PHARMACY
www.irjponline.com ISSN 2230 – 8407
Research Article

ANTIBACTERIAL SCREENING OF ANANDBHAIRAV RASA ON DIARRHOEA CAUSITIVE AGENTS

Kavita*
Department of R.S. & B.K., Smt Urmila Devi Ayurvedic College of Medical Sciences & Hospital, Hoshiyarpur, Punjab, India

Article Received on: 19 /10/12 Revised on: 20/11/12 Approved for publication: 02/12/12

*E-mail: kavita99kavita@yahoo.com

ABSTRACT
The use of herbal drugs for treating various diseases predates human history and used increasingly as dietary supplement to fight or prevent common disease. The antibacterial potentials of Ras preparation was investigated by preparing solvent extract of Anandbhairav Rasa and antibacterial activity tested against enteric bacterial pathogens such as E.coli, S. sonnei, B. cereus. All aquous and methanol extracts of Anandbhairav Rasa have shown sensitivity against E.coli , S. sonnei, B. cereus cultures but the sensitivity of aquous and methanol extract of Anandbhairav Rasa was highest in E.coli culture. Thus Anandbhairav Rasa help in preventing diarrhea.
Keywords: Antibacterial activity, Anandbhairav Rasa, Diarrhea

INTRODUCTION

In Rasendra Sar Sangraha, it is mentioned that Anandbhairav Rasa is efficacious in the treatment of Jwaratisar, Atisar and Jwar. From the modern point of view diarrhea & fever are commonly due to bacteria.

In the present study Anandbhairav Rasa has been selected for checking its antibacterial effect as it is frequently used in the treatment of diseases like Jwar (fever), Atisar (Diarrhea) and Jwaratisar (febrile diarrhea) which are infective in nature as per modern science. Furthermore if Ayurvedic fundamentals are considered it also advocates role of Krimi (infective organism) in the genesis of Diarrhoea[1,2].

Mandagni (improper digestive power) is most common cause of almost all diseases which leads to the formation of Ama (undigestive material)[3]. This Ama circulates in the body and releases various types of toxins which further suggest infective origin. Atisar is one such disease that arise from Ama[4].

Coming on to the drugs it is very clear that the ingredients of Anandbhairav Rasa are well known for their anti-infective action. On reviewing the literature it is found that Pippali[5], Marich[6], Jatikosh[7] are abundantly mentioned as Krimihar, Jantuhar agents by almost all texts. Apart from that Vatsnabh[8], Sunthi[9] were indicated in Kustha. If we compare Kustha to Leprosy, it is again bacterial in origin. Pharmacological action is chiefly based on their pharmacological properties. Pharmacological properties of all the ingredients of Anandbhairav Rasa are shown in the following table.

Table 1: Pharmacological properties of all the ingredients of Anandbhairav Rasa

Ingredients	Rasa	Guna	Virya	Vipaka
Hingula	Madhur, Takta / Tikta, Katu Kashaya,	Ushna	-	-
Gandhaka	Katu, Tikta, Kashaya, Madhur	Snigdha, Ushna, Sara	Ushna	Katu/ Madhur
Tankana	Katu	Laghu, Ruksha, Tikshna	Ushna	-
Vatsnabh	Madhur	Laghu, Ruksha, Tikshna, Ushna, Sukshma, Vikasi Ashukari,	Ushna	Madhur
Sunthi	Katu	Laghu, Snigdha	Ushna	Madhur
Pippali	Katu	Laghu, Snigdha	Ushna	Madhur
Marich	Katu	Laghu Tikshna,	Ushna	Katu
Jatikosh	Katu, Tikta	Laghu, Tikshna,	Ushna	Katu

Most of the ingredient drugs posses Katu Rasa which is well known for destroying Krimi[10]. Tikta Rasa also mentioned as Krimighna[11] by Acharya Charak.

For treatment of Krimi Ayurveda suggests three important measures, among them one method is Prakrtivighat[12] i.e. destroying the Krimi by using drugs which are opposite to their survival i.e. Katu, Tikta, Kashaya, Kshar and Ushna Dravya[13].

Antimicrobial status of these drugs has also allure modern scientists. Limyati et al[14], Usha & Saroja[15], Bhat & Broker[16], Subrahmanyam et al[17] have shown Sunthi, Jatikosh as antimicrobial agents.

MATERIALS AND METHOD

After the theoretical assessment of the four references pertaining to Anandbhairav Rasa as quoted in Rasendra Sar Sangraha two of these were selected. One reference from Sannipat Jwar Chikitsa and another from Jwaratisar Chikitsa were selected for this study. Hingula is the main ingredient of these formulations Most Rasa texts described to purify Hingula by the seven times impregnation of Nimbu Swaras or Adraka Swaras, or Lakuch Swaras, or Mahishi Dugdha. But Rasendra Sar Sangraha described to purify Hingula by the impregnation of Nimbu Swaras and Mahishi Dugdha. So in this study the researcher purified Hingula by seven impregnations of Nimbu Swaras and then by seven impregnations of Mahishi Dugdha. Thus two samples prepared by this method named as AB1 and AB3.

In the reference of Rasendra Sar Sangraha 2/105-107 author advised to take Ananadbhairav Rasa with the juice of *Zinziber officinale*. But in today's busy schedule nobody have time to take medicine with *Zinziber officinale* extract. So keeping this fact in mind another two samples were prepared with the impregnation of *Zinziber officinale* extract. Hingula Shodhan in these preparations were done by Adraka Swaras and Lakuch Swaras according to the reference of Rasendra Sar Sangraha 1/239. Thus these samples were named as AB2 and AB4.

Thus four samples of Anandbhairav Rasa were prepared as shown in the following table.

Table 2: Ingredients of all the sample of Ananadbhairav Rasa

Sample AB1	Sample AB2	Sample AB3	Sample AB4
Hingula (purified by 7 times impregnation of Nimbu Swaras & 7 times impregnation of Mahishi Dugdha)	Hingula (purified by 7 times impregnation of Adraka Swaras & 7 times impregnation of Lakuch Swaras)	Hingula (purified by 7 times impregnation of Nimbu Swaras & 7 times impregnation of Mahishi Dugdha)	Hingula (purified by 7 times impregnation of Adraka Swaras & 7 times impregnation of Lakuch Swaras)
Vatsnabh	Vatsnabh	Vatsnabh	Vatsnabh
Tankana	Tankana	Tankana	Tankana
Sunthi	Sunthi	Sunthi	Sunthi
Pippali	Pippali	Pippali	Pippali
Marich	Marich	Marich	Marich
Jatikosh	**Jatikosh**	**Gandhaka**	**Gandhaka**
Impregnation- Nimbu Swaras	Impregnation- Adraka Swaras Nimbu Swaras	Impregnation- Nimbu Swaras	Impregnation- Adraka Swaras Nimbu Swara

The antibacterial activity of Aqueous and methanol extracts of Anandbhairav Rasa were tested on different species of common pathogenic bacteria. The pathogenic strains of different species of bacteria were procured from 'Institute of Microbial Technology' (IMTECH), Chandigarh. The stock cultures were maintained by sub-culturing.

Drug selected for the study

Four samples of Anandbhairav Rasa were selected to study the bactericidal effect against E.coli, S. sonnei, B. cereus. To compare the drug sensitivity streptomycin and Gentamycin were also used.

Preparation of aqueous extracts of four samples

10 g each of four samples were taken and dissolved in 100ml of distilled water separately and were boiled for 2 hour on low heat. After dissolving the Anandbhairav Rasa in distilled water, it was filtered by Wattman's filter paper. The filtrate of aqueous extract was evaporated to dry on water bath. Dry sample of extract was sterilized and stored at 4°C. All extracts were obtained by this method were reddish brown in colour.

Preparation of methanol extracts of four samples

The method employed was 'Continuous extraction by Soxhlet Appratus' (Trease & Evans' Pharmacognosy).The powdered 10 gm sample was placed in a thimble and plugged with cotton wool. 100ml methanol was taken in flask and attached with soxhlet apparatus.

The extraction of sample was obtained by boiling the solvent followed by percolation. So, it was carried out at the boiling point of methanol i.e. at 60° C. Methanol was gradually refluxed into thimble and siphoned into the flask. The sample has taken minimum 5cycle for complete exhaustion. The time for each cycle was nearly about 20 to 60 minutes. After complete exhaustion, the material was collected and readjusted the assembly for next sample. The wet extract of sample was collected in glass petridish and evaporated to dryness at 40°C on a water bath. The extract was cooled in desiccators for 30 minutes and weighed the extract. All extracts obtained by this method were dark brown in color.

Preparation of nutrient agar media

500 ml of distilled water was taken in a beaker. All ingredients were added in to the beaker and mixed thoroughly and heated on hot plate to dissolve all ingredients. After proper assimilation of all ingredients 500 ml of distilled water was added to make the volume of media 1000ml. After that 200 ml nutrient agar was distributed in 5 conical flasks and plugged the conical flask by cotton plug.

Now the media was sterilized in Autoclave at 121°C, 15 lbs pressure for 15 minutes. Then the flask was allowed to cool until the flasks can be held by hand. Now sterilized media was poured into petri-dishes in a cabinet fitted with UV tubes. Remained media was stored in refrigerator for further use.

Revival of microbial cultures by spreading method

Three nutrient agar plates were marked, 1st for E.coli, 2nd for B. cereus and 3rd for S. sonnei with a wax pencil. 95 percent alcohol was taken into a beaker and dipped the bent glass rod in it. All three ampoules of freeze dried bacteria were incised at cotton plug side. 100ml of distilled water was mixed in the each ampoule with the help of syringe. A lapful bacterial solution was transferred in the center of appropriately labeled nutrient agar plate .Now the inoculated plate was placed on the turntable. The glass rod was removed from the beaker and the bent portion of rod was sterilized in the Bunsen burner flame. Subsequently the rod was cooled for 10-15 seconds. The cover of Petri dish was removed and the turntable was spin. The sterile bent rod was lightly touched to the agar surface and moved it back and forth while the turntable was rotated for spreading the culture over the agar surface. The Petri dish cover was replaced when the turntable was stopped spinning. The bent rod was immersed in alcohol and reflamed to sterilize it.

Steps were repeated to inoculate the other two plates with their respective bacterial cultures. All the 3 plates were incubated in an inverted position at 35°C for 24 to 48 hours. After 24 hrs bacterial growth was observed in each petri plate.

Sub-culturing of pure culture by streak-plate method

All the plates were labeled on the bottom, with the name of the bacteria to be inoculated, with a wax marking pencil. Bacterial colony containing petri plate was grasped in the left hand. The loop was sterilized holding in the right hand, the lid of petri plate containing bacterial colonies was removed. the tip of the loop was touched to the surface of a selected discreat colony and the inoculums was placed on the agar surface at the edge farthest from me and the inoculums was streaked from side to side in parallel lines across the surface of area. The inoculating loop was reflamed to destroy existing organism. All the plates were incubated at 35°C, in an inverted position, for 48 hours. After incubation, each of the three plates was examined for the growth of the colonies.

A confluent growth was seen where the initial streak was made, the growth was less dense away from the streak, and discrete colonies were obtained farthest away from the streak (i.e. end of the streak).

Evaluate antibacterial effect of Anandbhairav rasa (aqueous & methanol extract)

The nutrient agar plates were labeled as E.coli, S. sonnei & B. cereus with a wax pencil and then the plate was divided into 8 sectors for 8 samples with the help of a marker pen. Separate plates were prepared for control. The inoculating loop was sterilized by holding it in the hottest portion of the Bunsen burner flame until the entire wire became red hot. The loop was allowed to cool for a few seconds. The tip of the loop was touched to the surface of the agar streak plate. The lid of the agar plate was lifted and the culture was picked off from the surface of agar streak plate. Now inoculums were mixed in 1 ml distilled water. The inoculating loop was re-flamed to destroy existing organisms. 0.5 ml of inoculum was spread on the agar plate with the help of spreader and allowed to dry at room temperature for 30 min. Solution of each sample was made by adding 100mg of aqueous extract and 100mg of methanol extract in 1ml of distilled water and 1ml of methanol respectively. A hole of 2 mm diameter was made with the help of sterilized cork boxer one in each of the 8 sectors of one petri plate. By same method 3 petri plates with 8 wells were prepared for different three types of bacterial culture and 3 petri plates were prepared for control group (distilled water and methanol). 0.1 ml of samples (distilled water and methanol) were applied in each well. Disc of streptomycin & gentamycin were placed in each petri plate to check the comparative sensitivity. The cultured plates were incubated at 35°C for 48hours.

After incubation, the growth of pure colonies & inhibition zone was observed.

RESULT

All aqueous extracts of Anandbhairav Rasa have shown sensitivity against E.coli, S. sonnei, B. cereus cultures but the sensitivity of aqueous extract of Anandbhairav Rasa was highest in E.coli culture.

Methanol extract of all four samples of Anandbhairav Rasa showed sensitivity in all bacterial cultures but inhibition zone was maximum in E.coli culture and minimum in S. sonnei culture.

If we compare sensitivity of all samples of Anandbhairav Rasa against E.coli then we find Methanol extract of AB1 exhibited highest inhibition zone in E.coli culture.

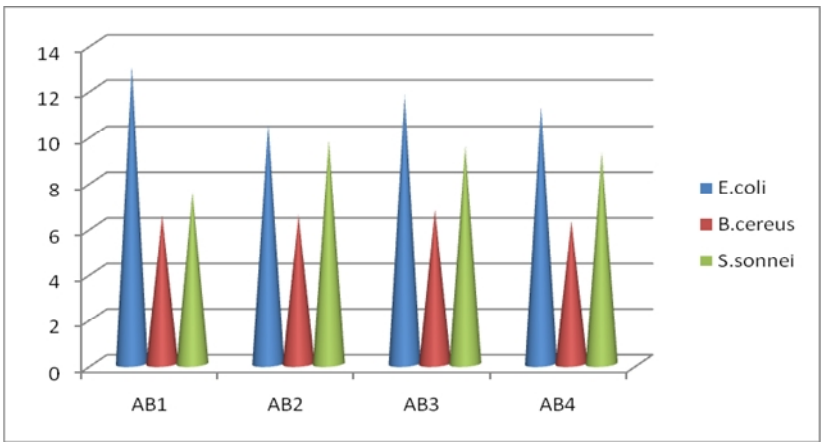
All aqueous extract of Anandbhairav Rasa showed almost equal sensitivity in B. cereus culture but if we compare all methanol extracts of Anandbhairav Rasa then we found methanol extract of AB1 showed highest sensitivity than others.

On comparing sensitivity of all samples in S. sonnei culture Methanol extract of AB4 exhibited highest inhibition zone and methanol extract of AB3 exposed least inhibition zone.

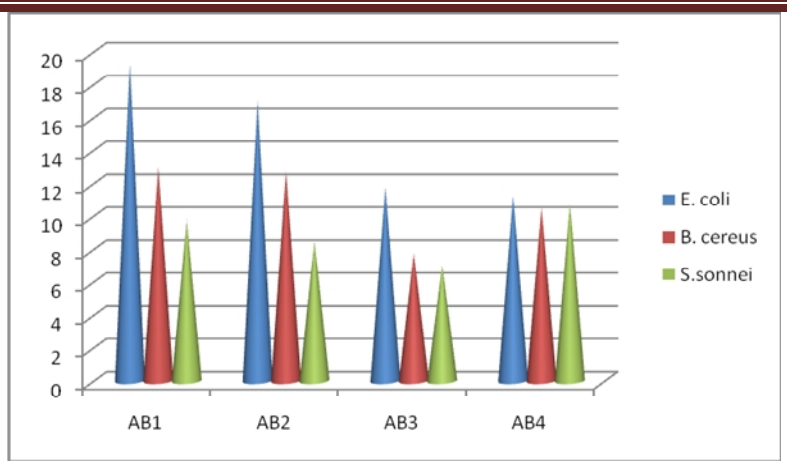
All of the samples of Anandbhairav Rasa exhibited bactericidal effect but in comparison to Streptomycin and gentamycin the bactericidal effect as shown by inhibition zone of Anandbhairav Rasa was minimum.

Table 3: Showing inhibition zone caused by all samples of Anandbhairav Rasa, streptomycin and gentamycin.

Name of the Drug	Name of the Organism		
	E.coli (Inhibition zone)	B. cereus (Inhibition zone)	S. sonnei (Inhibition zone)
Aquous extract of AB1	13mm	6.5 mm	7.5 mm
Aquous extract of AB2	10.5mm	6.5 mm	9.75 mm
Aquous extract of AB3	11.75 mm	6.75 mm	9.5 mm
Aquous extract of AB4	11.25 mm	6.25 mm	9.25 mm
Methnol extract of AB1	19.25 mm	13 mm	9.75 mm
Methnol extract of AB2	17 mm	12.75 mm	8.5 mm
Methnol extract of AB3	11.75 mm	7.75 mm	7 mm
Methnol extract of AB4	11.25 mm	10.5 mm	10.5 mm
Streptomycin	23.5 mm	32.5 mm	27.5 mm
Gentamycin	24.75 mm	13.5 mm	27.5 mm



Graph 1: Showing inhibition zone of all aquous samples of Anandbhairav Rasa against E.coli, S. sonnei, B. cereus.



Graph-2: Showing inhibition zone of all Methanol samples of Anandbhairav Rasa against E.coli, S. sonnei, B. cereus.

While comparing the effect of all samples of Anandbhairav Rasa it was observed that methanol extract of AB1 showed maximum inhibition zone against all the three test organism.

All aqueous extracts of Anandbhairav Rasa have shown sensitivity against E.coli , S. sonnei, B. cereus cultures but the sensitivity of aqueous extract of Anandbhairav Rasa was highest in E.coli culture. Methanol extract of all four samples of Anandbhairav Rasa showed sensitivity in all bacterial cultures but inhibition zone was maximum in E.coli culture and minimum in in S. sonnei culture.

All of the samples of Anandbhairav Rasa exhibited bactericidal effect but in comparison to streptomycin and gentamycin the bactericidal effect as shown by inhibition zone of Anandbhairav Rasa was less. While comparing the effect of all samples of Anandbhairav Rasa, it was observed that methanol extract of AB1 showed maximum inhibition zone against all the three test organism.

CONCLUSION

All aqueous extracts of Anandbhairav Rasa have shown sensitivity against E.coli, S. sonnei, B. cereus cultures but the sensitivity of aqueous extract of Anandbhairav Rasa was highest in E.coli culture.

Methanol extract of all four samples of Anandbhairav Rasa showed sensitivity in all bacterial cultures but inhibition zone was maximum in E.coli culture and minimum in S. sonnei culture.

While comparing the effect of all samples of Anandbhairav Rasa it was observed that methanol extract of AB1 showed maximum inhibition zone against all three test organism.

REFERENCES

1. Acharya Vagbhat, Astang Hridaya 8/1-3 Vidyotini Hindi commentary, Chaukhamba Sanskrit Sansthan, Varanasi, 2005
2. Acharya Madhava, Madhava Nidanam3/1-3, Shastri S., Upadhyay J., Sanskrit Hindi Commentary, Chaukhamba Sanskrit Samsthan, Varanasi, 2003.
3. Acharya Sushruta, Sushruta Samhita Uttar Tantra 56/10, Ambika Datta Shastri, Hindicommentary, Chaukhambha Sanskrit Sansthana, Varanasi, 2001.
4. Acharya Vagbhat, Astang Hridaya Chikitsa 8/164, Vidyotini Hindi commentary, Chaukhamba Sanskrit Sansthan, Varanasi, 2005
5. Bhava Misra, Bhav Prakash Haritakyadi Varg 58, Vidyotini Hindi comm., Chaukhambha Sanskrit Sansthan, Varanasi. 2000
6. Bhava Misra, Bhav Prakash Haritakyadi Varg 60, Vidyotini Hindi comm., Chaukhambha Sanskrit Sansthan, Varanasi. 2000
7. Bhava Misra, Bhav Prakash Karpuradi Varg 57, Vidyotini Hindi comm., Chaukhambha Sanskrit Sansthan, Varanasi. 2000
8. Sharma S. N., Shastri K. N., Rasa Tarangini 24/29, Hindi commentary, Motilal Banarasi Das Publication, Delhi, 2004
9. Bhava Misra, Bhav Prakash Haritakyadi Varg 52, Vidyotini Hindi comm., Chaukhambha Sanskrit Sansthan, Varanasi. 2000
10. Acharya Agnivesha, Charak Sutra 26/42 Pz 506, Vidyotini Hindi commentary, Chaukhamba Bharati Academy,Varanasi, 2005
11. Acharya Agnivesha, Charak Sutra 26/42 Pz 506, Vidyotini Hindi commentary, Chaukhamba Bharati Academy,Varanasi, 2005
12. Acharya Agnivesha, Charak Viman 7/14 Pz 727, Vidyotini Hindi commentary, Chaukhamba Bharati Academy,Varanasi, 2005
13. Acharya Agnivesha, Charak Viman 7 Pz 728, Vidyotini Hindi commentary, Chaukhamba Bharati Academy,Varanasi, 2005
14. Limyati et al., 1994, The antimicrobial activity of Z. officinale is reported
15. Usha & Saroja, 2000-2001 The aqueous extract of ginger arrested the growth of M.tuberculosis in vivo.
16. Bhat & Broker, 1953 M. fragrans extract showed mild antibacterial activity against pathogenic Staphylococci.
17. Subrahmanyam et al., 1957 The fruits extract reported to be inhibitory to E. coli, Acrobactoraerogens, L. casei, Staph. faecalis, Staph. aureus and Sh. sonnei.

Source of support: Nil, Conflict of interest: None Declared