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Original Article

Antimicrobial study of *Anandbhairav Ras (Atisar Adhikar)* on stock enteropathogenic bacteria w.s.r. to bacterial food poisoning

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ABSTRACT

Bacterial food poisoning is resulting from the ingestion of food contaminated by various bacteria. In Ayurveda infectious diseases and their epidemics have been discussed under the heading of Aupsargic Vyadhi and Janpadodhvans respectively. As bacterial food poisoning is resembles to Atisar (Diarrhoea), the ayurvedic *Kalp* described in *Atisar Adhyay* has benefited in bacterial food poisoning. *Anandbhairav Ras (Atisar)* is a drug belonging to *Atisar Chikitsa Adhyaya*. The study was design to evaluate in vitro anti-bacterial efficacy of *Anandbhairav Ras* against microorganism (pathogen) on stock enteropathogenic bacteria by using Well diffusion susceptibility testing. The in vitro antimicrobial study has studied in 9 groups and compared. All the 9 groups have studied on stock enteropathogenic bacteria *Bacillus cereus, E. coli, Salmonella Typhi* and *Staphylococcu aureus* and zone of inhibition has been measured in mm. In vitro antimicrobial efficacy of *Anandbhairav Ras* (ABR) on stock enteropathogenic bacteria, Ofloxacine 2 mg has showed maximum growth of inhibition followed by Ofloxacine 1 mg, ABR 8mg and ABR 4mg in *Staphylococcus aureus.* In vitro antimicrobial efficacy of *Anandbhairav Ras* on stock enteropathogenic bacteria has showed significant inhibition zone in both group study and positive against negative control, through the positive standard has more result than study group. Hence ABR can be used in infectious disease especially by enterogenic pathogen in therapeutic dose.

Keywords: *Anandbhairav Ras,* bacteria, antimicrobial study, zone of inhibition, agar plate, enteropathogenic

INTRODUCTION

Infectious diseases still account for a large proportion of death and disability worldwide. The Global Burden of Disease Study (GBDS) estimates that, in the year 2000 infectious diseases were responsible for 22% of all deaths and 27% of disability-adjusted life years (DALYs) worldwide[1]. Diarrheal diseases, pneumonia, and other infectious diseases are leading causes of death among children younger than five years in low and middle income countries and also in India[2]. Worldwide, food-borne diseases are a major health burden leading to high morbidity and mortality. The global burden of infectious diarrhoea involves 3-5 billion cases and nearly 1.8 million deaths annually[3]. Under the Integrated Disease Surveillance Project (IDSP) in India, food poisoning outbreaks reported from all over India in 2009 increased to more than double as compared to the previous year[4].
In ayurveda infectious disease (*Aupsargic Vyadh)i*[5] and epidemiology *(Janpadodhvans)*[6] has been discussed in detail. External Etiological Agent (Aagantuj) [is] one of the causes of most of the disease like Jwar, Atisar which is also consider to infectious[7]. Till 19[th] century the direct reference of bacteria has not found in any text of Ayurveda. Jeevanu which means the bacteria has described by Gannath Sen first time[8]. *Anandbhairav Ras (Atisar)* described almost all the text book of Ayurveda belonging to Rasshastra in *Atisar Chikitsa Adhyay*. It having the ingredient of *Suddha Hingula* (Cinnabar), *Suddha Vatsnabh* (*Aconitum ferox*), *Suddha Tankan* (Borex), *Marich*(*Piper nigram*) and *Pippali*(*Piper longam*). Ayurveda suggested that it should be taken with *Holarrhena antidysenterica* (*kutaj*) powder for diarrhoea (*Atisar*) [9]. Most of the ingredient of *Anandbhairav Ras (Atisar)* having *Katu Ras, Ushna Virya* and *Laghu, Ruksha Guna* in properties by which it can act on bacteria. All the drugs has showed antimicrobial efficacy individually except *Vatsnabh*. Hence *Anandbhairav Ras (Atisar)* is selected for this study.

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Aims and Objective:

1. To evaluate the in vitro antimicrobial efficacy of *Anandbhairav Ras* against enterogenic pathogenic Bacteria.
2. To compare the antimicrobial efficacy of *Anandbhairav Ras* against enterogenic pathogen with standard (Ofloxacin).

MATERIALS AND METHOD

Materials required for this study is Agar plate, inoculating loop, incubator, sterile borer, Anandbhairav *Ras*, Tween 80 solution, Ofloxacin, a syringe and scale. Antimicrobial Susceptibility Testing method used in this study is following:

Preparation of Muller-Hinton (MH) Agar plate- MH agar is considered the best medium to use for routine susceptibility testing of non-fastidious bacteria for the following reasons: It shows acceptable batch-to-batch reproducibility for susceptibility testing· It supports satisfactory growth of most non-fastidious pathogens A large body of data and experience has been collected concerning susceptibility tests performed with this medium .It is noted that the use of media other than Mueller-Hinton agar may result in erroneous results. Also noted that only the aerobic or facultative bacteria that grow well on un-supplemented MH agar should be tested using this protocol. Fastidious organisms require MH agar supplemented with additional nutrients and require that modification to this protocol be made. Neither the supplements nor the procedural modification are discussed in this basic protocol. Mueller-Hinton agar medium is the only susceptibility test medium that has been validated by NCCLS. Mueller-Hinton Agar is used for **Well diffusion susceptibility testing**, according.

Formula for Mueller-Hinton agar per litre of purified water

Beef, Infusion from	**300.0 g**
Casamino acid, technical	**17.5 g**
Starch	**1.5 g**
Agar	**17.0 g**

The components listed above were suspended in 1 litre of purified water .Then the all were mixed thoroughly, Heated with frequent agitation and boiled for 1 minute to completely dissolve the components .Then they were autoclave at 121°C for 15 minutes, dispensed as desired, allowed to solidify at room temperature, and then stored at 4 to 8°C. Mueller-Hinton agar is stable for approximately 70 days from the date of preparation. Muller Hinton agar was poured into 4 Petri dishes for each organism.

Mc Farland turbidity standard- A Mc Farland 0.5 standard was prepared and quality controlled prior to beginning susceptibility testing. If tightly sealed to prevent evaporation and stored in the dark, the standard can be stored for up to 6 months. The Mc Farland standard is used to adjust the turbidity of the inoculums for the susceptibility test.

Preparation of inoculums- Each culture to be tested should be streaked onto a non-inhibitory agar medium to obtain isolated colonies. After incubation at 35ºC overnight, select 4-5 well-isolated colonies with an inoculating needle or loop and transfer the broth to a sterile saline or non-selective broth and vortex thoroughly. The bacteria suspension should then be compared to the 0.5 Mc Farland standards. The turbidity standard should be agitated on a vortex mixture immediately prior to use. If the bacteria suspension doesn't appear to the same density as Mc Farland 0.5, the turbidity can be reduced by adding sterile saline or broth or increased by adding more bacterial growth.

Bacterial staining and well formation- Within 15 minute after adjusting the turbidity of the inoculums suspension, dip a sterile cotton swab into the suspension. Pressing firmly against the inside wall of the tube just above the fluid level, rotate the swab to remove the excess liquid. Streak the swab over the entire surface of the medium three times, rotating the plate approximately 60 degree after each application to ensure an even distribution of the inoculums. The Mueller-Hinton plate should be swabbed over the entire surface of the medium three times. Agar test plates of each test organism were prepared. The Petri dish incubated for 72 hrs at 37ºC to get active strain. After this 9 wells were made in each plate with sterile borer (5mm). Agar plugs were removed.

Preparation of Test Sample

A. *Anandbhairav Ras* 4 mg/well- *Anandbhairav Ras* 800 mg was dissolved in 10 ml Tween 80 solution

B. *Anandbhairav Ras* 8 mg/well- *Anandbhairav Ras* 1600 mg was dissolved in 10 ml Tween 80 solution

C. (*Anandbhairav Ras* 4 mg +*Kutaj Phala twak churna* 384 mg)/well- *Anandbhairav Ras* 800 mg and *Kutaj Phala twak* Extract 27.43 gm (35.71 %) was dissolved in 10 ml Tween 80 solution

D. (*Anandbhairav Ras* 8 mg + *Kutaj Phala twak churna* 768 mg) /well- *Anandbhairav Ras* 1600 mg and *Kutaj Phala twak* Extract 54.86 gm (35.71 %) was dissolved in 10 ml Tween 80 solution

E. *Kutaj Phala twak churna* 384 mg/well- *Kutaj Phala twak* Extract 27.43 gm (35.71 %) was dissolved in 10 ml Tween 80 solution

F. *Kutaj Phala twak churna* 768 mg/well- *Kutaj Phala twak* Extract 54.86 gm (35.71 %) was dissolved in 10 ml Tween 80 solution

Preparation of Standard Sample

G. Ofloxacine 1 mg- Ofloxacin 200 mg was dissolved in 10ml tween 80 solution.

H. Ofloxacine 2mg- Ofloxacin 400 mg was dissolved in 10ml tween 80 solution.

I. Tween 80 solution

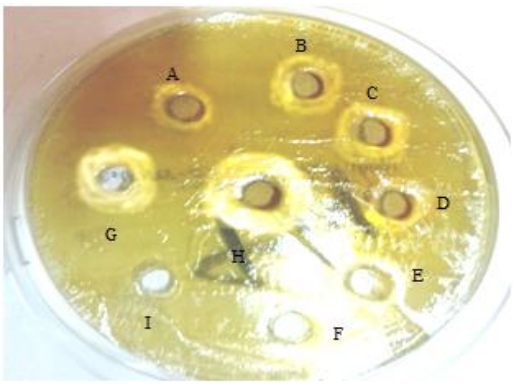
Application of sample in well to Inoculated Agar Plates- Control, standard and test sample is applied in the different well having 0.05 ml /well dose. The plates are inverted and placed in an incubator set to 35º C within 15 minutes after the sample are applied. The plates should not be incubated in an increased CO_2 atmosphere, because the interpretive standards were developed by using ambient air incubation, and CO_2 will significantly alter the size of the inhibitory zones of some agents.

Result interpretation- After 24 hours of incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculums were correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. The diameters of the zone of complete inhibition (as judged by the unaided eye) were measured, including the diameter of the disc. Zones were measured to the nearest whole millimeter, using sliding calipers, which is held on the back of the inverted Petri plate. The Petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light.

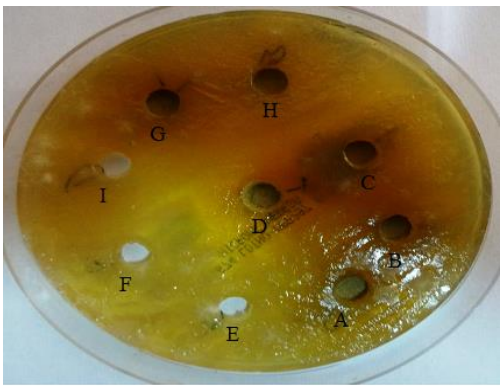
RESULTS AND OBSERVATION

The in vitro antimicrobial study has studied in 9 groups ((ABR 4mg, ABR 8mg, ABR 4mg+ *kutaj* 384 mg, ABR 8mg+ *kutaj*768 mg, *kutaj* 384 mg, *kutaj* 768 mg, Ofloxacine 1 mg, Ofloxacine 2 mg, Tween 80)) and compared. All the 9 groups have studied on stock enteropathogenic bacteria *Bacillus cereus, E. coli, Salmonella Typhi* and *Staphylococcus aureus* and zone of inhibition has been measured in mm.

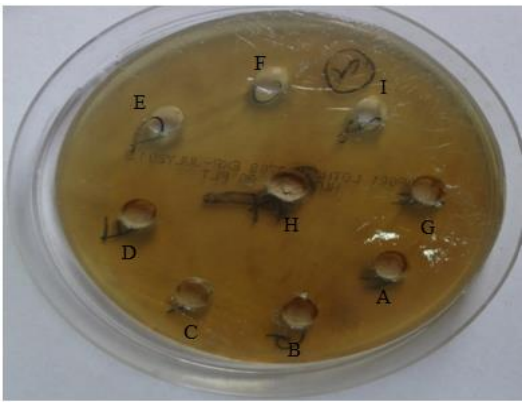
Zone of inhibition of ABR on stock enteropathogenic bacteria



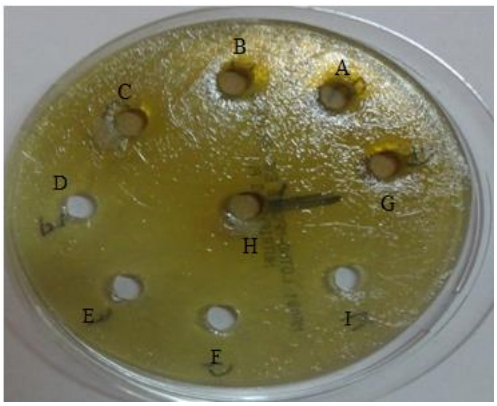
Bacillus cereus



E coli



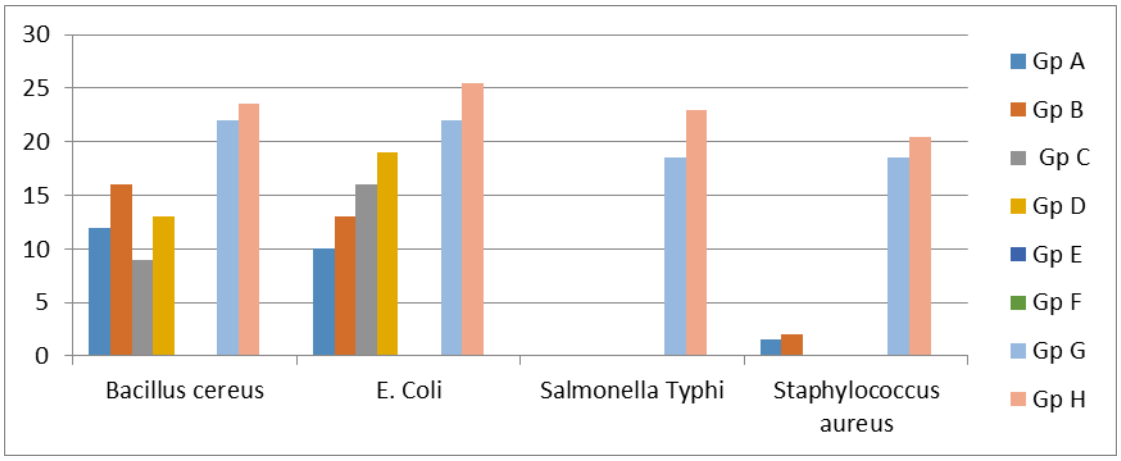
Salmonella typhi



Staphylococcus aureus

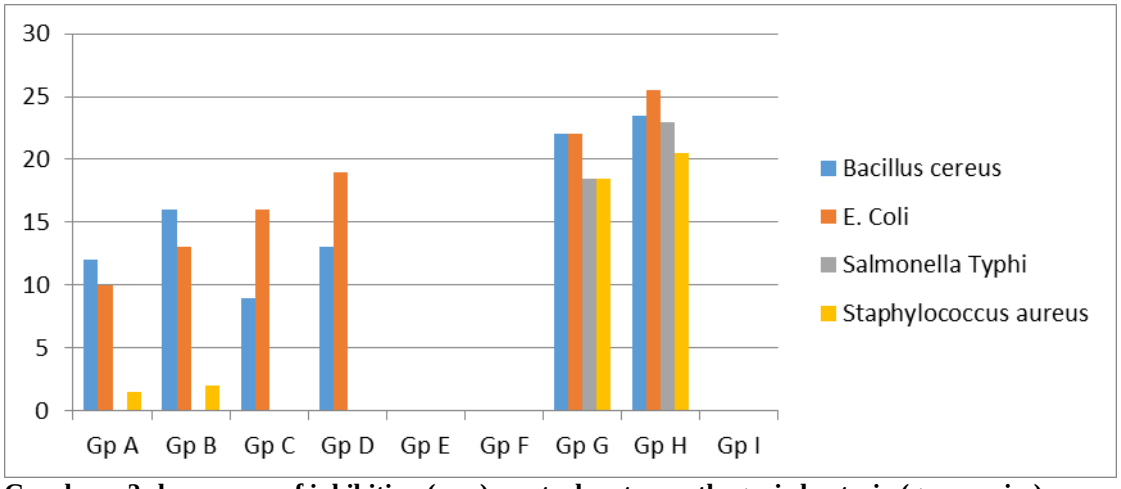
Table no. 1. Shows zone of inhibition (mm) on stock enteropathogenic bacteria

Groups	Zone of inhibition (mm)			
	Bacillus cereus	*E. Coli*	*Salmonella Typhi*	*Staphylococcus aureus*
ABR 4mg(Gp A)	12	10.0	0.0	1.5
ABR 8mg(Gp B)	16	13.0	0.0	2.0
ABR 4mg+ *kutaj* 384 mg (Gp C)	09.0	16.0	0.0	0.0
ABR 8mg+ *kutaj* 768 mg (Gp D)	13.0	19.0	0.0	0.0
kutaj 384 mg (Gp E)	0.0	0.0	0.0	0.0
kutaj 768 mg (Gp F)	0.0	0.0	0.0	0.0
Ofloxacine 1 mg (Gp G)	22.0	22.0	18.5	18.5
Ofloxacine 2 mg (Gp H)	23.5	25.5	23.0	20.5
Tween (Gp I)	0.0	0.0	0.0	0.0



Graph no. 1 shows zone of inhibition (mm) on stock enteropathogenic bacteria (bacteria wise)

The in vitro antimicrobial efficacy of ABR on stock enteropathogenic bacteria shows the maximum zone of inhibition in *E coli* Bacteria followed by *Bacillus cereus. Salmonella Typhi and Staphylococcus aureus* shows the less result than *E coli* and *Bacillus cereus*.



Graph no. 2 shows zone of inhibition (mm) on stock enteropathogenic bacteria (group wise)

The in vitro antimicrobial efficacy of ABR on stock enteropathogenic bacteria shows the significant zone of inhibition in all groups except *kutaj* 384 mg, *kutaj* 768 mg and negative control group. Ofloxacin 2mg shows the maximum zone of inhibition followed by ofloxacin 1 mg. ABR 8mg + *kutaj* 768 mg, ABR 4mg + *kutaj* 384 mg and ABR 8mg shows the moderate effect while ABR 4 mg shows the somewhat effect.

DISCUSSION

The in vitro antimicrobial efficacy of ABR on stock enteropathogenic bacteria has showed the maximum zone of inhibition in *E coli* Bacteria followed by *Bacillus cereus. Salmonella Typhi* and *Staphylococcus aureus* shows the less result than *E coli* and *Bacillus cereus*. It has also showed the significant zone of inhibition in all groups except *kutaj* 384 mg, *kutaj* 768 mg and negative control group. Ofloxacin 2mg has showed the maximum zone of inhibition followed by ofloxacin 1 mg. ABR 8mg + *kutaj* 768 mg, ABR 4mg + *kutaj* 384 mg and ABR 8mg shows the moderate effect while ABR 4 mg shows the somewhat effect. Most of the ingredient having Katu, Tikt Ras, Ushna Virya in property which is helpful to inhibit the growth of microorganism within living system by reducing the Kleda (wetness). Antimicrobial studies of ingredients of ABR showed that those are more potent sensitive to bacteria especially enterogenic pathogen.

CONCLUSION

In vitro antimicrobial efficacy of *Anandbhairav Ras* on stock enteropathogenic bacteria has showed significant inhibition zone in both group study and positive against negative control, through the positive standard has more result than study group. Hence ABR can be used in infectious disease especially by enterogenic pathogen in therapeutic dose.

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