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IN VITRO ANTI BACTERIAL STUDY OF *ANANDBHAIRAV RAS* BY SELF CREATED FOOD POISONING

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ABSTRACT

In Ayurveda infectious diseases and their epidemics have been discussed under the heading of Aupsargic Vyadhi and Janpadodhvans respectively. Abundant of herbal, mineral and herbomineral preparations have found in Ayurveda which have being continuously using for infectious disease by Ayurvedic practiontioer. Anand Bhairav Ras (ABR) is one of them. ABR contains pure Hingula, pure Vatsnabh, Tankan, Marich and Pipali in equal part[1]. The study was design to evaluate in vitro anti bacterial efficacy of *Anandbhairav Ras* against microorganism (pathogen) in self created bacterial food poisoning. The sample of artificial food poisoning was prepared by taking decomposed Meat Soup, Egg, Egg omelet, Milk and Potato and then after developing in vitro bacterial Food Poisoning in Artificial Stomach Media. Then the bacterial stain was taken from Artificial Food Poisoning and identifies the bacteria using gram stain method. The in vitro antimicrobial study has studied in 9 sub-groups. *ABR* 4 mg/well, *ABR* 8 mg, *ABR* 4 mg + *Kutaj Phala twak churna* 384 mg, *ABR* 8 mg + *Kutaj Phala twak churna* 768 mg, *Kutaj Phala twak churna* 384 mg, *Kutaj Phala twak churna* 768 mg, Ofloxacine 1 mg, Ofloxacine 2mg/well and Tween 80 solution. Ofloxacine 2 mg shows maximum zone of inhibition followed by *kutaj* 768 mg, *kutaj* 384 mg, ABR 4mg+ *kutaj* 384 mg, ABR 8mg+ *kutaj* 768 mg, ABR 8mg, Ofloxacine 1 mg and ABR 4mg in meat soup, egg sample, egg omelet sample, milk sample, potato sample. Hence ABR can be used in infectious disease especially by enterogenic pathogen in therapeutic dose.

KEYWORDS: *Anand Bhairav Ras*, Antimicrobial Drug, *Ayurveda*, Enteric Pathogen, Bacteria.

INTRODUCTION

Microorganism comprises a single cell (unicellular), cell clusters, or multicellular relatively complex organisms. Microorganisms are very diverse; they include bacteria, fungi, algae and protozoa; microscopic plants (green algae); and animals such as rotifers and planarians. Some microbiologists also include viruses, but others consider these as nonliving. Microorganisms are unicellular, but this is not universal, since some multicellular organisms are microscopic. Some unicellular protists and bacteria, like Thiomargarita namibiensis, are macroscopic and visible to the naked eye. Pathogenic microbes are harmful, since they invade and grow within other organisms, causing diseases that kill humans, animals and plants. Today, infectious diseases still account for a large proportion of death and disability worldwide and in certain regions remain the most important cause of ill health. 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 (WHO, 2002).[2] 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.[3] In ayurveda infectious disease and its epidemic has been discussed under the heading of Aoupsargic Vyadhi[4] and Janpadodhvans[5] respectively. Aagantuj[6] is one of the causes of most of the disease like Jwar, Atisar which is also consider to infectious. Till 19th century the direct reference of bacteria has not found in any text of Ayurveda. Jeevanu is means the bacteria has described by Gannath Sen first time.[7] So many herbal, mineral and herbomineral preparations has been found in Ayurvedic text for such type of infectious pathology and ABR is one of them. ABR described almost all the text book of Ayurveda belonging to Rasshastra in Atisar Chikitsa Adhyay. It having ingredient like Hingula, Vatsnabh, Tankan, Marich, Pipali and most of the content proved potent for their antimicrobial property.

AIMS AND OBJECT

To Evaluate in Vitro Anti Bacterial Efficacy of *Anandbhairav Ras* against Microorganism (Pathogen) in Self Created Bacterial Food Poisoning.

MATERIALS AND METHOD

Materials required for this study is conical flask, Gastric juice, Incubator, Agar plate, inoculating loop, sterile borer, *Anandbhairav Ras*, Tween 80 solution, Ofloxacin, a syringe, a scale and microscope. Method used in this study is following:

1. Preparing of food sample

1. **Meat Soup**: 100 gm chicken liver cooked in 500 ml water till the white colour of soup will appear. Then that chicken meat soup was filtered and by measuring that which remain 300ml.
2. **Egg**: Take the one chicken egg.
3. **Egg omelet**: The chicken egg has broken and its white yellow material has fried on pan. Thus we get omelet.
4. **Milk**: Take the dairy milk and then boiled it.
5. **Potato**: Take the potato and then boiled it.

After this the food samples were kept on room temperature for 24 hours.

2. Develop in vitro bacterial Food Poisoning

Artificial preparation of gastric fluid

0.5% Hcl, 0.2% Pepsin, 0.4% Renin mixed in distill water and then 0.1M Kcl and 0.1M Nacl was added. Thus we have prepared the artificial gastric fluid.

Artificial preparation of stomach environment

After 24 hours of preparing the sample, all samples added in artificial gastric fluid separately and sack well. Then the samples kept in BOD incubator at 37°C.

3. Taking the sample from Artificial Food Poisoning

The bacteria's were take out after 12 hours of growing periods in artificial stomach and was grow on agar plate.

4. Identification of bacteria-

Procedure

1. using a sterile inoculating loop, add 1 drop of sterile water to the slide. Then inoculate the sample on the slide
2. Air dry and Heat fix.
3. Cover the smear with Crystal Violet (primary stain) for 1 min.
4. Gently wash off the slide with water.
5. Add Gram's Iodine (mordant) for 1 min.
6. Wash with water.
7. Decolorize with 95% ethanol. This is the "tricky" step. Stop decolorizing with alcohol as soon as the purple colour has stopped leaching off the slide (time will vary depending on thickness of smear).

Immediately wash with water. Be sure to dispose of all ethanol waste in the appropriately labeled waste container.

8. Cover the smear with Safranin for 30 seconds.
9. Wash both the top & the bottom of the slide with water.
10. Blot the slide with bibulous paper.
11. Using the 10X objective lens, focus first on the line and then on the smear.

Table no. 1 shows gram staining of bacteria.

Sr. N.	Sample	Observation	Result
1	Meat soup	Both purple and pink color are observed	gram positive and gram negative bacteria are present
2	Egg	------*do*-------	------*do*-------
3	Egg omelet	------*do*-------	------*do*-------
4	Milk	------*do*-------	------*do*-------
5	Potato	------*do*-------	------*do*-------

Bacteria identified in artificial in vitro bacterial food poisoning

Meat Soup: *E Coli, Staphylococcus aureus, Helicobacter pylori, Bacillus cerus, Salmonella typhi.*

Egg: *Aeromonas hydrophila, E Coli, Staphylococcus aureus, Helicobacter pylori, Bacillus cerus, Salmonella typhi.*

Egg omelet: *Aeromonas hydrophila, E Coli, Staphylococcus aureus, Helicobacter pylori, Bacillus cerus, Salmonella typhi.*

Milk: *E Coli, Staphylococcus aureus, Helicobacter pylori, Bacillus cerus.*

Pototo: *E Coli, Staphylococcus aureus, Helicobacter pylori, Bacillus cerus.*

Table no.2 shows the test to identification of bacteria in artificial in vitro bacterial food poisoning.

S.No.	Test	Microorganism identified
1	Indole test	*E.Coli*
2	Methyl Red Test	*E.Coli*
3	Citrate Utilization Test	*Proteus myxofaciens, Raoultella planticola*
4	TSI	*Salmonella enterica*serovar Typhi
5	Urease test	*Helicobacter pylori*
6	SIM	*Helicobacter pylori*
7	Gelatin Hydrolysis Test	*Bacillus subtitles, Clostridium perfringens, Escherichia coli, Proteus vulgaris, Serratia liquefaciens, Staphylococcus aureus.*
8	Catalase Test	*Mycobacterium tuberculosis, Legionella pneumophila* and *Campylobacter jejuni*
9	Coagulase Test	*Staphylococcus Spesis*

5. Antimicrobial Susceptibility Testing

a. Bacterial staining and well formation on agar plate

After solidification the Agar medium, 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. The Petri dish incubated for 72 hrs to get active strain and then after well was made in the plates for 9 group and for each sample with sterile borer (5mm). Agar plugs were removed.

b. 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.

c. Preparation of Standard Sample (positive control)

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 (negative control).

d. 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 35ºC 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.

e. Result interpretation

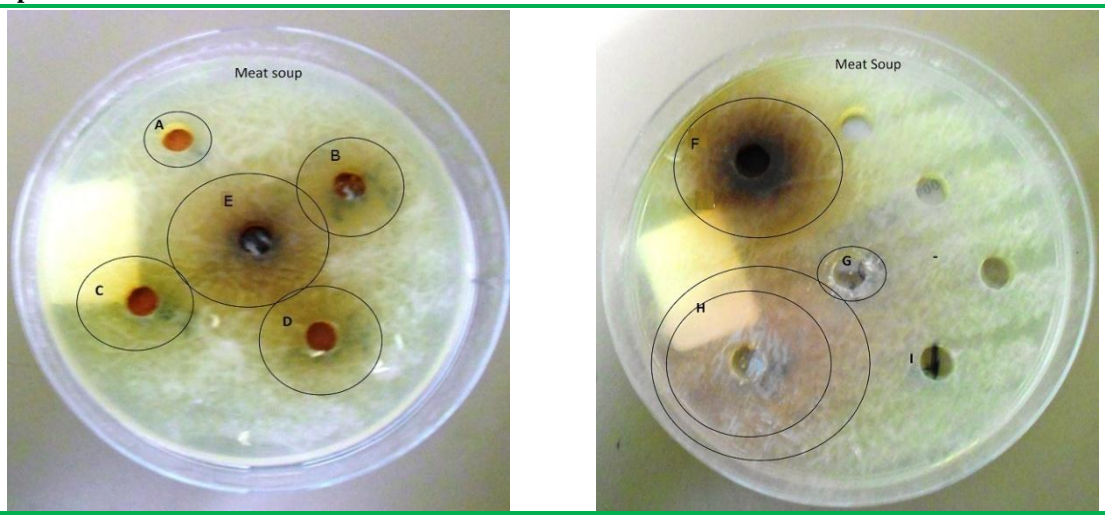
After 24 hours of incubation, each plate is examined. If the plate was satisfactorily streaked and the inoculums were correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. The diameters of the zone of complete inhibition (as judged by the 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

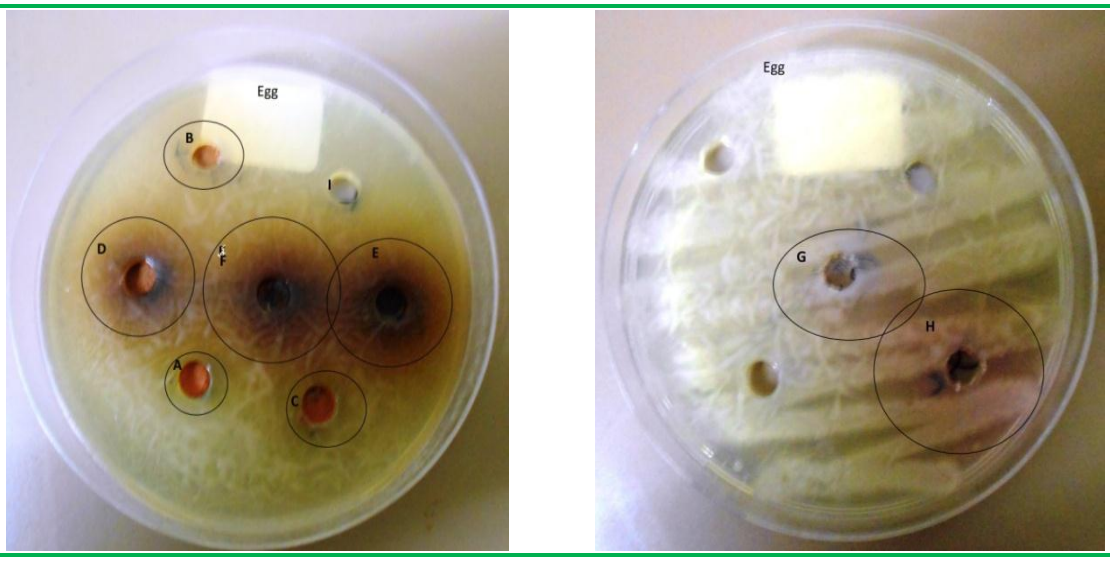
The in vitro antimicrobial study has studied in 9 groups (ABR 4mg, ABR 8mg, ABR 4mg+ *kutaj* 384 mg, ABR 8mg+ *kutaj*768 mg, *kutaj* 384 mg, *kutaj* 768 mg, Ofloxacine 1 mg, Ofloxacine 2 mg, Tween 80) and compared. All the 9 groups have studied on artificial cultured meat soup, egg, egg omelet, milk and potato samples. The bacterial colony has taken from artificial cultured samples of meat soup, egg, egg omelet, milk, potato after 12 hours and cultured agar plates and zone of inhibition has been measured in mm.

Zone of inhibition of ABR in artificial cultured in vitro bacterial food poisoning

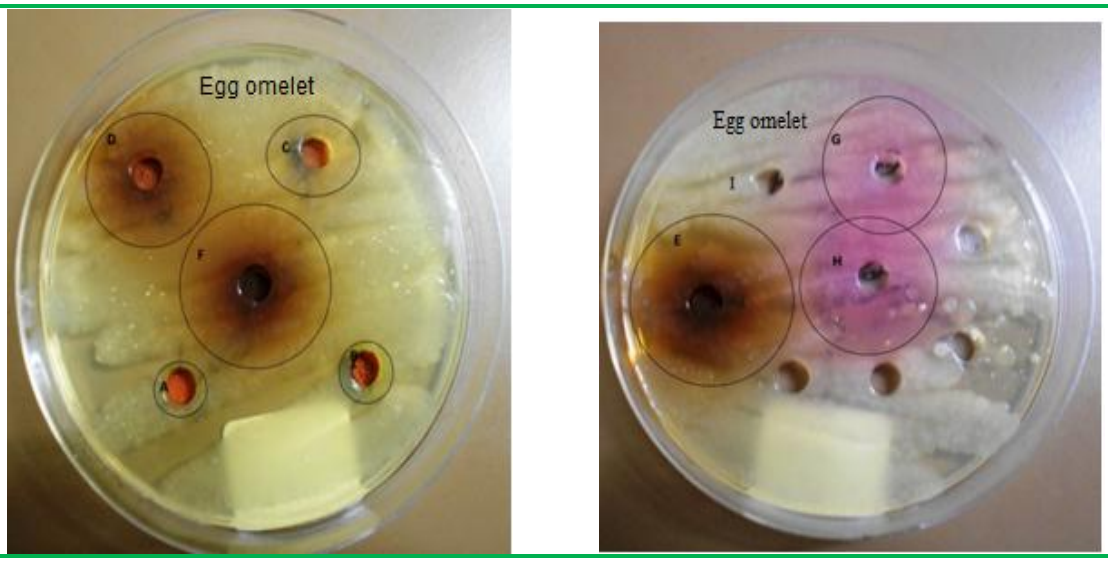
Meat soup



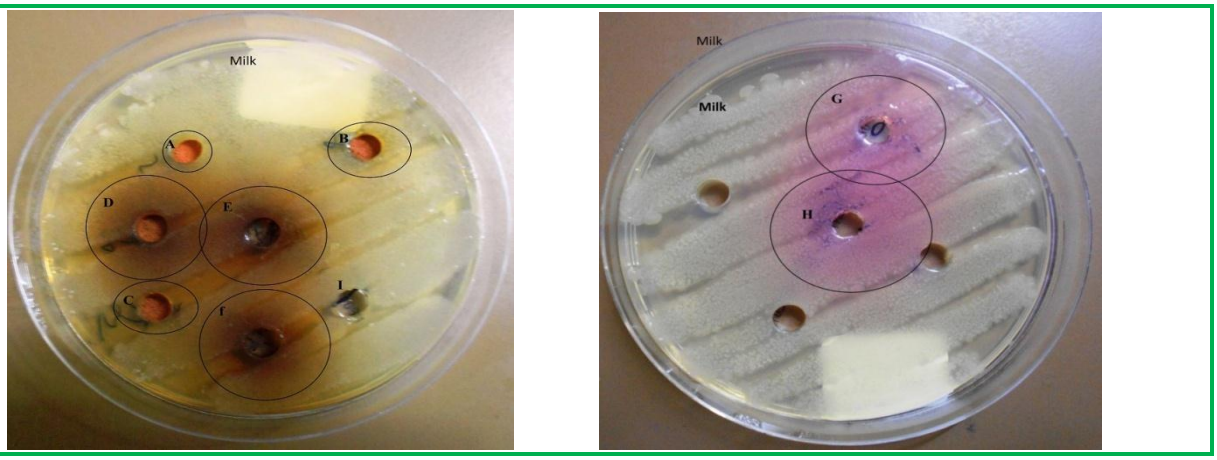
Egg



Egg omelet



Milk



Potato

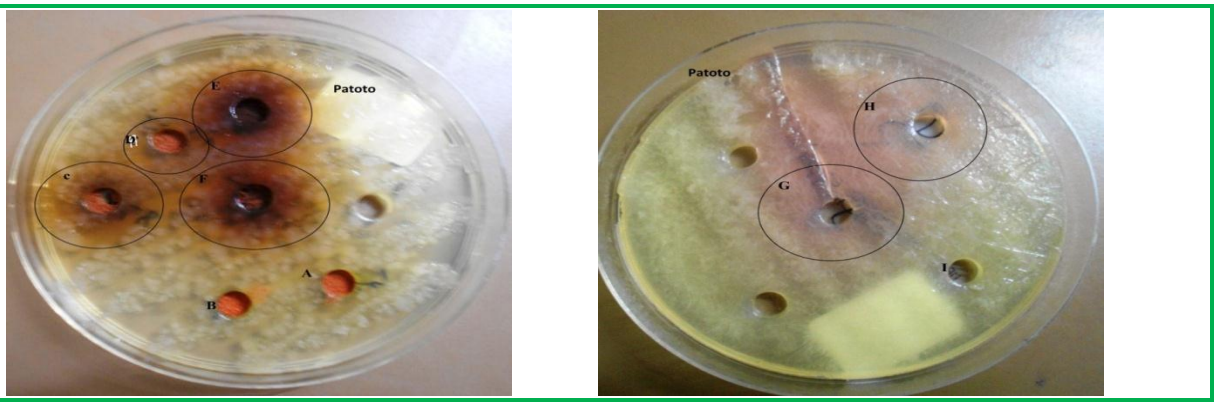
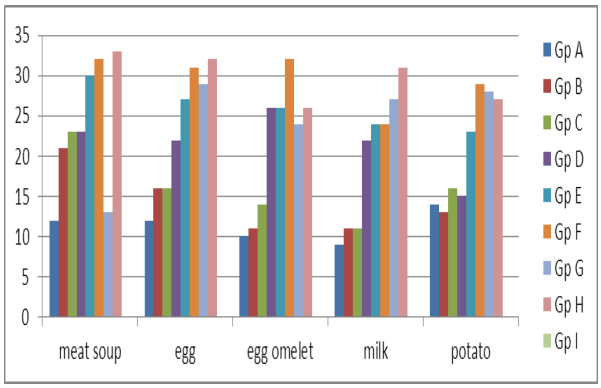


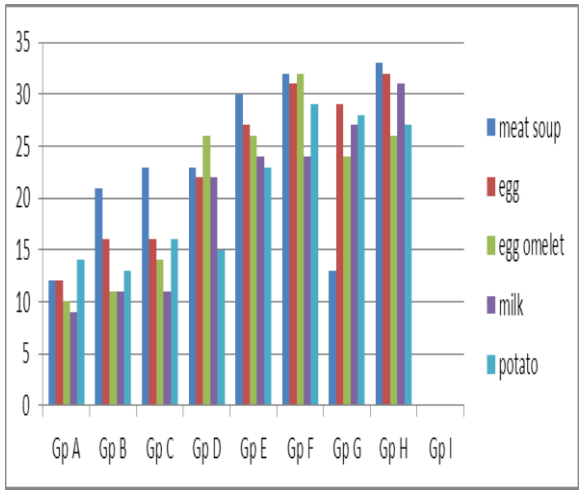
Table no. 3 shows zone of inhibition (mm) in artificial cultured bacterial food poisoning.

Groups	Zone of inhibition (mm)				
	Meat soup	Egg	egg omelet	Milk	Potato
ABR 4mg(Gp A)	12	12	10	9	14
ABR 8mg(Gp B)	21	16	11	11	13
ABR 4mg+ *kutaj* 384 mg (Gp C)	23	16	14	11	16
ABR 8mg+ *kutaj* 768 mg (Gp D)	23	22	26	22	15
kutaj 384 mg (Gp E)	30	27	26	24	23
kutaj 768 mg (Gp F)	32	31	32	24	29
Ofloxacine 1 mg (Gp G)	13	29	24	27	28
Ofloxacine 2 mg (Gp H)	33	32	26	31	27
Tween 80 (Gp I)	00	00	00	00	00



Graph no. 1 shows zone of inhibition (mm) in artificial cultured bacterial food poisoning (sample wise).

In vitro antimicrobial efficacy of *Anandbhairav Ras* in artificial cultured bacterial food poisoning shows the significant zone of inhibition in all the samples taken for study. The in vitro antimicrobial efficacy of ABR in artificial cultured bacterial food poisoning shows the more result in meat soup followed by egg and egg omelet. The potato sample shows the less result than all the samples including meat soup, egg, egg omelet and milk.



Graph no. 2 shows zone of inhibition (mm) in artificial cultured bacterial food poisoning (group wise).

The in vitro antimicrobial efficacy of ABR in artificial cultured bacterial food poisoning shows zone of inhibition in all groups except negative control group. Though results are varies. Ofloxacine 2 mg and *kutaj* 768 mg shows maximum zone of inhibition followed by Ofloxacine 1 mg and *kutaj* 384 mg. ABR 4mg+ *kutaj* 384 mg and ABR 8mg+ *kutaj* 768 mg shows moderate effects while ABR 8mg and ABR 4mg shows somewhat effect.

DISCUSSION

In vitro antimicrobial efficacy of *Anandbhairav Ras* in artificial cultured bacterial food poisoning shows the significant zone of inhibition in all the samples taken for study. The in vitro antimicrobial efficacy of ABR in artificial cultured bacterial food poisoning shows the more result in meat soup followed by egg and Egg omelet. The potato sample shows the less result than all the samples including meat soup, egg, Egg omelet and milk. It shows zone of inhibition in all groups except negative control group. Though results are varies. Ofloxacine 2 mg and *kutaj* 768 mg shows maximum zone of inhibition followed by Ofloxacine 1 mg and *kutaj* 384 mg. ABR 4mg+ *kutaj* 384 mg and ABR 8mg+ *kutaj* 768 mg shows moderate effects while ABR 8mg and ABR 4mg shows 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. In Siddha system of medicine most of the chronic diseases are cured by the medicines prepared from the metal and mineral products namely Parpam, Chendooram, Chunnam etc. The selected Siddha medicines for investigation mainly consist of Cinnabar. In the present study some clinically used herbo - mineral siddha drugs such as Linga chendooram-1, Linga chendooram - 2, Vajerakandi, Kantharasa villai, Sandamarutham and Rasa chunnam were investigated for antibacterial potential against *Escherichia coli*, *Salmonella typhi*, *Vibrio cholerae*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Study suggested that these herbomineral siddha preparations may be useful as an alternative medicine in the treatment of enteric bacterial pathogen.[8] The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of boric acid were obtained as 3.80 mg/mL, 3.80 mg/mL, 7.60 mg/mL and 7.60 mg/mL against the bacterial activities of *Staphylococcus aureus*, *Acinetobacter septicus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, respectively. The MICs and the MBCs of borax were obtained as 23.80 mg/mL, 23.80 mg/mL, 47.60 mg/mL, and 47.60 mg/mL against the above bacteria, respectively.[9] Piperine showed antimicrobial activity against all tested bacteria with zone of inhibition ranged from 8-18mm. maximum zone of inhibition was against Gram positive bacteria *Staphylococcus aureus* (18mm) and minimum against Gram negative bacteria *Escherichia coli* (8mm). Piperine showed maximum antifungal activity towards *Fusarium oxysporum* (14mm) and very least effect against *Aspergillus niger* (38mm).[10] The attempt was made to evaluate the antimicrobial activity of various solvent extracts of fruit of *Piper longum* L. against different gram positive and gram negative bacteria by using disk diffusion method. The petroleum ether extract was resistant towards all the tested bacterial strains while ethyl acetate was highly active. Among all the gram positive bacteria *Staphylococcus aureus* was highly sensitive with inhibition zone 24.33mm in presence of 500mg/ml ethyl acetate extract while in case of gram negative bacterial strains *Pseudomonas aeruginosa* and *Vibrio cholerae* were highly sensitive with inhibition zone 22.66mm. Hexane extract was least inhibitory towards all the bacterial strains.[11]

CONCLUSION

In vitro antimicrobial efficacy of *Anandbhairav Ras* in artificial cultural media- Ofloxacine 2 mg shows maximum zone of inhibition followed by *kutaj* 768 mg, *kutaj* 384 mg, ABR 4mg+ *kutaj* 384 mg, ABR 8mg+ *kutaj* 768 mg, ABR 8mg, Ofloxacine 1 mg and ABR 4mg in meat soup, egg sample, egg omelet sample, milk sample, potato sample. Hence ABR can be used in infectious disease especially by enterogenic pathogen in therapeutic dose.

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