



Review Article

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ANTIMICROBIAL PROPERTIES OF ANAND BHAIRAV RAS AND ITS INGREDIENTS: AN OVERVIEW

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ABSTRACT

In Ayurveda infectious diseases and their epidemics have been discussed under the heading of Aupsargic Vyadhi and Janpadodhvas respectively. Abundant of herbal, mineral and herbomineral preparations have been found in Ayurveda which have been continuously used for infectious disease by Ayurvedic practitioner. Anand Bhairav Ras is one of them. Anand Bhairav Ras (ABR) contains pure Hingula, pure Vatsnabh, Tankan, Marich and Pipali in equal part. Most of the ingredient are 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). Review of index Journals for antimicrobial properties of ingredients of ABR show that they are more sensitive to bacteria especially enterogenic pathogen. Hence ABR can be used in infectious disease especially caused by enterogenic pathogen in therapeutic dose. In this article we tried to highlight, evaluate and discussed the antimicrobial properties and sensitivity of ABR and its ingredients.

Keywords: Anand Bhairav Ras, Antimicrobial Drug, Ayurveda, Enteric Pathogen, Bacteria

INTRODUCTION

A microbe or microorganism is a microscopic organism that comprises a single cell (unicellular), cell clusters, or multi cellular 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 multi cellular 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)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 India². In Ayurveda infectious disease and its epidemic has been discussed under the heading of Aupsargic Vyadhi³ and Janpadodhvas⁴ respectively. Aagantuj⁵ is one of the causes of most of the disease like Jwar, Atisar which is also considered to be infectious. Till 19th century the direct reference of bacteria has not found in any text of Ayurveda. Jeevanu means the bacteria has described by Gannath Sen first time⁶. 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 is described in almost all the text book of Ayurveda belonging to Rasshastra in Atisar Chikitsa Adhyay. It has ingredient like Hingula, Vatsnabh, Tankan, Marich, Pipali and most of the content were proved potent for their antimicrobial property. Thus in this article the antimicrobial property of ABR and its ingredient were discussed and highlighted in detail.

Human Pathogenic Bacteria

Bacteria are often maligned as the causes of human and animal disease. However certain bacteria, the actinomycetes produce antibiotics such as streptomycin and nocardicin, others live symbiotically in the guts of animals (including humans), elsewhere in their bodies, on the roots of certain plants and converting nitrogen into a usable form⁷. A pathogen is usually defined as a microorganism that causes, or can cause, disease. We have defined a pathogen as a microbe that can cause damage in a host⁸. The classification of bacteria serves a variety of different functions. Because of this variety, bacteria may be grouped using many different types of schemes.

Gram stain and bacterial morphology

It allows a large proportion of clinically important bacteria to be classified as either Gram positive or negative based on their morphology and differential staining properties. Slides are sequentially stained with crystal violet, iodine, and then destained with alcohol and counter-stained with safranin. Gram positive bacteria stain blue purple and Gram negative bacteria stain red.

Growth Requirements

Microorganisms can be grouped on the basis of their need for oxygen to grow. Facultative anaerobic bacteria can grow in high oxygen or low oxygen content and are among the more versatile bacteria. In contrast, strictly

anaerobic bacteria grow only in conditions where there is minimal or no oxygen present in the environment. Bacteria such as bacteroides found in the large bowel are examples of anaerobes. Strict aerobes only grow in the presence of significant quantities of oxygen⁹.

Table 1: Gram Positive Bacteria

S. No.	Name	Morphology	O ₂ Requirements
1	<i>Staphylococci</i>	Cocci in grape-like clusters	Facultative anaerobe
2	<i>Streptococci</i>	Cocci in pairs, chains	Facultative anaerobe
3	<i>Pneumococci</i>	Diplococci, lancet shaped	Facultative anaerobe
4	<i>Enterococci</i>	Diplococci, lancet shaped	Facultative anaerobe
5	<i>Bacilli</i>	Rods, spore forming	Aerobic
6	<i>Clostridia</i>	Rods, spore formers	Anaerobic
7	<i>Corynebacterium</i>	Rods, nonspore forming	Facultative anaerobe
8	<i>Listeria</i>	Rods, nonspore formers	Facultative anaerobe
9	<i>Actinomyces</i>	Irregular, filamentous, form sulfur granules	Anaerobic

Table 2: Gram Negative Bacteria

S.No.	Name	Morphology	O ₂ Requirements
1	Enterobacteriaceae (<i>E. coli, klebsiella, salmonella, shigella</i>)	Rods	Facultative anaerobe
2	<i>Bacteroides</i>	Rods	Anaerobic
3	<i>Pseudomonas</i>	Rods	Aerobic
4	<i>Vibrio</i> (cholera)	Rods, curved shape	Micro aerophilic
5	<i>Campylobacter</i>	Rods, curved shape	Micro aerophilic
6	<i>Legionella</i>	Rods, poorly stained	Micro aerophilic
7	<i>Neisseria</i>	Cocci, kidney-bean shaped	Micro aerophilic
8	<i>Hemophilus</i>	Coccobacillary- pleomorphic	Facultative anaerobe
9	<i>Bartonella</i>	Small, pleomorphic rods	aerobic/micro aerophilic

Ingredient of Anand Bhairav Ras

ABR is having the ingredient of Suddha Hingula (Cinnabar), Suddha Vatsnabh (*Aconitum ferox*), Suddha Tankan (Borex), Marich (*Piper nigrum*) and Pipali (*Piper longum*) in equal quantity¹⁰⁻¹⁶.

Table 3: Ayurvedic properties of ingredients of ABR

S.No.	Ingredient	Ras	Gun	Virya	Vipak	Doshkarm
1	Hingula ^{17,18}	Katu, Tikt, Kasaya	-	Ushna	Katu	Kaph Pithar
2	Vatsnabh ^{19,20}	Katu, Tikt, Kasaya	Yogvahi Vyavayi, Vikasi, Ruksha, Tikshna, Laghu	Ushna	Madhur	Tridoshar
3	Tankan ²¹	Katu	Ruksha, Tikshna, Sara	Ushna		Kaphhar
4	Marich ^{22,23}	Katu	Ruksha, Tikshna, Laghu	Ushna	Katu	Vat Kaphhar
5	Pipali ²⁴	Katu	Laghu, Snigdha	Unushna	Madhur	Vat Kaphhar

Most of the Ras of ABR ingredients is Katu as per Acharyas but some exception are Katu, Tikt, Kasaya Ras of Hingula and Vatsnabh. Most of the ingredients of ABR have Laghu and Tikshna Guna. Vatsnabh have Vyavayi, Vikasi and Ruksha along with Laghu Guna. Pipali have Snigdha Guna along with Laghu. As per Ayurveda most of the ingredients of ABR have Ushna Virya, exception like pipali is Unushna. Most of the Acharyas mention Pipali having Madhur Vipak and Marich having Katu. Ayurveda mentioned Vat Kaph Har Dosh Karm of Marich and Pipali while Kaph Har Dosh Karm of Tankan, Tridoshar karm of Vatsnabh and Kaph Pithar Doshkarm of Hingula.

The therapeutic use of ingredient of Anand Bhairav Ras (Atisar) has varying according to various Acharyas. All

the ingredient of Anand Bhairav Ras (Atisar) is indicated for Mandagani. Most of the ingredient of Anand Bhairav Ras (Atisar) indicated in Pliha Roga, Aamvat, Jwar, Swas, Kas and Shul.

Antimicrobial Properties of Ingredients of Anand Bhairav Ras

Antimicrobial property of ABR (Atisar) is not studied still but antimicrobial property of ABR (Jwar) has been studied. All aqueous and methanol extracts of ABR (Jwar) have shown sensitivity against *E. coli*, *S. sonnei*, *B. cereus* cultures but the sensitivity of aqueous and methanol extracts of it was highest in *E. coli* culture³⁰. Most of the ingredients of ABR (Atisar) and ABR (Jwar) have similar except Shunthi and Jatikosh³¹.

Table 4: Therapeutic uses of ingredient of Anand Bhairav Ras (Atisar)

S. No.	Disease	Hingula ²⁵	Vatsnabh ²⁶	Tankan ²⁷	Marich ²⁸	Pipali ²⁹
1	Netra Rog	✓	✓	-	-	-
2	Pliha Rog	✓	✓	-	-	✓
3	Kustha	✓	-	-	-	-
4	Kamala	✓	-	-	-	-
5	Mandagni	✓	✓	✓	✓	✓
6	Avipak	✓	-	-	-	-
7	Prameha	✓	-	-	-	✓
8	Aamvat	✓	✓	-	-	✓
9	Jwar	✓	✓	-	-	✓
10	Gridhrasi	-	✓	-	-	-
11	Grihani	-	✓	-	-	-
12	Kusth	-	✓	-	-	✓
13	Shoth	-	✓	-	-	-
14	Swas	-	✓	✓	✓	✓
15	Kas	-	✓	✓	-	✓
16	Aadhaman	-	-	✓	-	-
17	Vran	-	-	✓	-	-
18	Shul	-	✓	-	✓	✓
19	Krimi	-	-	-	✓	✓
20	Aruchi	-	-	-	-	✓
21	Gulm	-	✓	-	-	✓
22	Arsh	-	-	-	-	✓
23	Pandu	-	✓	-	-	✓
24	Vat Rakt	-	✓	-	-	-

Hingula

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, Vajarakandi, 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 pathogen³².

Vatsanabh

Though *Aconitum ferox* has been highly used in most of the herbomineral preparations used for infectious disease like Jwar, Atisar, Kas, Swas etc. in Ayurveda, none of the Ayurvedic text has mention its antimicrobial (Krimighan) property.

Tankan

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, respectively³³.

Marich

Piperine showed antimicrobial activity against all tested bacteria with zone of inhibition ranged from 8-18 mm. maximum zone of inhibition was against Gram positive bacteria *Staphylococcus aureus* (18 mm) and minimum against Gram negative bacteria *Escherichia coli* (8 mm). Piperine showed maximum antifungal activity towards *Fusarium oxysporum* (14 mm) and very least effect against *Aspergillus niger* (38 mm)³⁴. Trikatu curna and its each component possess potent antimicrobial activity. The activity shown by aqueous extract is of considerable importance. The extract of Trikatu churna shows highest activity which is almost equal to the effects shown by standard ampicillin solution. The result shows that the Trikatu churna was very effective against *E. coli* and *S. aureus* but all the individual ingredients of Trikatu churna possess moderate antimicrobial activity³⁵. In aqueous extract, black pepper and turmeric showed good inhibitory activity against *Staphylococcus aureus* with zone of inhibition 25 mm to 30 mm and 26 mm to 28 mm respectively. In ethanolic extract, black pepper extract showed antibacterial activity against all test bacteria with zone of inhibition ranged between 15 mm and 22 mm³⁶. As the acetone and ethanol extracts of *P. nigrum* showed anti-*M. tuberculosis* activity, a mixture of these extracts (at 25 µg/ml each and 50 µg/ml each) was also tested. It was seen that these mixtures showed inhibitory activity against *M. tuberculosis*³⁷. In another study the methanolic extract of the *Piper nigrum* were obtained. The cold water extract shown good result 23 mm against *Escherichia coli* while less effective against *Pseudomonas aeruginosa* 15 mm. The cold water and methanolic extract have high activity against *Salmonella typhae*, *Escherichia coli* and low activity against *Pseudomonas aeruginosa* and *Enterococcus faecalis* respectively. Similarly the methanolic extract of *Piper nigrum* has shown no activity against *Staphylococcus aureus*. From the present research

it was concluded that all the extract of *Nigella sativa* and *Piper nigrum* have high activity against gram negative bacteria as compare to gram positive bacteria³⁸. Antimicrobial activity of black pepper (*Piper nigrum* Linn.) and its mode of action on bacteria were done. The extract of black pepper was evaluated for antibacterial activity. The results indicate excellent inhibition on the growth of gram positive bacteria like *Staphylococcus aureus*, followed by *Bacillus cereus* and *Streptococcus faecalis*. Among the gram negative bacteria *Pseudomonas aeruginosa* was more susceptible followed by *Salmonella typhi* and *Escherichia coli*³⁹.

Pipali

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 24.33 mm in presence of 500 mg/ml ethyl acetate extract while in case of gram negative bacterial strains *Pseudomonas aeruginosa* and *Vibrio cholerae* were highly sensitive with inhibition zone 22.66 mm. Hexane extract was least inhibitory towards all the bacterial strains⁴⁰. The alcoholic extracts of Siddha formulation and its ingredients were evaluated for antimicrobial and analgesic activities. It is one of the Siddha formulations, used in traditional Indian system of medicine, practiced against certain general health disorders such as carminative, appetizer, purgative etc. It is a powdered mixture of equi-proportions of fruits of *Phyllanthus emblica* (Euphorbiaceae), *Piper longum* (Piperaceae), *Terminalia chebula* (Combretaceae), roots of *Plumbago zeylanica* (Plumbaginaceae) and rock salt. The different concentrations of alcoholic extract of Siddha formulation, showed maximum activity against *Pseudomonas aeruginosa* and *Salmonella typhi*, moderate to maximum against *Staphylococcus aureus* but mild to moderate activity against *Proteus vulgaris* and *E. coli*⁴¹. The antimicrobial activity of *P. longum* extracts has been evaluated *in vitro* against two gram positive bacteria stains such as *Streptococcus faecalis*, *Streptococcus pyogenes* and two gram negative bacteria such as *E. coli* and *Salmonella paratyphi*. The gram negative bacteria *E. coli* showed maximum inhibition zone in ethanol extract of root (22 mm) where *Salmonella paratyphi* showed maximum inhibition zone in ethanol extract of stem (19 mm). The gram positive bacteria *Streptococcus faecalis* and *Streptococcus pyogenes* showed maximum inhibition zone in both the ethanol extracts of stem and fruit (18 mm, 20 mm resp.). *Piper longum* was reported as a strong antibacterial against *B. cereus* and *E. coli* which is traditionally used for chronic bronchitis and asthma⁴². An ethyl acetate fraction of Pippali extract was found active against *M. smegmatis* (3000 µg ml⁻¹) and *M. tuberculosis* (39 µg ml⁻¹). It also shows very significant activity against other bacterial strains like *E. coli* (152 µg ml⁻¹), *Staphylococcus aureus* (14 µg ml⁻¹), *Salmonella typhi* (180 µg ml⁻¹), *Enterococcus faecalis* (15 µg ml⁻¹), and

Pseudomonas aeruginosa (52 µg ml⁻¹)⁴³. Dry root of the plant *Piper longum* were extracted with n-hexane. The constituents were isolated and purified by column chromatography. The isolated constituents were found to show the better activity profile than the n-hexane extracts, which indicates that the isolated constituents might be responsible for the antibacterial activity. The minimum inhibitory concentration (MIC) value of piperine against *Bacillus cereus* and *Escherichia coli* was found to be 12.5 mg/ ml⁴⁴. Aqueous and methanol extracts of *Piper longum* (fruits) were tested against multiple drug resistant clinical isolates of *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella typhi*, *Shigella sonnei*, as well as reference-plasmid-harboring strains of *E. coli* and *Bacillus subtilis*. The crude methanol extract showed significant antibacterial activity with a minimal inhibitory concentration of 400 µg/mL against *Bacillus subtilis*. Methanol extract could reverse the antibiotic resistance in clinical isolates of *Shigella sonnei*, with a curing efficiency of 42 %. Aqueous extract showed strong antibiotic resistance reversal activities against R-plasmid harboring strains of clinical origin- *Enterococcus faecalis*, *Staphylococcus aureus*, *Salmonella typhi* with curing efficiencies of 64 %, 50 % and 32 % respectively⁴⁵. Methanol extracts of stem and leaves of *Piper longum* showed strong activities against gram-positive *Streptococcus b- haemolyticus* (18 mm). During the screening work, it was found that the ethyl acetate extract of root displayed highest activity against *Sarcina lutea* (22 mm) and *Shigella sonnei* (21 mm)⁴⁶. The screening of antimicrobial and anti tumor effects of *Piper longum* showed the better efficacy of these two extracts. Among the three bacteria tested, hot ethyl acetate extract showed more potency against *E. coli* than *Bacillus subtilis* and was found to be less active for *Staphylococcus aureus*. The antioxidant, antimicrobial and anti tumor effects were found to be higher for hot extracts than cold extracts⁴⁷. Twenty eight extracts prepared from the fruits of four species, viz. *Piper cubeba* Linn. f, *P. retrofractum* Vahl syn. *P. chaba* Hunter non Blume, *Piper longum* Linn and *P. nigrum* Linn were evaluated against bacterial pathogens, such as *Staphylococcus albus*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Bacillus megaterium* and one fungus *Aspergillus niger*. Compared to Streptomycin all the extracts exhibited a good antibacterial activity. Some of the extracts showed antifungal activity as well⁴⁸. Antibacterial activity of piperlongumine was evaluated against 18 clinically isolated strains, including identified strains belongs to *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. The antibacterial activity was more pronounced against *Klebsiella pneumonia* (24.00 ± 0.12 mm) and *Pseudomonas aeruginosa* (20.00 ± 0.12 mm) while it was moderate on *Staphylococcus aureus* (16.47 ± 0.18 mm). The isolated constituent was found to show better activity profile, which indicates that the isolated constituent might be responsible for the antibacterial activity⁴⁹.

DISCUSSION

Pathogenic microorganism is of the most common cause for epidemic around the world including India. There are so many bacterial disease which increases mortality and morbidity of human beings. In Ayurveda infectious disease and its epidemic has been discussed under the heading of Aoupsargic Vyadhi and Janpadodhvans respectively. Aagantuj is one of the causes of most of the disease like Jwar, Atisar which is also considered to be infectious. Till 19th century the direct reference of bacteria has not found in any text of Ayurveda. Jeevanu means the bacteria has been described by Gannath Sen first time. So many herbal, mineral and herbomineral preparations has been found in Ayurvedic text for such type of infectious pathology and Anand Bhairav Ras (Atisar) is one of them. Anand Bhairav Ras (Atisar) is described in almost all the text book of Ayurveda belonging to Rasshastra in Atisar Chikitsa Adhyay. It has the ingredient of Suddha Hingula (Cinnabar), Suddha Vatsnabh (*Aconitum ferox*), Suddha Tankan (Borex), Marich (*Piper nigrum*) and Pipali (*Piper longum*). Ayurveda suggested that it should be taken with *Holarrhena antidysenterica* (kutaj) powder for diarrhoea (atisar). Sushruta stated that the medicines or diet having Tikt, Katu Rasatmak properties are beneficial in disease caused by pathogenic microorganism⁵⁰. Charak also stated that Tikt and Katu Rasatmak medicines will be useful in the disease produced by systemic or local microorganism⁵¹. As most of the ingredient of Anand Bhairav Ras (Atisar) have Katu Ras, Ushna Virya and Laghu, Ruksha Gunas in properties with some exception so it can act as antimicrobial agent. As Vatsnabh have property of Yogvahi and Vikasi when treated it will help to distribute the drug in Sukshma Strotas (cellular level). Charak stated that Prakruti Vighat is a unique medicament used for disease produced by microorganism. In Prakruti Vighat, drugs having Katu, Tikt Ras, Ushna Virya and Gun and Katu Vipak etc. are utilized to decrease the kleda (wetness) produced by microorganism, hence growth of microorganism is inhibited⁵². There are so many herbal, mineral and herbomineral Ayurvedic preparations which have potent antimicrobial properties. Antibacterial activity of Shila Sindura is confirmed against *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus mutans* and *Candida albicans* as substantiated from both the methods i.e. Gradient plate technique and Kirby bauer method⁵³. Though *in vivo* and *in vitro* antimicrobial properties of ABR is not studied till date. The antimicrobial properties of individual ingredient of it have been studied in detail and showed significant result. Antimicrobial properties of Pipali have been studied more than any other ingredient of ABR while antimicrobial property of Vatsnabh is not studied till date. Most sensitive bacteria among these studies are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Again it is found that its ingredient is more sensitive in enterogenic pathogens than any other bacteria. Thus ABR and its ingredient have been found potent in antimicrobial property *in vitro* studies.

CONCLUSION

Anand Bhairav Ras can be used in infectious disease specially caused by enterogenic pathogen.

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