ORIGINAL ARTICLE

In vitro antimicrobial activity of ingredients of *Balchaturbhadra Yoga*

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Abstract

Introduction: *Balchaturbhadra Yoga* contains four ingredients, namely, *Pippali*, *Karkatshringi*, *Musta*, and *Ativisha*. In this study, the attempt was made to evaluate the antimicrobial activity of various solvent extracts of contents of *Balchaturbhadra Yoga* against different gram positive and gram negative bacteria. **Materials and Methods:** Using disk-diffusion method and single-dilution method, sensitivity of *Escherichia coli* ATCC 25992, *Enterococcus faecalis,* and *Vibrio cholera* foralcoholic as well as aqueous extract of *Pippali* and *Karkatshringi* was observed and minimum inhibitory concentration (MIC) was determined. **Result:** *E. coli* ATCC 25992 was sensitive to alcoholic as well as aqueous extract of *Pippali* and *Karkatshringi*. *E. faecalis* was sensitive to aqueous extract of *Karkatshringi*. *V. cholerae* was sensitive to both extract of *Karkatshringi*. MIC value of alcoholic extract of *Pippali* for *E. coli* was 312 µg/ml and MIC value of water extract of *karkatshringi* for *E. coli* was 625 µg/ml. MIC value of water extract of *Karkatshringi* for *E. faecalis* was 312 µg/ml and MIC values of aqueous as well as alcoholic extract of *Karkatshringi* for *V. cholera* were 125 µg/ml each. While antimicrobial activity of *Musta* and *Ativisha* were not found. **Conclusion:** In the present antimicrobial study of ingredients of *Balchaturbhadra Yoga,* the *Pippali* and *Karkatshringi* showed promising antimicrobial activity against *E. coli, E. faecalis,* and *V. cholerae.*

Key words: Antibacterial activity, *Atisara*, diarrhea, *Balchaturbhadra*

INTRODUCTION

Diarrhea is the third most common cause of death in under-five children, responsible for 13% deaths in this age-group, killing an estimated 300,000 children in India each year.[1] Infectious diarrhea is considered the second most common cause of morbidity and mortality worldwide.[2] Global annual burden of diarrhea is huge affecting 3-5 billion cases and causing approximately 2 million deaths a year,[3] most of these being in the developing world.[4] Overall, the prevalence being significantly higher in children below 2 years as compared to those 2-5 years.[5] Mostly, acute diarrhea is infectious in origin in childhood. Bacterial pathogens were identified in the majority of patients in developing countries. Etiological spectrum varies during different seasons and different geographic settings. Diarrheal disease remains an important cause of death and morbidity in developing countries with an estimated 1.5 million episodes and 1.5 million to 2.5 million deaths each year among children younger than 5 years.[6] It still continues to be a major cause of hospitalization and death in <5 years old children and has severe economic consequences.[7] It is also a major contributory factor in childhood malnutrition. The two most important consequences of diarrhea in children are malnutrition and dehydration. Malnutrition and diarrhea form a vicious cycle since malnutrition increases the risk and severity of diarrhea.[3] Mostly, acute diarrhea is infectious in origin in childhood. Bacterial pathogens were identified in majority of patients in developing countries. In the developed countries, it is estimated that over 50% of acute diarrhea are caused by viruses including *Rotavirus*, *Norwalk* virus, and *Corona* virus. Human *Rotavirus* is most important etiological agents of acquired diarrhea in infants and young children worldwide.[8] The bacterial agent that are known to

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cause diarrhea is *Escherishia coli* (20% of all cases of acute diarrhea are due to *E. coli, V. cholerae* (Cholera accounts for 5-10% cases), *Clostridium difficile*, *Shigella*, *Salmonella* (3-7% of childhood diarrhea), *Campylobacter,* and *Yersinia enterocolitica*.[3]

Microorganisms have developed resistance to many antibiotics, and this has created immense clinical problem in the treatment of infectious disease (Davis-`1994). This resistance has increased due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious disease. This situation has forced scientists to search for new antimicrobial substances molecules from various sources, such as medicinal plants (Karaman *et al*., 2003). Infection is considered as one of the main factors responsible for Diarrhea in children. Secondary metabolites produced by plants constitute a source of bioactive substances and nowadays the scientific interest has increased for new drugs of plant origin. The present study was conducted to investigate antimicrobial properties of the *Musta*, *Pippali*, *Ativisha,* and *Karkatshringi*. All these drugs have been described to have anti-diarrheal properties and antimicrobial properties in different texts.

MATERIALS AND METHODS

Plant Materials

The fruits of *Piper longum* L., rhizome of *Cyperus rotundus* L*,* root of *Aconitum heterophyllum* and galls of *Pistacia integerrima* were collected from Haridwar, Uttarakhand. The plant was identified and authenticated by the Professor N. K. Dubey, Department of Botany, Banaras Hindu University, Varanasi, with the voucher specimen no:

1. *P. integerrima* Stewart ex Brandis- Anacard. 2014/1
2. *C. rotundus* L.- Cyper 2014/1
3. *P. longum* L. - Piper 2014/1
4. *A. heterophyllum* Wall. Cat.- Ranun 2014/1.

Preparation of Extracts

For the study, dry extract of each drug was prepared in the laboratory of the Department of Medicinal Chemistry, IMS BHU. Aqueous extract of drugs was prepared by water decoction method, and alcoholic extract was prepared by Soxhlet method of extraction. Both the extracts were collected in separate sterile vials and preserved at 4°C temperature.

Test Organisms

E. coli ATCC 25992., *Enterococcus faecalis*, *Pseudomonas aeruginosa* TCC 10662., *Salmonella* Typhi, *Staphylococcus aureus*, *Salmonella paratyphi* A., *S. Paratyphi* B, *S. Typhimurium*, *V. cholerae* 01 classical were obtained from the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi, India.

Screening for Anti-bacterial

Antimicrobial susceptibility testing was carried out by dilution method, diffusion method. Diffusion method was done by two methods, Stoke's method and Kirby-Bauer method. In routine laboratory-modified Kirby-Bauer method was used as suggested by NCCLS (National Committee for Clinical Laboratory Services), USA, 2000.

Material Required for Antimicrobial Study and Minimum Inhibitory Concentration (MIC) Determination

Mueller-Hinton agar (pH 7.2 to 7.4), aqueous as well as alcoholic extract of *Musta, Pippali Ativisha,* and *Karkatshringi*, Luria-Bertani broth (pH 7.2), Sterile distilled water.

Procedures

M.H.A. plate taken and particular organism grown on plate, bacterial lawn made on plate (0.5 OD) (1.5×10^8 cfu/ml). Aqueous and alcoholic extract prepared as 80mg dry extract dissolved in 1 ml of sterile water and methanol, respectively. For alcoholic extract to be placed on the plate, disk-diffusion method used and for aqueous extract, serial double dilution method was used. Then, incubated overnight at 37°C.

Determination of MIC

The MIC of active extracts was determined by tube dilution method. Successive tubes filled with 15 ml nutrient broth containing 1000 µg/ml, 500 µg/ml, and 250 µg/ml up to 31.75 µg/ml respective concentrations of extracts were inoculated with 100 µl of the bacterial suspension containing 108 CFU/ml of respective test organisms. The tubes were incubated at 37°C in an incubator and observed for change in turbidity after 24 h. A tube containing nutrient broth without extract was taken as control. The least extract concentration which inhibited the growth of the test organisms was taken as MIC.

OBSERVATIONS AND RESULTS

After incubation, the inhibition was observed on *E. coli* ATCC 25992, *V. cholerae* 01 Classical, and *E. faecalis*.

E. coli ATCC 25992 was sensitive to alcoholic as well as aqueous extract of *Pippali* and *Karkatshringi* [Plate 1].

E. faecalis was sensitive to aqueous extract of *Karkatshringi* [Plate 2].

V. cholerae was sensitive to both extract of *Karkatshringi* [Plate 3].

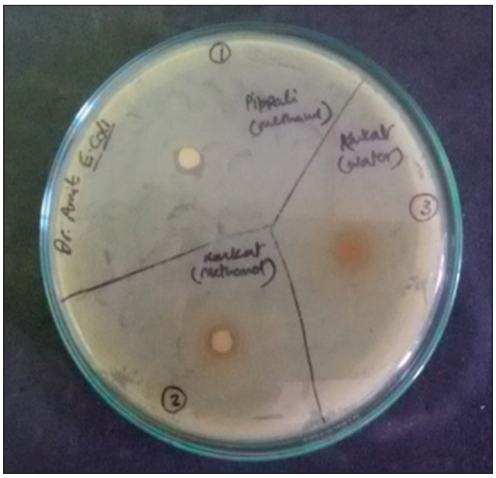

Plate 1: Sensitivity of *E. coli* ATCC 25992

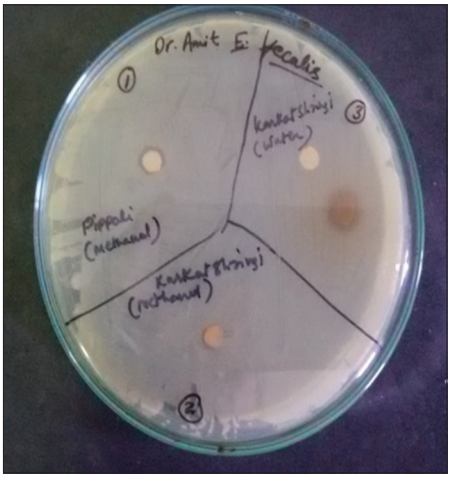

Plate 2: Sensitivity of *E. faecalis*

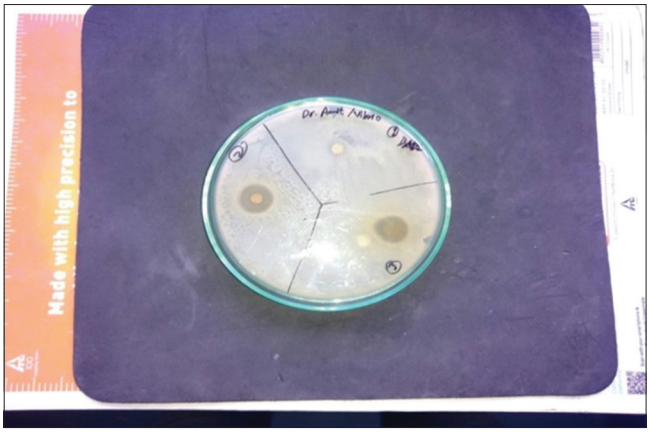

Plate 3: Sensitivity of *V. cholerae*

It may be possible that the alcoholic extracts of these drugs can show inhibitory effect on higher concentration or it may be possible that these drugs may show inhibitory effect.

Table 1 shows MIC value of alcoholic extract *Pippali* for *E. coli* is 312 µg/ml and MIC value of water extract of *karkatshringi* for *E. coli* is 625 µg/ml. MIC value of water extract of *Karkatshringi* for *E. faecalis* is 312 µg/ml and MIC values of aqueous as well as alcoholic extract for *V. cholerae* are 125 µg/ml each.

DISCUSSION

Among different bacteria included in the study some enteropathogens, i.e., *E. coli* ATCC 25992, *E. faecalis,* and *V. cholerae* were inhibited by *Pippali* and *Karkatshringi*. The MICs of alcoholic extract for *E. coli* were 312 µg/ml and 625 µg/ml, respectively. The MIC of water extract of *Karkatshringi* for *E. faecalis* was 312 µg/ml and MICs of aqueous as well as alcoholic extract of *Karkatshringi* for *V. cholerae* was 125 µg/ml. The Above finding suggests that out of four ingredients of *Balchaturbhadra Yoga, Pippali,* and *Karkatshringi* showed promising antimicrobial activity against *E. coli, E. faecalis,* and *V. cholerae.*

Although few studies have been carried out to study the antimicrobial effect of different components of the recipe, these studies have not covered the whole spectrum of bacteria, parasites, fungi, and viruses implicated in diarrhea disorders.

Antimicrobial study of '*Balchaturbhadradi Yoga'* was previously done by *Nirajet. al* at BHU in 2007.[9] That study showed that *E. coli* ATCC 25992 and *S. aureus* were sensitive to *Pippali*. The MIC value for *E. coli* was 80 µg/ml and MIC value for *S. aureus* was 40 µg/ml. *V. cholerae* was sensitive to *Karkatshringi* and inhibitory effect for *V. cholerae* was started from 40 mg/ml to minimum of 320 µg/ml (MIC). The present study provokes to explore further the antimicrobial effect of each and every component on the spectrum of the enteropathogens including bacteria, parasites, fungi, and viruses, only then exact resolution can be drawn to label them as specific antimicrobial agents.

CONCLUSION

In the present antimicrobial study of ingredients of *Balchaturbhadra Yoga,* the *Pippali* and *Karkatshringi*

Table 1: MIC values of various extracts

Sensitive bacteria	Drug extract	MIC value
Escherichia coli	*Pippali* (alcoholic extract)	312 µg/ml
	Karkatshringi (alcoholic extract)	625 µg/ml
Enterococcus faecalis	*Karkatshringi* (aqueous extract)	312 µg/ml
Vibrio cholera	*Karkatshringi* (aqueous as well as alcoholic extract)	125 µg/ml each

MIC: Minimum inhibitory concentration, *V. cholera: Vibrio cholera, E. faecalis: Enterococcus faecalis, E. coli: Escherichia coli*

showed promising antimicrobial activity against *E. coli, E. faecalis* and *V. cholerae.*

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