

In Vitro Antimicrobial and Phytochemical Study of Plant Extract

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ABSTRACT

Background and Aim: Micro-organisms have developed resistance to many antibiotics and this have created immense clinical problem in the treatment of infectious disease. This resistance has increased due to indiscriminate use of commercial anti-microbial drugs commonly used in the treatment of infectious disease. Present study was conducted to investigate antibacterial properties as well as phytochemical study of extract of the *Musta*, *Pippali*, *Ativisha* and *Karkatshringi*.

Material and method- Methanolic extract of each drug was collected in separate sterile vial and preserved at temperature 4°C. Kirby-Bauer method was used as suggested by NCCLS (National Committee for Clinical Laboratory Services), USA, 2000 for antimicrobial study. For phytochemical study the dried samples were re-suspended in HPLC grade Methanol for HPLC analysis. Result- Six phenolic acids were detected in the four plant samples. The MIC of active extracts was determined by tube dilution method. After incubation the inhibition was observed on *Escherichia coli* ATCC 25992, *V. cholerae* 01 Classical and *Enterococcus faecalis*. *Escherichia coli* ATCC 25992 was sensitive to alcoholic as well as aqueous extract of *Pippali*.

Conclusion:- it means these plant extracts having high antioxidant property and antibacterial effect as well.

Key Words: Plant extract, Micro-organisms, Anti-microbial, Phytochemical.

INTRODUCTION

Bacterial pathogens were identified in majority of patients in developing countries. Etiological spectrum varies during different seasons and different geographic settings. 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 world-wide. [1] The bacterial agent that are known to cause diarrhea is *Escherichia 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*. [2]

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. [3] Infectious diarrhea is considered the second most common cause of morbidity and mortality worldwide. [4] Global annual burden of diarrhea is huge affecting 3-5 billion cases and causing approximately 2 million deaths a year. [5] Overall, the prevalence being significantly higher in children below 2 years as compared to those 2-5 years. [6] 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. [7] It still continues to be a major cause of hospitalization and death in <5 years old children and has severe economic consequences. [8]

Aim of study: Present study was conducted to investigate antibacterial properties as well as phytochemical study of extract of the *Musta*, *Pippali*, *Ativisha* and *Karkatshringi*. All these drugs have been described to have anti-diarrheal properties and anti-microbial properties in different texts. Present in-vitro study was initiated to evaluate the efficacy of these drugs on different pathogens causing diarrhea which may help in ascertaining the mode of action as well as further specific use of the present combination in future in vivo studies.

MATERIALS AND METHODS

Plant Materials: For this study 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.

Phytochemical Study: The phytochemical study was carried out in department of Mycology and plant Pathology, Institute of Agricultural sciences, BHU Varanasi. The dried samples were re-suspended in HPLC grade methanol for HPLC analysis. Shimadzu LC-10A (Japan) was used that was equipped with dual pump LC-10A binary system, UV detector SPD-10A, Phenomenex (Torrance, USA) C18 column (RP-Hydro, 4 µm, 250mm× 4.6mm). Shimadzu Class VP series software was used to integrate the data. Separation of phenolics was achieved with acetonitrile/water (1:1 v/v) containing 1% acetic acid in a linear gradient program (Singh et al., 2009). The solvent flow rate was 1.0 ml min⁻¹.

Anti-microbial Study: Anti-microbial susceptibility testing was carried out in department of Microbiology, Institute of Medical Sciences, BHU Varanasi, by-Dilution method and Diffusion method. Diffusion method was done by two methods 1. Stoke's method and 2. Kirby-Bauer method. In routine laboratory modified Kirby-Bauer method was used as suggested by NCCLS (National Committee for Clinical Laboratory Services), USA, 2000.

Test Micro-organisms: For antimicrobial study, *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.

Procedures:

1. M.H.A. plate taken and particular organism grown on plate:

- Bacterial Lawn made on plate (0.5 OD)(1.5×10⁸ cfu/ml)
- Aqueous and alcoholic extract prepared as; 80mg dry extract dissolved in 1ml of sterile water and methanol respectively.

- For alcoholic extract to be placed on the plate, disc diffusion method used and for aqueous extract serial double dilution method was used.
- Then incubated over night at 37⁰C.

Determination of minimum inhibitory concentration (MIC): The MIC of active extracts was determined by tube dilution method. Successive tubes filled with 15 ml nutrient broth containing 1000µg/ml, 500µg/ml, and 250µg/ml up to 31.75µg/ml

respective concentrations of extracts were inoculated with 100µl 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 RESULT

Phytochemical Study: Concentration of the phenolic compounds in the four plants is as follows

Table 1.

Sr. no.	Phenolic compounds	Musta	Pippali	Ativisha	Karkatshringi
1.	Shikimic acid	140µg/ml	1068µg/ml	498.5 µg/ml	-
2.	Gallic acid	31.9µg/ml	265µg/ml	-	-
3.	Syringic acid	-	4.5µg/ml	-	38.6 µg/ml
4.	Quercetin	8.3µg/ml	-	-	-
5.	Cinnamic acid	10.9µg/ml	1.4 µg/ml	-	22.5 µg/ml
6.	IAA	46.5µg/ml	92.8 µg/ml	-	-

Table 1 shows six phenolic acids were detected in the four plant samples. Out of the 6 phenolic standards used, *Ativisha* contained only one phenolic compound i.e. Shikimic acid whereas *Musta* and *Pippali* contained 5 compounds as shown in the table. Syringic acid was not detected in *Musta* and Quercetin was not detected in *Pippali*. Only two phenolic compounds i. e. Syringic and Cinnamic acids were detected in *Karkatshringi*.

Escherichia coli ATCC 25992, *V. cholerae* 01 Classical and *Enterococcus faecalis*. *Escherichia coli* ATCC 25992 was sensitive to alcoholic as well as aqueous extract of *Pippali* (Plate 1) and *Karkatshringi*. *E. faecalis* was sensitive to aqueous extract of *Karkatshringi* (Plate 2). *Vibrio cholerae* was sensitive to both extract of *Karkatshringi* (Plate 3). It may be possible that these alcoholic extracts of these drugs can show inhibitory effect on higher concentration or it may be possible that these drug my show inhibitory effect.

Antimicrobial Study: After incubation the inhibition was observed on

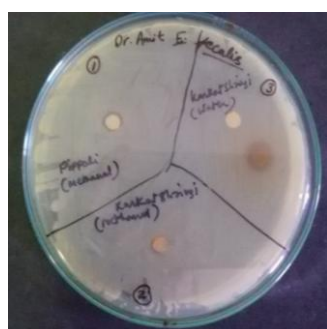


Plate -1

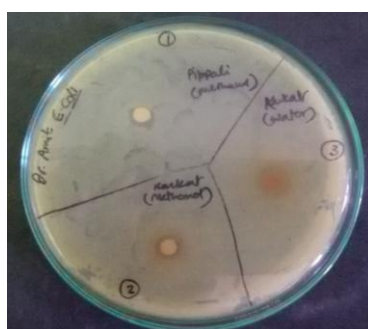


Plate -2

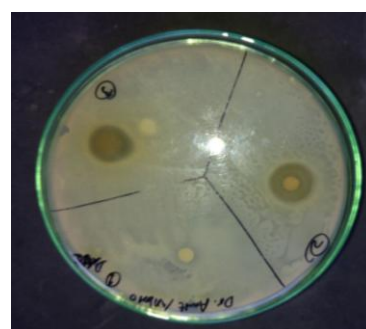


Plate -3

Table no: 2

	Sensitive Bacteria	Drug extract	MIC Value
1.	<i>E. coli</i>	a. <i>Pippali</i> (alcoholic extract) b. <i>Karkatshringi</i> (alcoholic extract)	a. 312µg/ml b. 625µg/ml
2	<i>E. faecalis</i>	<i>Karkatshringi</i> (aqueous extract)	312µg/ml
3.	<i>Vibrio cholera</i>	<i>Karkatshringi</i> (aqueous as well as alcoholic extract)	125µg/ml each.

Table no. 2 shows, MIC value of alcoholic extract *Pippali* for *E. coli* is 312µg/ml and MIC value of water extract of *karkatshringi* for *E. coli* is 625µg/ml. MIC value of water extract of *Karkatshringi* for *E. faecalis* is 312µg/ml and MIC values of aqueous as well as alcoholic extract for *Vibrio cholerae* are 125µg/ml each.

In the pictures provided, plate number 1 is for alcoholic extract of *Pippali*, plate number 2 for alcoholic extract of *Karkatshringi* and plate number 3 for aqueous extract of *Karkatshringi*.

DISCUSSION

Antimicrobial study of *Balchatur bhadradi Yoga* was previously done by Niraj *et al* at BHU in 2007. That study showed *Escherichia coli* ATCC 25992 and *Staphylococcus aureus* was sensitive to *Pippali*, MIC value for *E. coli* was 80µg/ml and MIC value for *Staphylococcus aureus* was 40µg/ml. *Vibrio cholerae* was sensitive to *Karkatshringi* and inhibitory effect for *Vibrio cholerae* was started from 40mg/ml to minimum of 320µg/ml (MIC).^[9]

In the present study antimicrobial study showed that *Escherichia coli* ATCC 25992 was sensitive to alcoholic as well as aqueous extract of *Pippali* and *Karkatshringi*. *Vibrio cholerae* was sensitive to both extract of *Karkatshringi*. *E. faecalis* was sensitive to aqueous extract of *Karkatshringi*. It may be possible that these alcoholic extracts of these drugs can show inhibitory effect on higher concentration or it may be possible that these drugs may show inhibitory effect. MIC value of alcoholic extract of *Pippali* for *E. coli* was 312µg/ml and MIC value of water extract of *Karkatshringi* was 625µg/ml. MIC value of *Karkatshringi* for *E. faecalis* is 312µg/ml and for *Vibrio cholerae* was 125µg/ml.

In phytochemical study, Out of the 6 phenolic standards used, *Ativisha* contained only one phenolic compound i.e. Shikimic acid where as *Musta* and *Pippali* contained 5 compounds as shown in the table 1. Only two phenolic compounds i.e. Syringic and

Cinnamic acids were detected in *Karkatshringi*. The beneficial effects derived from phenolics compounds have been attributed to their antioxidant activity.^[9] It means *balchatur bhadradi yoga* have high antioxidant property.^[10]

CONCLUSION

Six phenolic acids were detected in the four plant samples. Out of the 6 phenolic standards used, *Ativisha* contained only one phenolic compound i.e. Shikimic acid whereas *Musta* and *Pippali* contained 5 compounds as shown in the table 1. Syringic acid was not detected in *Musta* and Quercetin was not detected in *Pippali*. Only two phenolic compounds i. e. Syringic and Cinnamic acids were detected in *Karkatshringi*.

Out of four test drugs, *Escherichia coli* ATCC 25992 were sensitive to alcoholic as well as aqueous extract of *Pippali* and *Karkatshringi*. *Vibrio cholerae* was found sensitive to both extract of *Karkatshringi*. *E. faecalis* was sensitive to aqueous extract of *Karkatshringi*. The present study has revealed encouraging results but study needs further evaluation in large sample size.

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