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Asian Journal of Pharmaceutical Education and Research

Vol -5, Issue-3, July-September 2016 **SJIF Impact Factor 4.101**

ISSN: 2278-7496

RESEARCH ARTICLE

COMPARATIVE IN-VITRO ANTIMICROBIAL ACTIVITY OF HYDROALCOHOLIC EXTRACT OF *EMBELIA RIBES (ERE) AND ZIZIPHUS XYLOPYRUS* (ZRE)

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Article Received on 21/05/2016

Revised on 18/06/2016

Accepted on 22/06/2016

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ABSTRACT:

In the present paper we have focus on the worldwide use of traditional plants for the treatment of infectious diseases caused by microorganisms. In early twentieth century, the discovery of antibiotics provide a tool against the microbial infections but due to multiple drug resistance and adverse effects on host including hypersensitivity, immune suppression and allergic reactions shown by antibiotics creates a need of natural medicines with safe and better therapeutic effect. In present work, Hydroalcoholic extracts of *Embelia ribes* and *Ziziphus xylopyrus* shows antibacterial activity against *Escherichia coli* with zone of inhibition lying in the range of 10 to 17 mm and 11 to 19 mm respectively and against *Candida albicans* with zone of inhibition lying in the range of 8 to 12 mm and 12 to 20 mm respectively. Zone of inhibition of extracts were compared with that of different standards like Ciprofloxacin for antibacterial activity and fluconazole for antifungal activity. The results showed that the remarkable inhibition of the bacterial growth was shown against the tested organisms. Hence, these plants can be used to discover bioactive natural products that may serve as leads in the development of new pharmaceuticals research activities.

Keywords: Antimicrobial activity, Hydroalcoholic, *Embelia ribes, Ziziphus xylopyrus*

INTRODUCTION:

A herb is a plant or part of a plant valued for its medicinal, aromatic, or savoury qualities. Herbs can be viewed as biosynthetic chemical laboratories, producing a number of chemical compounds. Herbal remedies or medicines consist of portions of plants or unpurified plant extracts containing several constituents, which often work together synergistically. Herbal medicine or herbalism is the use of herbs or herbal products for their therapeutic or medicinal value. The use of medicinal plants as a source for relief from illness can be traced back over five mellenia to written documents of the early civilization in China, India and the north east, but it is thoughtless as art as old as mankind.[1] Among the estimated 250'000- 500,000 plant species, only a small percentage have been investigated phytochemically and the fraction submitted to biological or pharmacological screening. Compound of natural or synthetic origin has been the source of innumerable therapeutic agents. [2,3]

Microorganisms have potential to cause diseases. Human body is very prone to viral, bacterial and fungal infections. The discovery of antibiotics in the early twentieth century provided an increasingly important tool to combat bacterial diseases. But due to the indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases multiple drug resistance has been developed.[4] In addition to this problem, antibiotics are sometimes associated with adverse effects on the host including hypersensitivity, immune suppression and allergic reactions.[5]

Embelia ribes Burm. is a threatened woody shrub belongs to the family Myrsinaceae, which is sparsely distributed in the moist deciduous forests of the Western Ghats, India, SriLanka,Malaysia and South China.[6] The fruit is bitter in taste, good appetizer, cures tumors, ascites, bronchitis, jaundice and mental disorders . Seeds are used as antibiotic, anthelmintic, antituber-culosis, alterative and stimulative.[7] The whole plant is used in the treatment of anti-inflammatory to relive rheumatism and fever.[8] E. ribes grows in semi-evergreen and deciduous forests at an altitude of 1,500 m, throughout India. It is considered to be vulnerable due to excessive harvesting, because of its many uses (it is used in 75 ayurvedic preparations).

Ziziphus Xylopyrus (family Rhamnaceae) is such a plant which is commonly found in various parts of north-western India, Uttar Pradesh, Bihar and Central and South India. As per the ethnomedicinal information, various parts of this plant possess several medicinal

properties. The fruit powder with pinch ginger powder thrice in a day is useful for stomachache and indigestion. It also possess antidepressant, antimicrobial and anthelmintic activities as per the available information. [9, 10]

MATERIALS AND METHODS

Preparation of extract

The seed and fruit of *Embelia ribes* and *Ziziphus xylopyrus* were crushed and air dried at room temperature. The dried Fruit were coarsely powdered and successfully extracted with ethanol (80%) using Soxhlet extractor at a temperature of 55-60 ^{0}C for a period of 72 hrs. The solvents was distilled off at lower temperature under reduced pressure and concentrated to dryness (crude extract). The dried extract was weighed and then stored in a freezer. The crude extract was used for the experiments.

Phytochemical Studies

The extracts were subjected to phytochemical screening tests for the detection of various constituents using conventional protocol.[11]

ANTIMICROBIAL ACTIVITY

Pathogenic fungus used

The pathogenic fungus used in the current study is obtained from Microbial Culture collection, National Centre for cell science, Pune, Maharashtra, India.

Media preparation (broth and agar media)

Composition of nutrient agar media;

Potatoes extract	- 200gms
Dextrose	- 20 gms
Agar	-15 gms
Distilled Water	-to make 1000ml
pH (at 25°C)	- 5.6±0.2

Method of preparation

This agar medium was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients are transferred to flask containing required quantity of distilled water and heat to dissolve the medium completely.

Sterilization culture media

The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch^2 (121^{o}C) for 15 minutes.

Preparation of plates

After sterilization, the molten agar in flask was immediately poured (20 ml/ plate) into sterile Petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37^{o}C overnight to check the sterility of plates. The plates were dried at 50^{o}C for 30 minutes before use.

Revival of the microbial cultures

The microbial cultures used in the study were obtained in lyophilized form. With the help aseptic techniques the lyophilized cultures are inoculated in sterile nutrient broth and potato dextrose broth for fungus than incubated for 24 hours at 37^{o}C. After incubation the growth is observed in the form of turbidity. These broth cultures were further inoculated on to the nutrient agar and potato dextrose agar plates with loop full of bacteria and further incubated for next 24 hours at 37^{o}C to obtain the pure culture and stored as stocks that are to be used in further research work.

Antimicrobial sensitivity

The antimicrobial sensitivity test is employed on to the fungus used under present study with the anti fungal gel formulation. For this experiment 6 mm diameter Whatman filter paper discs were impregnated with stock of 100 mg/ml then dried in aseptic conditions.[12] A nutrient agar plate is seeded with particular bacteria and potato dextrose agar plat with particular fungus with the help of spread plate technique prior and left for 5 minutes. Now the drug impregnated filter paper discs were place in the center of preinoculated culture plates then incubated for 24 hours at 37^{o}C. After incubation, plates were observed to see the sensitivity of extracts towards test bacteriums at particular concentration in the form zone of inhibition.

Antibiogram Studies

Broth cultures of the pure culture isolates of microorganisms *E. coli* and *candida albicans* which are sensitive towards the 25 mg/ml concentration of extract used in present study were prepared by transferring a loop of culture into sterile nutrient broth and incubated at 37^{o}C for 24-48hours. A loop full was taken from these broths and seeded onto sterile nutrient agar and potato dextrose agar plates through sterile cotton swab to develop diffused heavy lawn culture.

The well diffusion method was used to determine the antimicrobial activity OF Hydroalcoholic extract of *Embelia ribes (ERE) and Ziziphus xylopyrus* (ZRE) using standard procedure. There were 3 concentration used which are 25, 50 and 100 mg/ml for both the extract. The plates were incubated at 37^{o}C for 24 hr. and then examined for clear zones of inhibition around the discs impregnated with particular concentration of drug.

Antibacterial Activity of Hydroalcoholic extract of *embelia ribes (ERE) and ziziphus xylopyrus* (ZRE)

Microbial Cultures

For the studies of antimicrobial effect of one antibacterial and one antifungal used. Microbial strains procured from Microbial Culture collection, National Centre for cell science, Pune, Maharashtra, India. The lyophilized cultures of microbial strain upon culturing in nutrient and potato dextrose broth for 24-48 hours at 37^{o}C in an incubator resulted into turbid suspension of activated live microbial cell ready to be used for microbiological study. From the broth of respective revived cultures of micro organism loop full of inoculum is taken and streaked on to the nutrient and potato dextrose agar medium and incubated again at same culture conditions and duration that yielded the pure culture colonies on to the surface of the agar culture that are successfully stored in refrigerated conditions at 4^{o}C as stock culture to be used for further experimentation.

Antimicrobial Studies

The lawn cultures was prepared with the pathogenic microorganism used under present study and sensitivity of microorganism towards the extract studied at the concentration of 25mg/ml using well diffusion method.

RESULTS AND DISCUSSIONS

Antibiogram studies

The present investigation in this research work, the antimicrobial activity evaluated against bacterial and fungal microbial pathogen used under present study. The gel formulation used to suitably dilute upto the concentrations of 25, 50 and 100 mg per ml and applied on to the test organism using Kirby Bauer filter well diffusion method. Results of the experiment are being concluded in the Table 1 and 2.

Antimicrobial activity on *Escherichia coli*

Escherichia coli was inhibited by the standard antifungal used in present work i.e., Ciprofloxacin, at all the concentration (25, 50 and 100 mg/ml) used in the study for comparison. The resulting zone of inhibition against *Escherichia coli.*

Table 1: Antimicrobial activity of extract on *Escherichia coli*

S. No	Name of drug	Zone of inhibition		
		25mg/ml	50 mg/ml	100mg/ml
1.	Ciprofloxacin	17±0.22	24±0.12	29±0.11
2.	Extract-1	10±0.22	13±0.22	17±0.22
3.	Extarct-2	11±0.12	14±0.10	19±0.20

Extract-1- *Embelia ribes,* Extarct-2- *Ziziphus xylopyrus*

In present work, Hydroalcoholic extracts of *Embelia ribes* and *Ziziphus xylopyrus* shows antibacterial activity against *Escherichia coli* with zone of inhibition lying in the range of 10 to 17 mm and 11 to 19 mm respectively.

Antimicrobial activity on *Candida albicans*

Candida albicans was inhibited by the standard antifungal used in present work i.e., fluconazole, at all the concentration (25, 50 and 100 mg/ml) used in the study for comparison. The resulting zone of inhibition against *Candida albicans.*

Table 2: Antimicrobial activity of extract on *Candida albicans*

S. No	Name of drug	Zone of inhibition		
		25mg/ml	50 mg/ml	100mg/ml
1.	Fluconazole	22±0.22	27±0.12	32±0.11
2.	Extract-1	8±0.12	10±0.14	12±0.14
3.	Extarct-2	12±0.21	15±0.13	20±0.17

Extract-1- *Embelia ribes,* Extarct-2- *Ziziphus xylopyrus*

In present work, Hydroalcoholic extracts of *Embelia ribes* and *Ziziphus xylopyrus* shows antifungal activity against *Candida albicans* with zone of inhibition lying in the range of 8 to 12 mm and 12 to 20 mm respectively.

CONCLUSION

Fungal infections of the skin are one of the often faced with dermatological diseases in worldwide. In present work, Hydroalcoholic extracts of *Embelia ribes* and *Ziziphus xylopyrus* shows antibacterial activity against *Escherichia coli* with zone of inhibition lying in the range of 10 to 17 mm and 11 to 19 mm respectively and against *Candida albicans* with zone of inhibition lying in the range of 8 to 12 mm and 12 to 20 mm respectively. The present results will form the basis for selection of plant species for further investigation in the potential discovery of new natural bioactive compounds. Further studies which aimed at the isolation and structure elucidation of antibacterial active constituents from the plant have been initiated.

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