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Research Article

ANTIMICROBIAL ACTIVITY OF SOLVENT EXTRACTS *OF POLYGONATUM CIRRHIFOLIUM* (MAHAMEDHA) AND *HABENERIA INTERMEDIA* (RIDDHI)

Reetu Sharma*, Ranjit Singh
Dept. of Pharmaceutical Science, Adarsh Vijendra Institute of Pharmaceutical Sciences, Shobhit University, Gangoh Saharanpur, Uttar Pradesh, India
**Corresponding author: ritusharma.pharma43@gmail.com*
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ABSTRACT
Antimicrobial drugs have caused a dramatic change not only of the treatment of infectious diseases but of the mankind as a whole. Antimicrobial chemotherapy made remarkable advances, However, in reality emerging and re-emerging infectious diseases have left us facing a counter charge from infections. Infections with drug resistant organisms remain pertinent problem in clinical practice that is difficult to solv. (In the present study, the antimicrobial activity of the polar (methanolic) plant extracts viz. *Polygonatum cirrhifolium* (Mahamedha) and *Habeneria intermedia* (Riddhi) were determined at 200µg/ml. The results showed significant antimicrobial activity against the pathogens studied viz. *Staphylococcus epidermidis*, *Aspergillus niger* and *Candida albicans*. The potent antimicrobial extracts were having MIC values in the range of 150-200 µg/ml.

Keywords: Solvent extracts, Antimicrobial activity, *Polygonatum cirrhifolium* (Mahamedha), *Habeneria intermedia* (Riddhi), Minimum inhibitory concentration, Skin infecting pathogens.

1. INTRODUCTION
India is called "Botanical Garden of the world" as it is the largest producer of medicinal herbs. India is one of the world's 12 biodiversity centers with the presence of over 45000 different plant species. India's diversity is unmatched due to the presence of 16 different agro-climatic zones, 10 vegetation zones, 25 biotic provinces and 426 biomes (habitats of specific species). Of these, about 15000-20000 plants have good medicinal value. Plants based antimicrobials have enormous therapeutic potential. They are effective in the treatment of infectious diseases while simultaneously mitigating many of the side effects that are often associated with synthetic antimicrobials. In the ethno-pharmacological approach, local knowledge about the potential uses of the plants is very useful as compared to the random approach where indigenous knowledge is not taken into consideration. Compounds inhibiting microorganisms, such as benzoin and emetine have been isolated from plants. Plants are capable of synthesizing an overwhelming variety of low molecular weight organic compounds usually unique and complex in structure. Phytochemicals produced in plants are secondary compounds responsible for metabolic activities and defense in purpose. Phytochemicals are produced by specific biochemical pathways, which occur inside the plant cells. At least 12,000 phytochemicals (secondary metabolites) have been isolated from plants, a number estimated to be less than 10% of the total. Translation of ethnobotanical information for isolation and identification of phytochemicals is required for revealing the pharmacological status of the important phytochemicals [1] In the present study, the antimicrobial activity of polar and non-polar solvent extracts of *Polygonatum cirrhifolium* (Mahamedha) and *Habeneria intermedia* (Riddhi) were studied.

2. MATERIAL AND METHODS
2.1. Preparation of plant extracts
Whole plants of *Polygonatum cirrhifolium* (Mahamedha) and *Habeneria intermedia* (Riddhi) were washed with distilled water, dried under shade and pulverized. The method [2] was adopted for preparation of plant extracts with little modifications. Briefly, 20 g portions of the powdered plant material were soaked separately in

different solvents i.e. hexane, chloroform, methanol, and 50% v/v hydro-alcohol, and water on the basis of increasing polarity for 72 h. Each mixture was stirred every 24 h using a sterile glass rod. At the end of extraction, each solvent was passed through Whatman filter paper No. 1 (Whatman, England). The filtrates obtained were concentrated in vacuo using water bath at 30°C.

3. DETERMINATION OF ANTIMICROBIAL ACTIVITY

3.1. Culture Media

For antibacterial test, Soyabean Casein Digest agar/broth and Sabouraud's dextrose agar/broth of Hi Media Pvt. Bombay, India was used for antifungal test.

3.2. Inoculum

The bacteria were inoculated into Soyabean casein digest broth and incubated at 37°C for 18 h and suspension was checked to provide approximately, 10^5 CFU/ml. The same procedure was done for fungal strains and the strains were inoculated into Sabouraud's dextrose broth but the fungal broth cultures were incubated at 48-72 h.

3.3. Microorganisms used

The pathogenic strains used in the study were *Staphylococcus epidermidis* ATCC 12228) *Candida albicans* (ATCC 10231and *Aspergillus niger* (ATCC 16404 These strains were procured from National Chemical Laboratory (NCL), Pune, Maharashtra, India

3.4. Determination of diameter of zone of inhibition by well diffusion method

The agar well diffusion method (3) was modified. Soyabean Casein Digest agar medium (SCDM) was used for bacterial cultures. The culture medium was inoculated with the bacteria separately suspended in nutrient broth. Sabouraud's dextrose agar/broth was used for fungal cultures. The culture medium was inoculated with the fungus separately suspended in Sabouraud's dextrose broth. A total of 8 mm diameter wells were punched into the agar and filled with plant extracts and solvent blanks. Solvents used were hexane, chloroform, methanol, 50% v/v hydro-alcohol, and water. Standard antibiotic (Azithromycin, 1 mg/ml) was simultaneously used as the positive control. The plates were then incubated at 37°C for 18 h. The antibacterial activity was evaluated by measuring the diameter of zone of inhibition observed. For assaying, antifungal activity of plant extracts, Sabouraud's dextrose agar/ broth medium plates were used. The extracts were prepared in 200µg/mL by taking the crude extracts taken in specific concentration dissolved in sterilized N- saline. The same procedure as that for determination of antibacterial property was adopted and then after the diameter of zone of inhibition was observed after 48-72 h. Fluconazole (1mg/ml) was used as standard for determination of antifungal activity. The procedure for assaying antibacterial and antifungal activity was performed in triplicates to confirm the readings of diameter of zone of inhibition observed for each of the test organism.

3.5. Determination of Minimum Inhibitory Concentration (MIC)

MIC value of potent plant extracts of both the plants viz. *Polygonatum cirrhifolium* (Mahamedha) and Habeneria *intermedia* (Riddhi) was determined by the method adopted [4, 5] with some modifications. Plant extract was prepared in highest concentration (200µg/ml) in sterile distilled water and was serially diluted with N-saline (0.85 % NaCl) and similar quantity of bacterial suspension was added to different test tubes and incubated for 48 h. The inhibition of turbidity appeared in the minimum dose at which total growth of bacteria gets killed is known as minimum lethal concentration (MLC) while little turbidity appeared in the minimum amount of dose of plant extract which inhibits the growth of bacteria is known as Minimum Inhibitory Concentration (MIC).

4. RESULTS AND DISCUSSION

The results of the study showed the significant antimicrobial spectrum of the plant extracts of *Polygonatum cirrhifolium* (Mahamedha) and *Habeneria intermedia* (Riddhi). The antimicrobial activity of the plant extracts was determined at 200µg/ml. The potent antimicrobial extracts were having MIC values in the range of 150-200 µg/ml. The results of antimicrobial activity are shown in Tables 1-4. The results showed significant antimicrobial activity of polar extracts. The results of the antimicrobial activity of *Polygonatum cirrhifolium* and *Habeneria intermedia* corelate with the previous findings [6-8]. The study revealed that, the Astavarga plants extracts are having significant antimicrobial potential against the skin borne pathogens.

Table 1: Antimicrobial activity of solvent extracts of *Mahamedha (Polygonatum cirrhifolium)*

Diameter of Zone of inhibition (mm)			
Plant extracts/Positive Control/Solvent Blanks	**Test organisms**		
	S. epidermidis	***A. niger***	***C. albicans***
Methanolic extract	25.0± 0.022	28.0± 0.018	30.0± 0.015
Hydro-alcoholic extract	22.0± 0.025	25.0± 0.025	15.0± 0.045
Aqueous	18.0± 0.057	13.0± 0.65	15.0± 0.82
Hexane extract	11.0± 0.087	23.0± 0.089	11.0± 2.06
Chloroformic extract	10± 1.56	8.6± 2.05	5.0± 2.23
Positive control Azithromycin (1mg/ml)	38.0± 0.015	NT	NT
Positive Control Fluconazole (1 mg/ml)	NT	36.0± 0.015	32.0± 0.023
Methanol	NA	NA	NA
Hydro-alcohol	NA	NA	NA
Distilled water			
Hexane	NA	NA	NA
Chloroform	NA	NA	NA

**NA, No activity; NT, No Tested; p<0.05 (level of significance)*

Table 2: Antimicrobial activity of solvent extracts of *Riddhi (Habenaria intermedia)*

Diameter of Zone of inhibition (mm)			
Plant extracts/Positive Control/Solvent Blanks	**Test organisms**		
	S. epidermidis	***A. niger***	***C. albicans***
Methanolic extract	28.0± 0.021	18.0± 0.015	25.0± 0.015
Hydro-alcoholic extract	25.0± 0.018	12.0± 0.045	18.0± 0.056
Aqueous	22.0± 0.065	10.0± 0.78	12.0± 1.23
Hexane extract	10.0± 0.082	15.0± 0.089	8.0± 2.56
Chloroformic extract	8.0± 2.08	9.0± 2.56	5.0± 2.23
Positive control Azithromycin (1mg/ml)	38.0± 0.015	NT	NT
Positive Control Fluconazole (1 mg/ml)	NT	36.0± 0.015	32.0± 0.023
Methanol	NA	NA	NA
Hydro-alcohol	NA	NA	NA
Distilled water			
Hexane	NA	NA	NA
Chloroform	NA	NA	NA

**NA, No activity; NT, No Tested; p<0.05 (level of significance)*

Table 3: MIC values of solvent extracts of *Mahamedha (Polygonatum cirrhifolium)*

MIC values (µg/mL)			
Plant extracts/Positive Control/Solvent Blanks	**Test organisms**		
	S. epidermidis	***A. niger***	***C. albicans***
Methanolic extract	150.0	170.0	168.0
Hydro-alcoholic extract	153.0	159.0	170.0
Aqueous	172.0	170.0	170.0

Table 4: MIC values of solvent extracts of *Riddhi (Habenaria intermedia)*

MIC values (µg/mL)			
Plant extracts/Positive Control/Solvent Blanks	**Test organisms**		
	S. epidermidis	***A. niger***	***C. albicans***
Methanolic extract	152.0	165.0	175.0
Hydro-alcoholic extract	175.0	183.0	175.0
Aqueous	180.0	185.0	190.0

5. CONCLUSION

The plants are having medicinal importance as they contain phytochemicals and significant active principles of pharmacological significance. The present study was an attempt to determine the antimicrobial spectrum of Astavarga plants viz. *Polygonatum cirrhifolium* (Mahamedha) and *Habeneria intermedia* (Riddhi). The results concluded that, these plants extract have significant importance in terms of antimicrobial activity against the dreadful pathogens as studied.

Conflict of interests

The authors don't have any conflicts of interest.

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