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Antifungal activity of *Heterostemma tanjorense* (Wight and Arn.)

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ABSTRACTS

Antifungal activity of solvent extract of Heterostemma tanjorense (Wight and Arn.) have been investigated against human pathogenic fungi, such as Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Candida albicans and Fusarium moniliform The various solvents extracts were found to be effective against test organism but the ethyl acetate and ethanol extracts appeared to be most effective antifungal agents as compared to aqueous and chloroform extract. Infectious diseases represent a critical problem to health and they are one of the main causes of morbidity and mortality worldwide. The resistance to antibiotics and with the toxicity during prolonged treatment with several drugs due to this medicinal plants are widely used by the traditional medical practitioners for curing various diseases in their day to day practice. Since ancient times, plants have been an exemplary source of medicine. The presented review summarizes the information concerning the new profile of antifungal drugs obtaining from medicinal plants.

Key words: Antifungal activity, *Heterostemma tanjorense,* fungal extracts and traditional medicine.

INTRODUCTION

According to World Health Organization, more than 80% of the world's population relies on traditional medicine for their primary healthcare needs [14]. In developing countries, low income people such as farmers, people of small isolate villages and native communities use folk medicine for the treatment of common infectious diseases. These plants are ingested as decoctions, teas or juice preparations [6]. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. Making antibacterial therapy effective, safe and affordable has been the focus of interest during recent years. There are several reports on antimicrobial activity of different herbal extracts [1, 4,3,5,7 and 13]

The use of medicinal plants as a source for relief from illness can be traced back over five millennia to written documents of the early civilization in China, India and the Near east, but it is doubtless an art as old as mankind. Neanderthals living 60,000 years ago in present day Iraq used plants such as hollyback, these plants are still widely used in ethnomedicine around the world [11, 12].

The potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species, only a small percentage has been investigated phytochemically and the fraction submitted to biological or pharmacological screening is even smaller.

MATERIALS AND METHODS

Plant collection

The *Heterostemma tanjorense* (Wight and Arn.) was collected from follow land in around Srirangam, Trichy (Dt) brought into the laboratory for further processes. The collected samples were carefully stored in sterile polythene bags and used for the further study.

Sterilization of Plant Materials

The disease free and fresh plants were selected for this investigation. About 2gm fresh and healthy leaves were taken for each solvent including aqueous. Then, surface sterilized with 0.1% mercuric chloride and alcohol from few seconds. Again the plant materials were washed thoroughly with distilled water (Three times).

Preparation of Plant Extracts

Two grams of sterilized plant leaves were kept in the 10 ml organic solvents such as ethanol, ethyl acetate, chloroform and distilled water. Then these are grind with the help of mortar and pestle. The grind plant material was subjected to centrifugation, for 10-15min at 10,000rpm. The supernatant was collected and stored for further antibacterial screening purposes.

Selection of human pathogenic fungi

Totally five fungal strains such as, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus terreus*, *Candida albicans* and *Fusarium moniliform* were selected for the present investigation. The human pathogenic fungi were originally obtained from Microbial Germ Plasm Culture Collection Unit (MGPCCU), Sri Gowri Biotech Research Academy, Thanjavur used for the present investigation.

Preparation of Microbial Inoculums

The young microbial inoculums culture was prepared and used during the research period. The potato dextrose broth (PDB) was prepared and poured into several tubes. Then these tubes were sterilized. The pure microbial cultures inoculated in the tubes by using inoculation loops. After that these tubes were incubated at 27^{o}C for 48-72 hours for bacteria and fungi respectively. After incubation the cultures were used for the experiments.

Composition of Potato Dextrose Agar Medium

Potato (Peeled)	-	200g
Dextrose	-	20g
Agar	-	15g
Distilled water	-	1000ml
pH	-	5.6

Preparation of Potato Dextrose Agar Medium

The potato tubers were peeled and weighed for about 200g. The tubers were chopped into small pieces with the help of sterile knife. The chopped potatoes were transferred into a conical flask containing about 100ml of distilled water. The content was boiled for 20 min. The supernatant were decanted and filtered by muslin cloth and the filtrate was collected. Dextrose agar were transferred into the extract and shacked to dissolve the ingredients. The medium was made up to 1 liter by addition of distilled water. The pH of the medium was adjusted to 5.6 by using 1N hydrochloric acid or sodium hydroxide drop wise. Finally, the medium was poured into two conical flasks and cotton plugged and sterilized in pressure cooker for 20 minutes.

Screening for Antimicrobial Activity assay

Antifungal Activity assay (Agar - well diffusion method)

In the freshly prepared and sterilized potato dextrose agar medium, a pinch amount of streptomycin was added and mixed well. Then these 20 ml of medium was poured into each petriplate and allowed to solidify. The test fungal cultures were evenly spread over the appropriate media by using sterile cotton swab. Then a well 0.5cm was made in the medium by using sterile cork borer, 150µl of the each aqueous, Acetone and Petroleum ether plant extracts were transferred into separate wells. Then these plates were incubated at 27^{o}C for 48-72 hours. After incubation period the results were observed and measure the diameter of inhibitor zone around the each well.

Antibiotic sensitivity test on fungi (Positive control)
The antibiotic sensitivity test using standard antibiotics (Fluconazole, Griseofulvin and Amphotericin B) were analyzed by agar - well diffusion method.

Antifungal effects of solvents (Negative control)
The antifungal activity of ethanol, ethyl acetate, chloroform and aqueous were tested against the selected bacterial strains.

RESULTS AND DISCUSSION

In the present investigation, the antimicrobial properties of ethanol, ethyl acetate, chloroform extracts of medicinal plant viz., *Heterostemma tanjorense* were tested against human pathogenic bacteria. The antibacterial properties of the extracts on *Heterostemma tanjorense* were also comparatively analysed against standard antibiotics by antibiotic sensitivity test. [9] reported that skin disease diarrhea, diabetes, malaria, respiratory infection, fungal and bacterial infection are the common health problem in rural areas. In under developing countries numerous medicinal plants are used traditionally which are remedial against these disease.

The antifungal activity of ethanol, ethyl acetate, chloroform and aqueous extract of *Heterostemma tanjorense* were tested against human pathogenic strains such as, *Aspergillus flavus, Aspergillus niger, Aspergillus terreus, Candida albicans* and *Fusarium moniliform.*

The ethylacetate extract of *Heterostemma tanjorense* showed a maximum zone of inhibition against *Aspergillus niger, Aspergillus flavus, Aspergillus terreus* (30 mm) and moderate inhibition was found against *Candida albicans* (20mm).

The ethanol extract of *Heterostemma tanjorense* exhibited maximum antifungal activity against *Aspergillus flavus* (30 mm) and lesser fungal activity were *Candida albicans* and *Fusarium moniliform* (20 and 17 mm). The aqueous extract of *Heterostemma tanjorense* does not showed any activity against selected pathogenic fungi (Table- 1 and Fig-1). [2,10] have been screening the antifungal activity of medicinal plants against dermatophytes, but in this study first attmept was made to investigate the antifungal activity of medicinal plant *Cressa cretica* against dermatophytic fungi such as *Aspergillus flavus, A. niger, by M. gypseum, P. varioti, T. rubrum* casued different skin diseases like Tinea. Capitus, T. pedis, T. manum and T. corporis.

The antibiotic sensitivity test using standard antibiotics viz., ampicillin, penicillin and tetracycline were tested against pathogenic bacteria studied. The results of antibiotic sensitivity test were presented in table-2 and fig- 2. All the antibiotics used were exhibited antibacterial activity. The results confirmed that the solvent extracts of *Heterostemma tanjorense* exhibited a higher antibacterial activity against pathogenic bacteria. Similarly, when compared to the standard antibiotics, the solvent extracts of *Heterostemma tanjorense* showed lesser antifungal activity against fungi. Antifungal effect of ethanol, ethyl acetate, chloroform and distilled water, solvents revealed no activity against pathogenic fungi.

Table -1: Antifungal activity of *Heterostemma tanjorense*

S.No.	Test Organisms (fungal pathogens)	Zone of inhibition (diameter in mm)			
		Ethanol	Ethyl acetate	Chloroform	Distilled water
1.	*Aspergillus flavus*	30	30	20	5
2.	*Aspergillus niger*	25	30	15	-
4.	*Aspergillus terreus*	20	30	15	-
3.	*Candida albicans*	18	20	15	5
5.	*Fusarium moniliform*	15	17	12	0.5

Fig -1 Antifungal activity of *Heterostemma tanjorense*

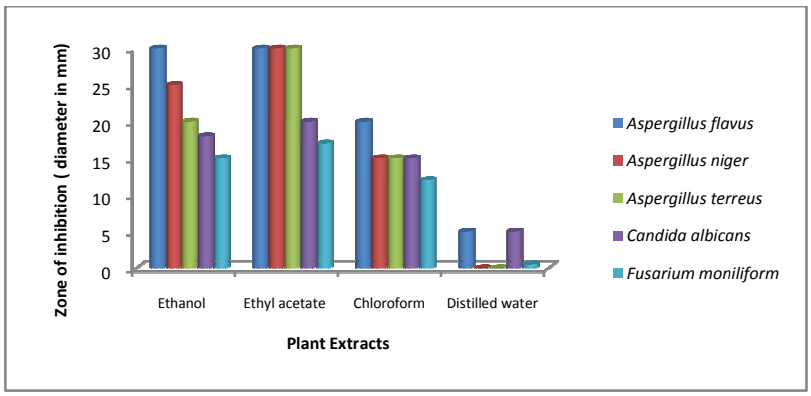
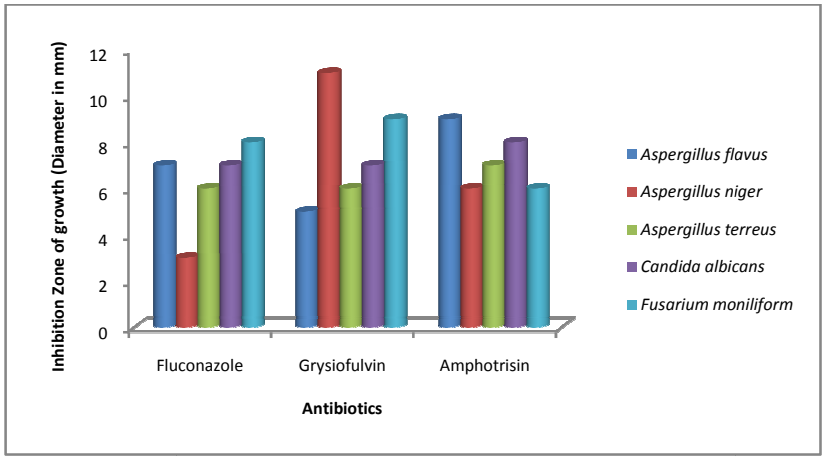


Table- 2: Antibiotic sensitivity test on fungi (Positive control)

S.No.	Test Organisms (Fungal pathogens)	Inhibition Zone of growth (Diameter in mm)		
		Fluconazole	Grysiofulvin	Amphotrisin
1	*Aspergillus flavus*	7	5	9
2	*Aspergillus niger*	3	11	6
3	*Aspergillus terreus*	6	6	7
4	*Candida albicans*	7	7	8
5	*Fusarium moniliform*	8	9	6

Fig -2:Antibiotic sensitivity test on fungi (Positive control)



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