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Research Article

Evaluation of anti-fungal activity of selected medicinal plant seed extracts of India

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Abstract

In the present study aqueous, acetone, petroleum ether and chloroform solvents extracts of 6 selected plant seeds (*Acacia catechu*, *Sida cordifolia*, *Momordica fotida*, *Albiziz procera*, *Mesua ferrea and Lantana camare*) are screened for their anti-fungal potential against 4 important fungal species(*Candida albicans*, *Cryptococcus luteotus*, *Aspergillus niger* and *Mucor heimalis*). Among the six plant seed extracts tested, petroleum ether and acetone extracts showed highest to moderate levels of anti-fungal activity when compared to standard anti-fungal drugs (fluconazole(1mg/ml) and Amphotericin B (150 Units/disc).). The chloroform extracts of *Albizia procera* seed extract failed to inhibit the growth of *Candida* and *Cryptococcus* species. Among the solvent extracts tested, petroleum ether recorded highest significant anti-fungal activity when compared to aqueous, chloroform and acetone extracts.

Keywords: Anti-fungal activity, fungi, petroleum ether, extracts.

1. Introduction

Pathogenic fungi are the most important infectious agents in plants and animals, causing major damage in the physiological process. In fruit and vegetables, there is an ample diversity of fungal species causing problems related to nutritional and character [1]. In addition, in some cases fungi they are indirectly accountable for allergic or toxic disorders among consumers because of the production of mycotoxins or allergens. Commonly, phytopathogenic fungi are restricted by synthetic fungicides; conversely, the use of these is progressively more limited due to the harmful effects of pesticides on human health and the environment [2]. The escalating demand of production and regulations on the use of agrochemicals and the emergence of pathogens resistant to the products employed, justifies the search for novel active molecules and new control approaches. Ever since ancient times, the plant kingdom has provided a variety of compounds of known remedial properties, like analgesics, anti-inflammatories, medicines for asthma, and others. In recent years, anti-microbial properties of plant extracts have been reported with increasing frequency from different parts of the Globe [3]. Several works have demonstrated in laboratory trials that different plant tissues, such as roots, leaves, seeds, stem and flowers possess inhibitory properties against bacteria, fungi and insects [4]. Currently, there is little evidence on the antimicrobial properties of the medicinal plants under investigation against phytopathogen fungi. So in this regard an attempt has been made to investigate six selected plant seeds of (*Acacia catechu*, *Sida cordifolia*, *Momordica fotida*, *Albiziz procera*, *Mesua ferrea and Lantana camare*) for their anti-fungal activity by using different solvent extracts on selected pathogenic fungi.

2. Materials and Methods

2.1 Collection of Plant material

The plants were collected from their natural habitat, form different parts of south and north India. The plant material was identified and authenticated.

2.2 Chemicals:

The entire chemicals used in the present study are of analytical grade.

2.3 Preparation of plant extract

The collected plant material (seeds) was carefully washed under running tap water followed by sterilized distilled water, and was air dried at room

temperature in laboratory for 10-25 days. These dried plant materials were then homogenized to a fine coarse powder using an electric blender and then stored in air tight containers until further use. Various organic solvents viz. water **[AQ]**, Acetone **[AE]**, Petroleum ether **[PE]**, and Chloroform **[CF]** were used for extractions. 100 gm of homogenized coarse powders of seeds were soaked in different conical flasks containing 100 ml of water **[AQ]**, Acetone **[AE]**, Petroleum ether **[PE]**, and Chloroform **[CF]** each and were allowed to stand for 30 min on a water bath with occasional shaking, which were then kept on rotary shaker at 200rpm for 24h [5-7]. Finally each sample extract (water **[AQ]**, Acetone **[AE]**, Petroleum ether **[PE]**, and Chloroform **[CF]**) were prepared by using Soxhlet apparatus and was filtered through sterilized Whatman No 1 filter paper and concentrated to dryness under vacuum at 40^0C using rotaevaporater. Thus the obtained dried extracts were lyophilized, labelled and stored at 4^0C in sterile bottles. [8-9].The extracted powder was dissolved in 10 % dimethyl sulfoxide (DMSO) for the further use. To detect various biologically active phytochemical constituents present in various solvent extracts the standard methods were followed [10-12].

2.4 Micro-organisms tested:

2.4.1 Fungal strains:

The investigated fungal strains are identified strains and were obtained from the National Chemical Laboratory (NCL), Pune, India. The test fungal strains include 2 yeasts viz. *Candida albicans*, *Cryptococcus luteotus* and 2 moulds viz. *Aspergillus niger* and *Mucor heimalis*. The fungal strains were grown on sabouraud broth and maintained on MGYP slants (yeast) and potato dextrose agar slants (mould) at 4°C.

2.4.2 Assay for antifungal activity:

2.4.2.1Preparation of inoculum

The test fungal strains were inoculated into sabouraud dextrose broth and incubated at 28°C on a rotary shaker. The inoculum size was maintained as per the 0.5 McFarland standard ($1x10^8$ cfu/ml). The activated inoculum was used for antifungal assay.

2.4.2.1 Preparation of test compound

The test extracts (AQ, AE, PE and CF) of selected plant species were diluted in 2 % dimethylsulphoxide (DMSO) and the stocks were prepared at the concentration of 10 mg/ml. The antifungal activity was evaluated at 250µg/disc concentration.

2.4.2.3 Antifungal susceptibility testing

The screening of test extracts of selected plant species for antifungal activity was determined by agar disc diffusion method [14]. The molten sabouraud dextrose agar media (Hi-Media) was inoculated with 200 µl of the inoculum ($1x10^8$cfu/ml) when the temperature of media reached 40-42°C and then poured into the Petri plate (Hi-Media). Sterile disc (7 mm) (Hi-Media) was saturated with 20 µl of the extract with the concentration of 250 µg/disc and allowed to dry. The disc was then introduced on the upper layer of the seeded agar plate. For each fungal strain, controls were maintained where pure solvents were used instead of the extract. The plates were incubated at 28°C for 48 h. The result of antifungal activity was obtained by measuring the diameter of the zone of inhibition. The experiment was performed under strict aseptic conditions for three times to minimize error. The antifungal agent fluconazole was included in the assays as positive control.

3. Results

3.1 Anti-fungal activity of six selected plants:

3.1.1 *Acacia catechu:*

All the seed extracts (AQ, AE, PE and CF) of *A.catechu* plant were tested for their anti-fungal activity against selected four fungal species (*Aspergillus niger*, *Mucor heimalis*, C*andida albicans* and *Crptococcus luteotus*). All the four extracts showed very high zones of inhibition against these selected organisms. Of all the extracts the PE extract of this plant has showed highest inhibitory activity against these fungal species. The highest zone of inhibition was noticed in *A.niger* (28 mm) (fig 1). The AQ extract has shown moderate zones of inhibition when compared to all the organic solvent extracts. All the Organic solvent seed extracts has shown competitive zones of inhibition against the selected fungal species when compared to the standard drugs Fluconazole (1mg/ml) and Amphotericin B (150 Units/disc).

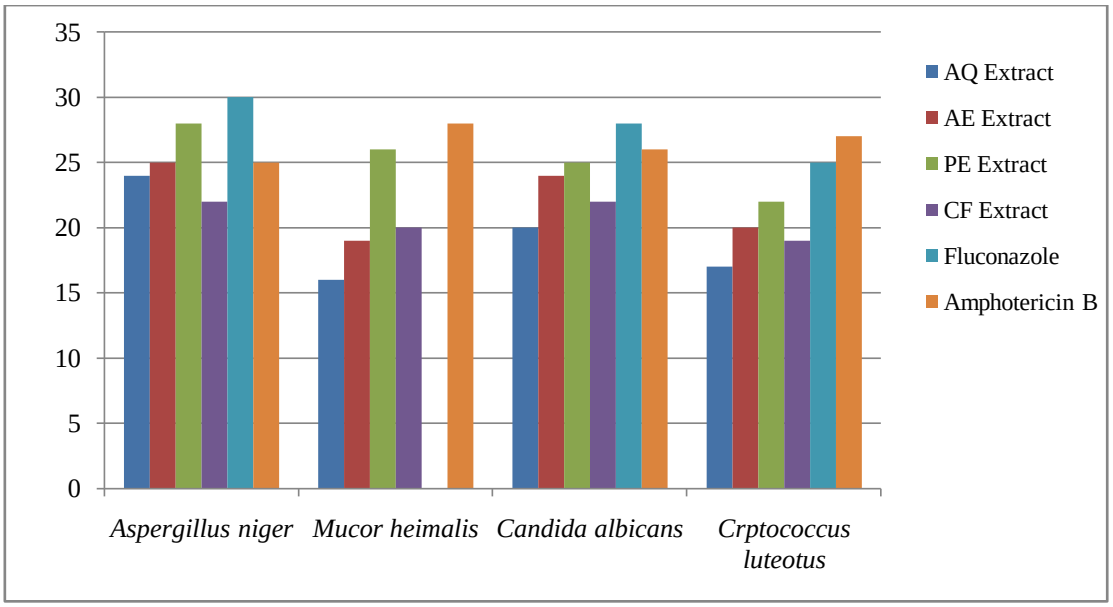

Fig 1: Anti-fungal activity of different solvent seed extracts of *Acacia catechu*

3.1.2 *Sida cordifolia:*

All the seed extracts (AQ, AE, PE and CF) of *Sida cordifolia* plant were tested for their anti-fungal activity against selected four fungal species (*Aspergillus niger*, *Mucor heimalis*, *Candida albicans* and *Crptococcus luteotus*). All the four extracts showed moderate to high zones of inhibition against these selected organisms. Of all the extracts the AE and CF extract of this plant has showed highest inhibitory activity against these fungal species. The highest zone of inhibition was noticed in *A.niger* (27 mm) with AE extract. The AQ extract has shown moderate to low zones of inhibition when compared to all the organic solvent extracts. The least zone of inhibition was observed in *Mucor heimalis* (9 mm) with AQ (fig 2).

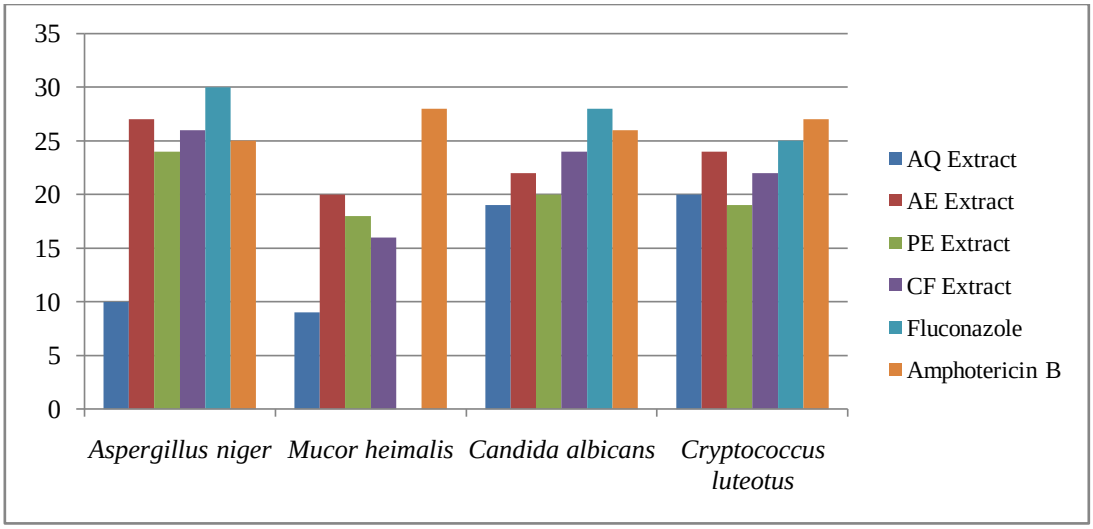

Fig 2: Anti-fungal activity of different seed extracts of *Sida cordifolia*

3.1.3 *Momordica foetida:*

All the seed extracts (AQ, AE, PE and CF) of *Momordica foetida* plant were tested for their anti-fungal activity against selected four fungal species (*Aspergillus niger*, *Mucor heimalis*, *Candida albicans* and *Crptococcus luteotus*). All the four extracts showed moderate to high zones of inhibition against these selected organisms. Of all the extracts the AE and CF extract of this plant has showed highest inhibitory activity against these fungal species. The highest zone of inhibition was noticed in *A.niger* (24 mm) and the least zone of inhibition was noticed in the same species with 8 mm with AQ extract. The AQ extract has shown very low zones of inhibition when compared to all the organic solvent extracts. The PE extract showed moderate zones of (20, 14, 18 and 19 mm) on this fungi species (*Aspergillus niger*, *Mucor heimalis*, *Candida albicans* and *Crptococcus luteotus*) (fig 3).

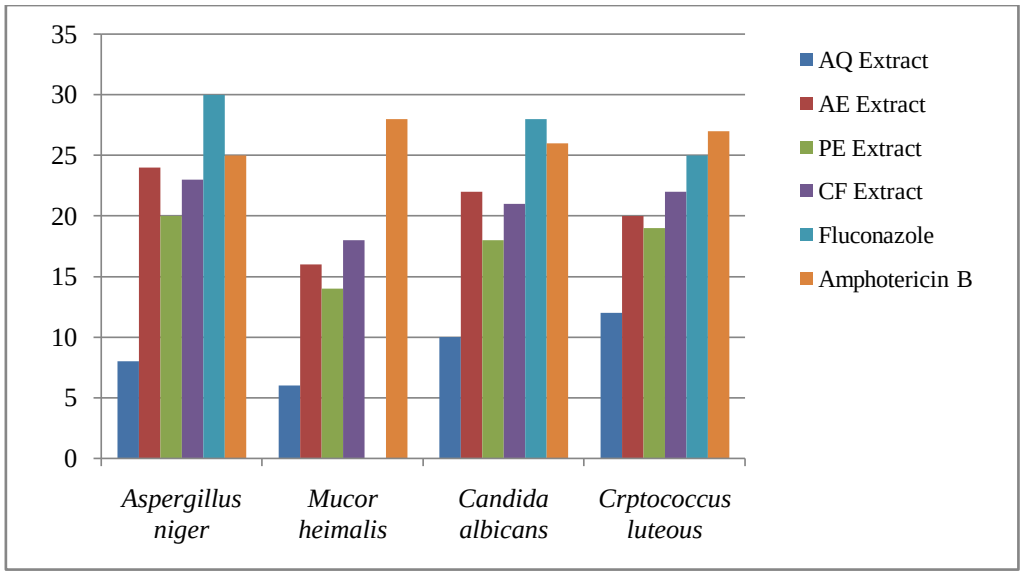

Fig 3: Anti-fungal activity of Different solvent seed extract of *Momordica foetida*

3.1.4*Albizia procera:*

All the seed extracts (AQ, AE, PE and CF) of *Albizia procera* plant were tested for their anti-fungal activity against selected four fungal species (*Aspergillus niger*, *Mucor heimalis*, *Candida albicans* and *Crptococcus luteotus*). All the four extracts showed very low zones of inhibition against these selected organisms. The highest zone of inhibition was noticed in *A.niger* (11 mm) with PE extract. Only the PE extract has shown zone of inhibition of 10 mm for *Candida albicans* and the rest of the extracts showed no inhibition on this *Candida albicans.* The PE extract showed no inhibitor activity on *Mucor heimalis* (fig 10). The CF extract failed to inhibit the growth of *Candida* and *Cryptococcus.*

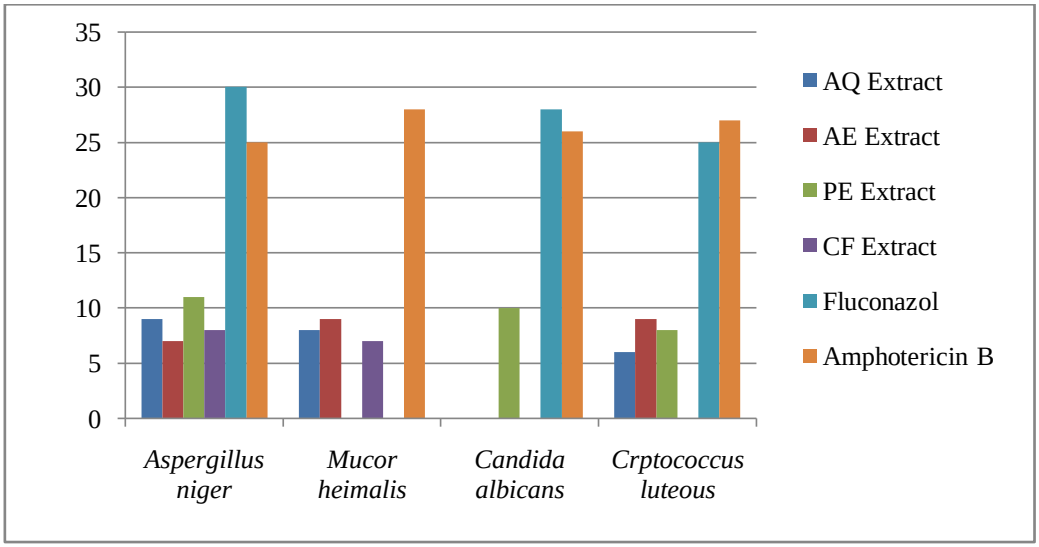

Fig 4: Antifungal activity of different solvent seed extracts of *Albizia procera*

3.1.5 *Mesua ferrea:*

All the seed extracts (AQ, AE, PE and CF) of *Mesua ferrea* plant were tested for their anti-fungal activity against selected four fungal species (*Aspergillus niger*, *Mucor heimalis*, *Candida albicans* and *Crptococcus luteotus*). All the three organic extracts (AE, PE and CF) showed very low zones of inhibition against *Mucor heimalis* and reported no inhibition activity on the other fungal species. The AQ extracts of this plant showed no inhibitory activity against these selected fungal species. The highest zone of inhibition was noticed in *Mucor heimalis* (12 mm) with CF extract. (fig 5).

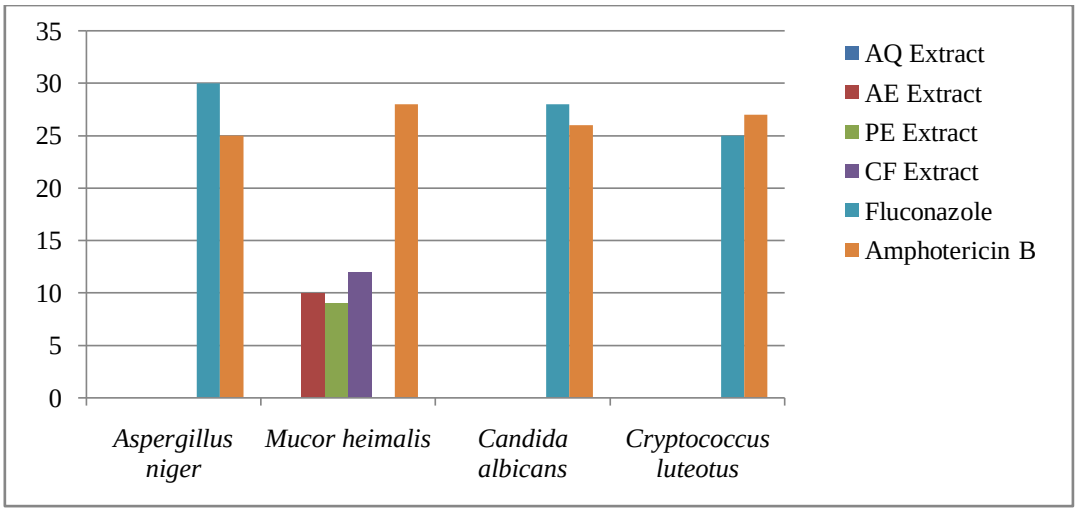

Fig 5: Anti-fungal activity of different solvents seed extracts of *Mesua ferrea*

3.1.6 *Lantana camare*

All the seed extracts (AQ, AE, PE and CF) of *Lantana camare* plant were tested for their anti-fungal activity against selected four fungal species (*Aspergillus niger*, *Mucor heimalis*, *Candida albicans* and *Crptococcus luteotus*). All the four extracts showed moderate to high zones of inhibition against these selected organisms. Of all the extracts the PE extract of this plant has showed highest inhibitory activity against these fungal species. The highest zone of inhibition was noticed in *A.niger* (28 mm) and 26 mm against *Mucor heimalis* in PE extract. The AQ extract has shown moderate zones of inhibition when compared to all the organic solvent extracts.

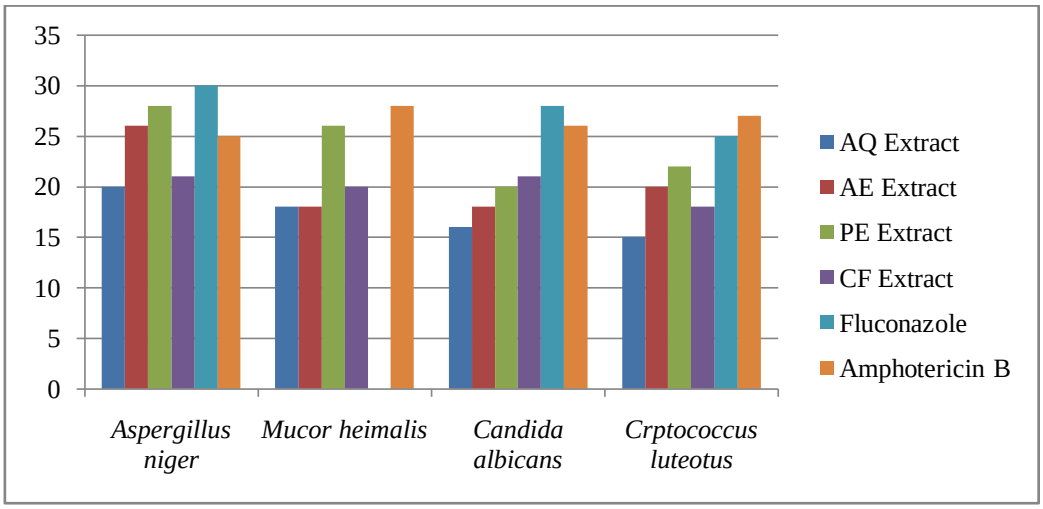

Fig 6: Anti-fungal activity of different solvents seed extracts of *Lantana camare*

4. Discussions

Plants are important source of potentially useful structures for the development of new chemotherapeutic agents. The first step towards this goal is the *in vitro* anti-fungal activity assay. Many reports are available on the antiviral, antibacterial, antifungal, anthelmintic, antimolluscal and anti-inflammatory properties of plants. Some of these observations have helped in identifying the active principle responsible for such activities and in the developing drugs for the therapeutic use in human beings. However, not many reports are available on the exploitation of antifungal or antibacterial property of plants for developing commercial formulations and their applications in medicine on their seed extracts. So in this regard we conducted the study on different seed extracts of six medicinal plant species.

In the present investigation, the AQ, AE, PE and CF seed extracts from six medicinal plants are studied for their anti-bacterial and anti-fungal activities. All the seed extracts of the selected plants showed the presence of different bioactive compounds which are very helpful in the anti-bacterial and anti-fungal activity. So the results obtained for the anti-bacterial and anti-fungal studies conducted on the selected bacteria and fungi are due to the presence of these bioactive compounds. The results of present investigation clearly indicate that the antifungal activity vary with the species to species and plants to

plant material used. Thus, the study ascertains the value of plants used in ayurveda, which could be of considerable interest to the development of new drugs.

5. Conclusions

The present investigation is an important step in developing plant based pesticides which are ecofriendly for the management of the seed borne fungi and development of commercial formulation of botanicals. Further investigation will be done for developing commercial formulation based on field trail and toxicological experiment. It is important observation that all the biomolecules are polar in nature with their solubility more to water, methanol and ethanol.

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