

IN VITRO ANTIFUNGAL ACTIVITIES OF THE AQUEOUS AND METHANOL EXTRACT OF *ABRUS PRECATORIUS* LINN (*FABACEAE*) SEEDS

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Summary

The aqueous and methanol extractions of seed of *Abrus precatorius* Linn were evaluated for the antifungal activity against selected fungal sps viz. *C. albicans*, *C. tropicalis*, *C. krusei*, *C. kefyri*, *C. glabrata*, *C. guilliermondi*, *Aspergillus niger*, *Aspergillus fumigates* and *Aspergillus flavus* using agar well diffusion method, minimum inhibitory concentration. Methanol extract of the seeds of *Abrus precatorius* Linn revealed higher antifungal activity against *C. albicans*, *C. tropicalis*, *C. krusei*, *Aspergillus fumigates* and *Aspergillus flavus* where as intermediate activity was recorded against *C. kefyri*, *C. glabrata*, *C. guilliermondi*, and *A. niger*. On the other hand aqueous seeds of the above plant recorded higher antifungal activity against *C. krusei*, *C. guilliermondi* and *A. fumigates* and an intermediate antifungal effect on *C. albicans*, *C. tropicalis*, *C. kefyri*, *C. glabrata*, *Aspergillus niger* and *Aspergillus flavus*. The results obtained in the present study suggest that the methanol and aqueous extract of the seeds of *Abrus precatorius* Linn revealed the scope to develop a novel broad spectrum of antifungal herbal formulation.

Keywords: *Abrus precatorius* Linn, Antifungal activity, aqueous extract, methanol extract

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Introduction

Infectious disease are the leading cause of death worldwide. Antibiotic resistance has become a global concern (1). Many infectious disease have been known to be treated with herbal remedies throughout the history of mankind. There are between 250000 and 500000 plant species on the planet earth. However, a small percentage of which are utilized for treatment of diseases (2). The increasing failure of chemotherapeutics and antibiotic resistance exhibited by pathogenic microbial infectious agents has led to the screening of several medicinal plants for their potential antimicrobial activity (3,4). Several plants used in folk medicine have been studied for antimicrobial activity to develop a source of new antifungal compounds with fewer side effects, a wider spectrum of action and lower cost (5).

The plant *Abrus precatorius* Linn a medicinal plant belongs to the genus *Abrus* (Family: *Fabaceae*), commonly called “Indian liquorice, Jequirity, Crab eye” in English and in vernacular “Kundumani”. Crab’s eye is possibly native of India (6) or of Guinea in Africa (7), but today has naturalized throughout most of the tropics (6). The alternate, pinnately compound leaves are 5 to 10 cm long and have five to 20 pairs of leaflets. The racemes have tight clusters of white to purple flowers (8). The most notable thing about this species is the 6-mm, spherical red and black seeds. Crab’s eye traditionally used against leucoderma, wounds, alopecia, asthma, tubercular glands, leprosy, fever, ulcer and tumor (9).

The active metabolites in the seed of *Abrus precatorius* Linn include abrin, abrus agglutinin, glycyrrhizin gallic acid, trigonelline, precatorine and lipolytic enzymes. Glucine, Coumestrans, resin asparagines, saponins, alkaloids and sterols (10,11). Gallic acid, glycyrrhizin and trigonelline are potent antioxidants (12). The leaves and roots contain glycyrrhizin, the principal component of licorice. These tissues prepared in various ways are used to treat coughs and a number of other ailments (13). The seeds are considered abortifacient (14), aphrodisiac, diuretic, emetic, laxative, purgative, refrigerant, sedative and used in various ailments to cure headache, snakebite, blennorrhagia, boil, cancer, cold, colic, conjunctivitis, convulsion, fever, gastritis, gonorrhoea, jaundice, malaria, night-blindness, ophthalmia and rheumatism. Various African tribes use powdered seeds as oral contraceptives (15). Dry seeds of *Abrus precatorius* are powdered and taken one teaspoonful once a day for two days to cure worm infection (16). Seeds have also the potential of good insecticide (17).

Bioactivity studies on *Abrus precatorius* Linn of seeds established its antifertility activity (18), ureterotonic effect (19), antidiarrhoeal effect (20), antitumor activity (21), anti-inflammatory activity (22), hypoglycemic, hypolipidemic effect (23) and effectiveness in the treatment of *Shistosoma haematobium* infection. The present study was carried out to test the antifungal efficacy of the seeds extract of *Abrus precatorius* Linn with reference to fungal spp.

Materials and methods

Plant material

The plant material of the seeds of *Abrus precatorius* Linn seeds were freshly collected in January-February 2009 in and around Edhapattu village (Villupuram Dt, Tamilnadu, India) and were cleaned with distilled water and shade dried at room temperature. The plant material was authenticated and a voucher specimen (Voucher number VCB209) of the plant was kept at the Department of Botany, Voorhees College, Vellore, Tamilnadu (India).

Preparation of extracts

The powdered seeds (200 g) of *Abrus precatorius* Linn were extracted separately to exhaustion in a Soxhlet apparatus using Aqueous (90°C) and methanol (50°C) solvent systems. All the extracts were filtered through a cotton plug followed by Whatman filter paper No.1 and then concentrated by using a rotary evaporator at low temperature (40-50°C) and reduced pressure to get 4.3g and 5.4g yield from aqueous and methanol fractions respectively. The extracts were preserved in airtight containers and kept at 4-5°C until further use. All the extracts were tested for antifungal activity against the fungal spp.

Test organisms

The fungal spp used for the test were *Candida albicans* (MTCC 227), *C.tropicalis* (MTCC 750), *C.krusei* (ATCC 6258), *C.kefyr* (ATCC 4235), *C. guilliermondi* (ATCC 6260), *C.glabrata* (ATCC 2001), *Aspergillus niger* (MTCC 277), *Aspergillus fumigates* (MTCC 343) and *Aspergillus flavus* (MTCC 418). All the stock cultures were obtained from Microbial Type Cell Culture (IMTECH, India).

Culture media and inoculums preparation

Sabouraud dextrose agar /broth (Himedia, India.) were used as the media for the culturing of fungal strains. Loops full of all the fungal cultures were inoculated in the Sabouraud dextrose broth (SDB) at 37°C for 72 hrs.

Antifungal activity study

A. Agar well diffusion method

The extracts obtained from the leaves were used for studying their antifungal activity. A loop full of fungal strain was inoculated in 30 ml of sabouraud dextrose broth in a conical flask and incubated for 72 hrs to get active strain by using agar well diffusion method (24). The media was poured into petridishes. After solidification 0.25 ml of test strains were inoculated in the media separately. Care was taken to ensure proper homogenization.

The experiment was performed under strict aseptic conditions. After the medium was solidified, a well was made in the plates with sterile borer (6mm). The extract compound (100µl) was introduced into the well and plates were incubated at 37°C for 72 hrs. All samples were tested in triplicates.

Microbial growth was determined by measuring the diameter of zone of inhibition. Controls with Ketoconazole was kept for all test strains except *C.kefyr* for which Itraconazole used as control and the control activity was deducted from the test and results were recorded.

B. Determination of Minimum inhibitory concentration (MIC)

Antifungal activity was measured using a dilution technique(25).The plant extract (100 mg) was solubilized in 1 ml of dimethyl sulfoxide (DMSO) and serially two fold diluted in Yeast Nitrogen Base Phosphate (YNBP) broth (Himedia, India) to obtain a concentration range of 15.6-1000 mg/ml. YNBP broth containing only DMSO diluted in the same way, which did not influence fungal growth, were included as controls. The fungal strains were suspended in sterile physiological Tris buffer (pH 7.4, 0.05 M), homogenized and adjusted to an optical density of 0.05 at 530 nm (equivalent to 1×10^6 CFU/ml). This suspension was used as the inoculums for the test in the agar plates.

Fungal suspensions (100 μ l) were inoculated using a micropipette. The minimal inhibitory concentration (MIC) was defined as the minimal concentration of the plant extract which completely inhibited the visible growth (turbidity) of the fungus in tubes. The minimal fungicidal concentration (MFC) was defined as the minimal concentration of the extract which completely inhibited the visible growth of the fungus on solid media in petriplates that were incubated at 37°C for 72 hrs.

Statistical analysis

Data are expressed as means \pm S.D statistical analysis was performed with SPSS(8th version) Least standard deviation test were used for analysis of variance (ANOVA) and Post hoc test respectively. Difference on statistical analysis of data were considered significant at $P < 0.05$.

Results and discussion

The results obtained are presented in Table 1-2 and fig.1. In the present study the anti-fungal activity of plant extracts (aqueous and methanol) was evaluated against nine fungal *spp*. In the first stage, aqueous and methanol, seeds extracts of *Abrus precatorius* Linn applied on one isolate of each fungal species. Methanol seeds extract of *Abrus precatorius* Linn showed high antifungal activity against *C.albicans*, *C. tropicalis*, *C. krusei*, *Aspergillus fumigates* and *Aspergillus flavus*. The aqueous seed extracts showed high antifungal activity against *C. krusei*, *C. guilliermondi* and *Aspergillus fumigates*.whereas both extract showed an intermediate activities against *C.kefyr*, *C.glabrata* and *A.niger*.

Aqueous Extract

The results revealed the details of mean MICs of aqueous seeds extract against 9 isolates of fungal *spp*. The lowest MIC was recorded (31.25mg/ml) for *C.krusei*, *C. guilliermondi* and *Aspergillus fumigates* when comparared with that of other species *viz.* *C.albicans*(250 mg/ml), *C.tropicalis* (500mg/ml), *C.kefyr* (500mg/ml), *C.glabrata* (250mg/ml), *A.niger*(250 mg/ml) and *Aspergillus flavus*(250 mg/ml).

Methanol extract

The results revealed the details of mean MICs of methanol seeds extract against 9 isolates of fungal species. The lowest MIC was recorded (31.25mg/ml) for *C.albicans*, *C.tropicalis*, *C.krusei*, *Aspergillus fumigates* and *Aspergillus flavus*. When compared with that of other species viz. *C.kefyr* (62.5mg/ml), *C.glabrata* (125 mg/ml), *C. guilliermondi*(62.5 mg/ml) and *A.niger*(500 mg/ml).

Table 1. Antifungal activities of seeds extract of *Abrus precatorius* Linn.

Name of the micro organisms	Aqueous extract	Methanol extract	Control
<i>C.albicans</i>	8.76±0.18 ^a	14.86±0.23 ^b	18.98±0.54 ^{#c}
<i>C.tropicalis</i>	7.66±0.20 ^a	12.26±0.26 ^b	24.00±0.51 ^{#c}
<i>C.krusei</i>	14.36±0.35 ^a	15.16±0.31 ^a	17.68±0.31 ^{#b}
<i>C.kefyr</i>	7.70±0.15 ^a	13.03±0.12 ^b	15.68±0.34 ^{*b}
<i>C.glabrata</i>	8.63±0.17 ^a	9.26±0.06 ^a	18.04±0.56 ^{#b}
<i>C.guilliermondi</i>	13.66±0.40 ^{ab}	12.26±0.12 ^{ab}	14.03±0.56 ^{#b}
<i>A.niger</i>	7.76±0.08 ^a	8.13±0.14 ^a	18.37±0.31 ^b
<i>A.fumigatus</i>	11.66±0.16 ^a	14.16±0.14 ^b	19.01±0.12 ^c
<i>A.flavus</i>	8.70±0.23 ^a	9.30±0.11 ^a	4.06±0.88 ^b

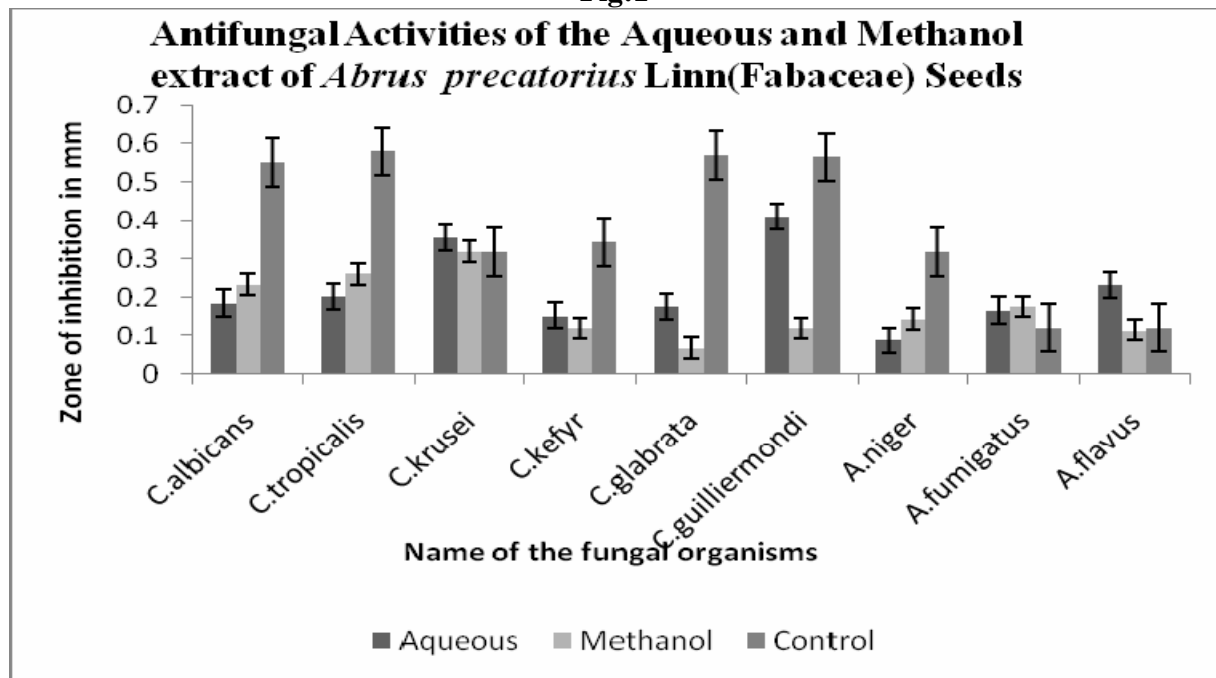
Different superscripts in the same Column are significantly different p<0.05 level (Least standard deviation) means followed by ±SEM, (* = Itraconazole , # = ketoconazole).

Herbal and alternative medicine is popular in the general population worldwide. A great number of modern drugs are still derived from herbs (26). The aqueous and methanol extract of *Abrus precatorius* Linn displayed considerable antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*. In the present study the methanol seeds extract revealed higher degree of antifungal activity for (MIC 31.25mg/ml) *C.albicans*, *C.tropicalis*, *C.krusei*, *Aspergillus fumigates* and *Aspergillus flavus* when compared with that of other fungal spp tested. However, the antifungal activity of aqueous seeds extract recorded less potent in comparison to methanol seed extract. Similar studies elsewhere recorded antibacterial activity against *Staphylococcus aureus* and *Staphylococcus epidermidis*(27).The antifungal activity of *Abrus precatorius* Linn against test strains such as *C.albicans* ,*C.tropicalis*, *C.krusei* , *C.kefyr* ,*C.glabrata* *C.guilliermondi* , *Aspergillus niger*, *Aspergillus fumigates* and *Aspergillus flavus* compared to control can be attributed to the chemical profile of the extracts containing saponins ,alkaloids etc.

Table 2. Minimum Inhibitory Concentration (MIC) of aqueous and methanol extracts of *Abrus precatorius* Linn.

S.No.	Name of the organisms	Minimum inhibitory concentration(mg/ml)	
		AE	ME
1	<i>C.albicans</i>	250	31.25
2	<i>C.tropicalis</i>	500	31.25
3	<i>C.kefyr</i>	500	31.25
4	<i>C.krusei</i>	31.25	62.50
5	<i>C.glabrata</i>	250	125
6	<i>C.guilliermondi</i>	31.25	62.5
7	<i>A.niger</i>	250	500
8	<i>A.fumigatus</i>	31.25	31.25
9	<i>A.flavus</i>	250	31.25

Key words: AE → Aqueous extract; ME → Methanol extract

Fig:1

Conclusion

The demonstration of broad spectrum of antifungal activity by *Abrus precatorius* Linn may help to discover new chemical classes of antibiotic substances that could serve as selective agents for infectious disease chemotherapy and control. This investigation has opened up the possibility of the use of this plant in drug development for human consumption possibly for the treatment of fungal infection. The effect of this plant on more pathogenic organisms and toxicological investigations and further purification however, needs to be carried out.

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