In vitro EVALUATION OF VARIOUS EXTRACTS OF ABRUS PRECATORIUS L. AGAINST HUMAN PATHOGENIC FUNGI

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<u>ABSTRACT</u>

Abrus precatorius L. of Fabaceae is widely distributed in India. The plant have been reported for neuromuscular effects, neuroprotective, abortifacient, antiepileptic, anti-viral, anti-malarial and antifertility properties. *Candida albicans* causes candidosis and *Epidermophyton floccosum* causes dermatophytosis. It also invades skin and nails. It is a infectious agent in immunocompromised patient with Behcet's syndrome. Aqueous, ethyl alcohol and ethyl acetate hot extracts of leaves, bark, roots & seeds were investigated for their antifungal activity. The growth inhibition was determined using food poisoning method against human pathogenic fungi. It was observed that ethyl acetate extract showed maximum inhibition than ethyl alcohol extract followed by aqueous extract against the selected human pathogenic fungi.

Keywords: Abrus precatorius L., antifungal activity.

INTRODUCTION

Medicinal plants are part and parcel of human society to combat diseases from the down of civilization (Narendra Garaniya & Atul Bapodra, 2014). Plant based drugs are useful for the treatment of the diverse range of disease and are gaining popularity because of several advantages such as fewer side effect, better patient tolerance, relatively less expensive and acceptance due to a long history of use, especially herbal medicines provide rational means for the treatment of many diseases that are incurable in other system of medicine. According to World Health Organization (WHO) almost 80% people of the developing countries depends on traditional and folk medicines. Search for newer drugs from plants has been on the rise since many of the micro-organisms including fungi are posing serious health related disorders. Probably this may be due to the prolonged and indiscriminate use of antibiotics increase in number of immunocompromised patients. In addition, many of the existing drugs cause various side-effects. The vast plant biodiversity of our country is presently under explored and

many plants could be the source of novel drugs (S.Madhumathi, et.al.2000). Medicinal herbs are an important source for the therapeutic remedies of various ailments (Moses A.G. and Maobe, 2013).

Plants which have been used as medicine over hundreds of years, constitute and obvious choice for study. It is interesting to determine whether their traditional uses are supported by actual pharmacological effects or merely based on folklore. Biologically active compounds from natural sources have always been of great interest to scientists working on infectious diseases. In the recent years there has been a growing interest to evaluate plants possessing antimicrobial activity for various diseases. (Clark & Hufford, 1993). A number of studies have been reported dealing with antimicrobial screening of extracts of medicinal plants. (Malcom & Sofawora, 1969). The great interest in the use and importance of Indian medicinal plants by the WHO in many developing countries has led to intensified efforts on the documentation of ethnomedicinal data of medicinal plants (Dhar et.al.1968).

Fungi cause important human disease especially in tropical regions. Despite the existence of potent antifungal agents, resistant or multi-resistant strains are continuously appearing, imposing the need for a permanent search and development of antifungal compounds.

Candida albicans infects skin causing candidosis while *Epidermophyton floccosum* is one of the common causes of dermatophytosis infecting skin (Tinea corporis, tinea cruris, tinea pedis and nail onychomycosis). Candidosis and dermatophytosis are the most common forms of fungal infection in many countries (Odds, 1988, Ribbon 1988). Treatment demands the use of antifungal agents such as Griseoflulvin and Amphotericin B (Koening, 1995). However, the high cost of this kind of treatment, especially in developing countries, the long period of therapy and the possibility of the emergence of resistant strains may hinder the eradication of these diseases (Baker et.al. 1989, Willocks et.al.1991). The screening the traditional medicinal plants may offer potential resources since there is widespread in rural areas, with much of the population relaying on them (Gadhi, C.A. et.al. 2001).

Abrus precatorius L. is woody twinning plant with characteristics toxic red seeds with black at the base. It is beautiful, much-branched, slender, perennial, deciduous woody, climbing herb commonly known as 'Gunj'. The qualitative phytochemical studies of different parts of plant extract showed that, the leaves were found to contain sweet testing compounds such as abrusoside and glycyrrhizin and seed contain protein toxin, abrin which is deadly when ingested even at a small dose.

Materials and Methods:

Collection of plant material:

The plant parts of *A. precatorius* L. collected from different regions of Marathwada particularly Nanded District and was immediately identified botanically on the spot in the field by using Flora of Marathwada (Naik, 1998). The plants parts collected were shredded and dried completely at 50^oC for 72h. After drying, the plant materials were grinded well using mechanical blenders into fine power and transferred into air tight container with proper labeling (Charles A, et.al., 2011).

Cultures:

The fungal human pathogen cultures of *Candida albicans* and *Epidermophyton floccosum* were obtained from Department of Microbiology, Government Medical College, Aurangabad and Department of Pathology, Dr.S.C. Govt. Medical College, Nanded. The cultures were maintained on the medium suggested by the respective laboratory and sub-culturing was done fortnightly. The cultures were incubated in an incubator for growth and later were stored in refrigerator.

Extraction of plant material:

The plant part powder was added to distilled water/ ethyl alcohol/ ethyl acetate and was allowed to boil for further 4-5 minutes on a water-bath under hood. 10ml of ethanol was used for every gram of powder. The extract was cooled and contents were homogenized thoroughly in a mortar and pestle. The extract was filtered by passing through several layers of muslin cloth. The residual ground powder was re-extracted by boiling in solvent used earlier for 3 minute to ensure the complete removal of contents. The extracts were pooled, centrifuged at 5000 rpm and the volume was adjusted to represent 10 ml/gram of fresh weight of tissue (ml/gfw).

Plant Extract for Antifungal Properties:

Antifungal activity of the plant extracts (free from alcohol/ ethyl acetate and converted into aqueous) was evaluated by well-diffusion method expressed by zone of inhibition mm in diameter for *Candida albicans, Epidermophyton floccosum*.

The bioassay was carried out by using 1ml of inoculum $(1X10^{6} \text{ colony forming units})$ prepared from an overnight culture for given test fungi. 1ml of the resultant spore /cell suspension was poured in the petri plate and the plates were poured with respective medium to seed each prepared plate. The medium was allowed to solidify. Using a sterilized cork borer, wells of 5mm diameter were made in the solidified inoculated medium and the plate area uniformly. The wells were filled with 0.5ml of extract. Plates were then incubated aerobically at 37 ± 2 °C for 72 h for fungi.

Similarly, wells containing standard concentration of Amphotericin B were used to compare the antifungal property of the plant extract. 1gm of Amphotericin B (Hi-media, Mumbai) was dissolved separately in sterile distilled water and 0.5ml was used to fill the wells.

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RESULTS AND DISCUSSION:

Effect of A. precatorius aqueous extracts.

The aqueous extracts of *A. precatorius* exhibited antifungal activity in terms of perfect inhibition of spore germination of test fungi namely *C. albicans* and *E. floccosum* showed that 10% aqueous extracts were more inhibitory to both human pathogenic fungi and with increase in dilution zone of inhibition of fungi was decreased. Seed extract showed maximum inhibition followed by root and bark extract and it was least in leaf extract. The 10% seed extract gave a 17 mm zone of inhibition in case of E. Floccosum which was more than control i.e. Amphotericin B.

Effect of A. precatorius alcoholic extracts.

The alcoholic extract of *A. precatorius* exhibited antifungal activity in terms of perfect inhibition of spore germination of test fungi namely *C. albicans* and *E. floccosum* showed that 10% alcoholic extracts was more inhibitory to both human pathogenic fungi and with increase in dilution zone of inhibition of fungi was decreased. Seed extract showed maximum inhibition followed by root and bark extract and it was least in leaf extract. The seed extract was more effective in both the test organisms compared to control.

Effect of A. precatorius ethyl acetate extracts :

The ethyl acetate extract of *A. precatorius* exhibited antifungal activity in terms of perfect inhibition of spore germination of test fungi namely *C. albicans* and *E. floccosum* showed that 10% ethyl acetate extracts was more inhibitory to both human pathogenic fungi and with increase in dilution zone of inhibition of fungi was decreased. Seed extract showed maximum inhibition followed by root and bark extract and it was least in leaf extract. All the extract were more effective than control in both the fungi.

It is clear that from the above results the seed extract of the plant showed highest activity in ethyl acetate. However, the activity of leaf was relatively less than the activity of bark of the plant. Similarly, the activity of bark was comparatively less than the root extracts. These results confirm the observations made by various workers with different plant and plant parts. (Cotton, 1996, Taylor, 1996, Grierson & Afolayan, 1999). *A. precatorius* L. is a unique source of many potential phytochemicals which makes this plant very important and versatile for its large number of medicinal properties i.e. antidiabetic, nero-protective, anti-microbial and analgesic (Narendra Garaniya & Atul Bapodra, 2014).

Hence from the above results, the seeds of the plant *A. precatorius* can be used for Integrated Paste Management to control growth of human pathogenic fungi and seeds can be utilized to develop antifungal agents.

Sr. No.	Plant parts	Zone of inhibition (mm)								
		C. albicans				E. floccosum				
		10%	5%	2.5%	С	10%	5%	2.5%	С	
1	Leaves	7	6	5	14	12	9	7	12	
2	Bark	9	8	7	14	14	12	10	12	
3	Root	12	11	10	14	15	14	12	12	
4	Seeds	14	10	10	14	17	15	13	12	

 Table 01

 Effect of A. precatorius L. aqueous extracts on growth of test fungi

C – Amphotericin

 Table 02

 Effect of A. precatorius L. alcoholic extract on growth of test fungi

Sr. No.	Plant parts	Zone of inhibition (mm)								
		C. albicans				E. floccosum				
		10%	5%	2.5%	C	10%	5%	2.5%	С	
1	Leaves	9	8	7	-14	16	14	11	12	
2	Bark	11	9	7	14	17	15	12	12	
3	Root	13	11	9	14	20	17	15	12	
4	Seeds	16	14	11	14	20	20	17	12	

C-Amphotericin

Table 03

Effect of A. precatorius L. ethyl acetate extracts on growth of test fungi

Sr. No.	Plant parts	Zone of inhibition (mm)								
		C. albicans				E. floccosum				
		10%	5%	2.5%	С	10%	5%	2.5%	С	
1	Leaves	18	13	11	14	20	18	16	12	
2	Bark	18	16	14	14	24	23	21	12	
3	Root	20	17	15	14	26	24	21	12	
4	Seeds	24	21	19	14	27	25	25	12	

C-Amphotericin

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