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Biosorption Characteristics and Novel Antifungal Activity of *Abrus precatorius* Seed Extract

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Antifungal activity of *Abrus precatorius* was investigated against a panel of opportunistic fungi *Fusarium solani* and *Alternaria solani*. Its biosorption capacity was also characterized in Kaolin clay and wastewaters. Appreciable inhibition zones were formed by crude, supernatant, residue and dialysed samples of *Abrus* against both the fungi whereas inhibition was retained in 2nd peak of gel filtration. The crude extract exhibited pronounced activity against *Fusarium solani* with 22 mm zone size and minimum inhibitory concentration (MIC) 10.36 µg/mL. Ethanolic extract also showed significant inhibition with MIC 9.76 µg/mL. The plant seed extracts prepared in distilled water, 5 % NaCl and potassium phosphate buffer showed high coagulative effect in Kaolin clay suspension (turbid water). The plant seed protein coagulated well the particles in the samples. It is worth noting that crude buffer extract showed high activity among all the extracts that is comparable to previously used chemical sorbent (alum) in both low and high turbidity. Based on our findings, we suggest that *Abrus precatorius* holds promise as a candidate for broad spectrum disease protection, so a resource for new biofungicide and also serves as a good starting material for the synthesis of environment friendly natural coagulant and disinfectant.

Keywords: *Abrus precatorius*, Antifungal activity, Seed extracts, Bio-fungicide, Water treatment.

INTRODUCTION

Plants have formed the basis of sophisticated traditional medicine systems that have given rise to many important drugs still in use today[1]. Over 200 or more natural peptides or their analogues have been found with varying activities against pathogenic fungi[2]. Antifungals work by exploiting differences between mammalian and fungal cells to kill the fungal organism without dangerous effects on the host[3]. Considering the limited number of the antifungal agents presently available, characterization of additional sources with different modes of action is of pivotal importance[4].

Abrus precatorius is a medicinal plant and is generally regarded as antitubercular and antiplasmodial[5]. Leaves and roots of *Abrus* are used in various ways to treat epilepsy and a number of other ailments[6]. Agglutinin from *Abrus* showed antitumour activity[7] and immunostimulant activity. However, the plant has not been investigated for its antifungal potential. Furthermore, in order to enhance the applicability of biosorption in wastewater treatment, it is important to identify more natural resources that could uptake metals with high efficiency and specificity as well as to design better bioprocesses that effectively remove heavy metals from aquatic systems. This motivated us to study unexplored plant species and check for their ability to remove metals from polluted environment.

We report here antifungal and biosorption characteristics of *Abrus precatorius*. The results demonstrate that this plant is a good candidate for use against fungi and as a natural coagulant.

EXPERIMENTAL

The seeds of medicinal plant *Abrus precatorius* were collected and taxonomically identified from the Department of Botany, University of Agriculture, Faisalabad, Pakistan.

Seeds were washed, dried, powdered, treated with 95 % ethanol to remove lipid and ethanolic soluble contents and air dried. The plant extract was prepared in potassium phosphate buffer (pH 7) under liquid nitrogen. PMSF was added to a final concentration of 1 mM to inhibit the protease action. The seed mixture was centrifuged at 10,000 × g at 4 °C for 20 min. The supernatant was collected and stored at 4 °C till further analysis[8].

Partial purification: Crude extract was $(NH_4)_2SO_4$ precipitated at 80 % saturation level and centrifuged[9]. The residue was resuspended and dialyzed for 24 h against distilled water[10]. The samples with maximum antimicrobial activity were purified by gel filtration[11] on Sephadex G-100 column (2 × 30 cm), equilibrated and eluted with potassium phosphate (pH 7). Absorbance of the eluants was recorded at 280 nm. The fractions with maximum protein contents were pooled and applied for antifungal activity[12].

Antifungal assay: Pure cultures of pathogenic fungal strains, *Fusarium solani* and *Alternaria solani* were procured from the Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan. The strains were grown on potato dextrose agar (Oxoid, U.K) medium; chloramphenicol solution (100 µL per 30 mL) was added to prevent contamination[13]. The samples (100 µL) were applied on each disc laid flat on the growth medium and incubated at 28 °C for 48 h. Terbisil (Terbinafine, Saffron) was included as a reference in each assay. The extracts having antifungal activity exhibited clear zones that were measured by zone reader[10-12].

Minimum inhibitory concentration (MIC): MIC determination was performed by microdilution method. To 96 well microdilution plates, 100 µL PDA broths (Oxoid, U.K) was poured. Sample (100 µL) was added to first well and serially two-fold diluted to10.36 µg/mL after which 10 µL of fungal spores were added to each well and incubated at 27 °C for 48 h. Extract-free well was used as a control. MIC values were recorded as the lowest concentration of the extract that completely inhibited the growth.

Coagulation activity assay: This method was used to explore the flocculation ability of seed coagulant proteins. Crude plant extract was prepared in extraction buffer (potassium phosphate), 5 % NaCl and distilled water and compared with activated carbon (5 %) and alum (5 %). Highly turbid crude extract solution (20 µL) was added to 1 mL Kaolin clay suspention in quartz cuvette and homogenized instantly. This was allowed to settle for 0-150 min and absorbance was noted at 500 nm on spectrophotometer U-2001[14]. The reduction in absorbance relative to the control defined the coagulation activity. Same procedure was repeated at low turbidity. The coagulation ability was also checked by using sewerage water sample at high and low turbidity on turbidometer.

RESULTS AND DISCUSSION

Antifungal activity: The crude seed extract, ammonium sulphate precipitated and gel filtration fractions from *Abrus precatorius* were evaluated against *Fusarium solani* and *Alternaria solani* and reported in terms of inhibition zones (Table- 1). Most potent inhibitory effect of crude seed extract of the plant with inhibition zone 34 mm was found against hyphomycete fungus *Alternaria solani*. It has been reported earlier that lipophilic extracts of *Vitex negudo* exhibited antifungal activity against *Alternaria alternata* by direct bioautography. The zones of inhibition of *V. negudo* were 28 mm in diameter[15] that were quite comparable to our findings. A possible explanation for the difference is that the compounds in extract of *V. negudo* that inhibited the growth of fungi might not be the same as present in *Abrus precatorius*. Lipophilic compounds that bind within or internal to cytoplasmic membrane[16], quinines[17] or thionines and effect the growth of filamentous fungi mainly by causing membrane permeabilization[9].

Although all the fractions displayed interesting activities against hyphomycete fungi but fraction of gel filtration exhibited reduction in the activity against both the fungi. The reason might be that some form of activator or cofactor was removed by chromatographic purification procedure that rendered the rest of compounds inactive.

The test samples from *Abrus* exhibited pronounced activity against an emerging pathogen *Fusarium solani*. The crude extract showed good inhibition zone of 22 mm with MIC 10.36 µg/mL. Ethanolic extract also showed antifungal capacity with MIC 9.76 µg/mL that might be due to the factor that it contains certain reactive organic compounds that cause inhibition of the growth of fungi at comparatively low concentrations[18]. Moreover ribosomal inactivating proteins (RIPs) have also been reported in *Abrus*. Some type I RIPs have been shown to inhibit fungal growth[19]. Likewise, RIPs have been found to have insecticidal properties against *Coleopteran* and *Lepidopteran* species[20,21]. It has also been suggested that as little as one RIP molecule per cell is capable of shutting down protein synthesis[22]. Thus this specie, like many other plants, has the potential to be used as fungicide.

Most potent inhibitory effect of crude seed extract with inhibition zone 34 mm was found against *Alternaria* while it was of 22 mm against *Fusarium*. Fraction of gel filtration exhibited low activity against both the fungi. Dialysed sample showed antifungal capacity against both the fungi that is quite comparable to terbisil.

Biosorption characteristics: Fig. 1 shows the results of coagulation effect of *Abrus precatorius* seeds in salt, water and buffer extracts in comparison with alum, activated carbon and distilled water. The findings indicated that plant seed proteins coagulated well the particles in the samples leading to a clear supernatant. The coagulation effect was far better at low turbidity but was best with distilled water extract (DWE) at high turbidity because floc-settlement was faster and was as good as that of alum (Fig. 2). This can be explained with the fact that *Abrus precatorius* seeds exhibited strong antimicrobial activity. It is worth noting that crude buffer extract (CBE) and alum showed comparable activity in both low and high initial turbidity. A significant difference in attenuation (OD500) was observed between the coagulants and the control. It is appreciating that results of kinetic experiment using CWE and CSE showed higher coagulation activity than activated

TABLE-1
ANTIFUNGAL ACTIVITIES OF DIFFERENT EXTRACTS OF *Abrus precatorius* AGAINST *Fusarium solani* AND *Alternaria solani*

Sample	*Fusarium solani*		*Alternaria solani*	
	Zone diameter (mm)	Representation	Zone diameter (mm)	Representation
Crude extract	22	+ +	34	+ + +
Ethanol extract	16	+	18	+
Residue after $(NH_4)_2SO_4$ precipitation	24	+ +	28	+ +
Dialysed	28	+ +	36	+ + +
Peak 1(2^{nd} fraction) of Gel filtration	18	+	18	+
Positive control (Terbisil)	32	+ + +	38	+ + +
Negative control (Autoclave water)	0	-	0	-

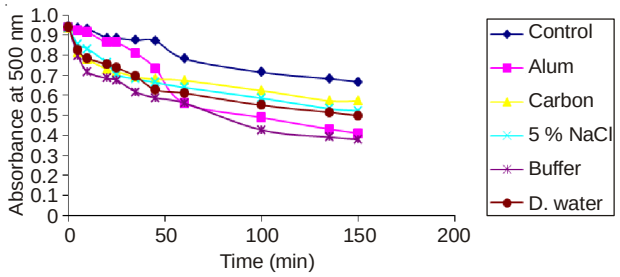

Fig. 1. Coagulation kinetics of the extracts of *Abrus precatorius* at low turbidity

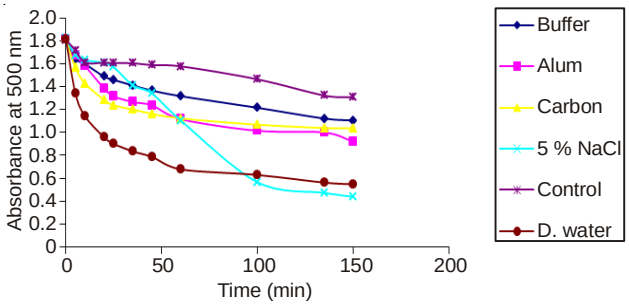

Fig. 2. Coagulation kinetics of the extracts of *Abrus precatorius* at high turbidity

carbon for low initial turbidity. The active component from an aqueous salt extraction was not only a protein, polysaccharide or lipid, but also an organic polyelectrolyte[23]. The proposed mechanism for turbidity removal by *Abrus* seed extracts in this study is a combination of particle-charge neutralization and micro-bridging or an electrostatic patch mechanism based on the results of Zeta potential measurements.

A coagulation kinetics study determines the settling rate behaviour of different coagulants and used to distinguish active and inactive coagulants in a short period of time. Natural biosorbents and flocculant proteins from different plants have the potential for the removal of different toxic and hazardous materials from the waste domestic and contaminated drinking water. Coulibaly *et al.*[24] exploited different fungi for wastewater treatment. Ekmekyapar *et al.*[25] used non-living lichen biomass of *C. rangiforms* and found it to be suitable biosorbent for removing Cu(II) from aqueous solutions. Southichak *et al.*[26] exploited *Phragmites australis* as a novel biosorbent for the removal of heavy metals from aqueous solution. *Moringa oleifera* seeds have also been used as natural flocculants to clarify drinking water[27] and for the removal of iron, manganese and *E. coli*[28].

Crude buffer extract of *Abrus* showed low absorbance which means high coagulation activity that is comparable to alum. D. water extract and crude salt extract exhibited higher coagulation effect than carbon.

Crude water extract and crude salt extract both showed low absorbance and higher coagulation activity than alum and carbon. Buffer extract showed coagulation activity comparable to alum.

A simple, scalable and a convenient coagulation activity assay is used which allows straight forward comparison of the characteristics and coagulation properties of water, buffer and salt crude extracts samples of *Abrus*. Specifically, the goal is to replace proprietary chemical coagulants such as aluminium sulphate (alum) with naturally derived coagulants from this plant and can be used as full or partial substitute for alum and activated carbon. There are several areas where this result could be applied, the most appealing of which is to develop technologies for treatment of surface waters subject to wide fluctuation in raw water turbidity and general quality and to make it destined for potable use and the treatment of wastewaters for agricultural re-use.

Conclusion

It is evident from this study that *Abrus precatorius* is a good source of antifungal activity. In view of these findings and the work done by many other researchers, it is proposed that *Abrus* should be placed on a high priority list for propagation and conservation.

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