Available online at www.ijtpr.com
International Journal of Toxicological and Pharmacological Research 2010, 2(1);26-29

ISSN: 0975 5130

Research Article

Antibacterial and Antifungal Activity of Methanolic Extract of *Abrus pulchellus* Wall and *Abrus precatorius* Linn - A Comparative Study

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ABSTRACT

The present study was carried out to investigate antibacterial and antifungal activity of seeds of *Abrus pulchellus* Wall and *Abrus precatorius* Linn. The powdered seed materials were extracted using methanol solvent. The antibacterial activity was tested against *Staphylococcus aureus* MTCC-902, *Escherichia coli* MTCC-405 and *Pseudomonas aeruginosa* MTCC-1934 by Agar well diffusion method. The antifungal activity was determined in terms of inhibition of mycotic infection of Jowar seeds using standard blotter method. *S. aureus* was inhibited to more extent than *E. coli* and *P. aeruginosa* as revealed by greater inhibition zone around the wells. Among extracts, *A. pulchellus* inhibited test bacteria to more extent than *A. precatorius*. Antifungal activity of extracts revealed inhibition of fungal growth on seeds. In extract treated seeds, 100% germination was recorded and seed infection was considerably lesser when compared to control (10% DMSO). Preliminary phytochemical analysis showed the presence of flavonoids, alkaloids and saponins in both the extracts. The antibacterial and antifungal activity of extracts may be due to the presence of phytochemicals in the crude methanolic extract. Further studies on isolation of active constituents responsible for the activities and field trials using extract treated seeds are under investigation.

Key words: *A. precatorius*, *A. pulchellus*, Agar well diffusion, Standard blotter method, Seed borne fungi

INTRODUCTION

Infectious diseases caused by bacteria, fungi, viruses, and parasites remain a major threat to public health, despite tremendous progress in human medicine. Their impact is particularly great in developing countries because of the relative unavailability of medicines and the emergence of widespread drug resistance [1]. Interest in natural products with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics [2]. Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. Higher plants, as sources of medicinal compounds, have continued to play a dominant role in the maintenance of human health since ancient times. Over 50% of all modern clinical drugs are of natural plant origin and natural products play an important role in drug development programs in the pharmaceutical industry [3]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids, and phenolic compounds [4]. Phytomedicines derived from plants have shown great promise in the treatment of various diseases including viral infections. Single and poly herbal preparations have been used throughout history for the treatment of various types of illness [5]. Plant derived natural products have received considerable attention in recent years due to their diverse pharmacological activities [6].

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Abrus precatorius Linn (Fabaceae) is distributed throughout India, ascending to an altitude of about 1050m in the outer Himalayas. It is called Indian Wild Liquorice, Jequirity, Crab's Eye and Precatory Bean in English. It is uterine stimulant, abortifacient and toxic. Seeds are teratogenic. A paste of seeds is applied on vitiligo patches. Along with other therapeutic applications, the *Ayurvedic Pharmacopoeia of India* has indicated the use of seeds in baldness. Seeds contain abrin, a toxalbumin, indole derivatives, anthocyanins, sterols, terpenes. Abrin causes agglutination of erythrocytes, haemolysis and enlargement of lymph glands. A nontoxic dose of abrin (1.25mcg/kg bodyweight), isolated from the seeds of red var., exhibited a noticeable increase in antibody-forming cells, bone marrow cellularity and alpha-esterase-positive bone marrow cells. Oral administration of agglutinins, isolated from the seeds, is useful in the treatment of hepatitis and AIDS. The seed extract exhibited antischistosomal activity in male hamsters. The methanolic extract of seeds inhibited themotility of human spermatozoa. The roots contain precol, abrol, glycyrrhizin (1.5%) and alkaloids—abrasine and precasine. The roots also contain triterpenoids—abruslactone A, methyl abrusgenate and abrusgenic acid. Alkaloids/bases present in the roots are also present in

Table-1: Phytoconstituents present in methanolic extract of *A. precatorius* and *A. pulchellus*

Phytoconstituent	*A. precatorius*	*A. pulchellus*
Tannins	-	-
Flavonoids	+	+
Alkaloids	+	+
Saponins	+	+
Glycosides	-	+
Steroids	+	-
Terpenoids	-	-

leaves and stems. *A. fruticulosus* Wall. Ex Wight and Arn. synonym *A. pulchellus* Wall., *A. laevigatus* E. May. (Shveta Gunjaa) is also used for the same medicinal purposes as *A. precatorius* [7]. *Abrus pulchellus* Wall. (Fabaceae) is a twinning shrub commonly known as Bili gulaganji in Kannada and Rosary pea in English. Leaves are pinnately compound, leaflets 9 to 12 pairs, oblong, leaf rachis 12 cm long, stipulate, adnate or free lateral stipules are present, entire margin, leaf apex obtuse, reticulate venation. Flowers are in axillary long racemes, calyx 5 lobed, fused, corolla rose/white. Fruit is a pod, flat appressed and pubescent. Seeds are pale yellow/white [8]. The present study was carried out to investigate antibacterial and antifungal activity of methanolic extract of *A. precatorius* and *A. pulchellus*.

MATERIALS AND METHODS

Collection and identification of plant material

The seeds of plant material of *Abrus pulchellus* (Voucher no. PK/SRNMN/301) and *Abrus precatorius* (PK/SRNMN/302) were collected from a local vendor, authenticated in department of Botany, S.R.N.M.N College of Applied Sciences, Shivamogga and the voucher specimen were deposited for future reference.

Extraction and Phytochemical analysis

For extraction, about 50g of the dried and powdered seed material was taken and added to 100ml of methanol. The mixtures were sonicated for 30 min, and then left at room temperature overnight. The extracts were filtered over Whatman No 1 filter paper, and the filtrates were concentrated under reduced pressure to pasty mass [9,10]. The methanol extract was subjected to chemical tests to screen the presence of various secondary metabolites [11, 12].

Table-2: Antibacterial activity of extract of *A. precatorius* and *A. pulchellus*

Treatment	Zone of inhibition in mm		
	E. coli	***P. aeruginosa***	***S. aureus***
A. precatorius	1.5	1.7	1.8
A. pulchellus	1.9	1.9	2.2
Standard	2.4	2.5	2.8
Control (DMSO)	-	-	-

Results are average of three trials

Preparation of extracts

DMSO was used to prepare the extracts for analyses. The condensed extracts obtained after extraction were dissolved in 10% DMSO. Extracts at concentration 20mg/ml of DMSO were used to screen antibacterial and antifungal activity.

Antibacterial activity of methanol extracts by Agar well diffusion method

The antibacterial activity was tested against *Staphylococcus aureus* MTCC-902, *Escherichia coli* MTCC-405 and *Pseudomonas aeruginosa* MTCC-1934 by Agar well diffusion method [13]. The test bacteria were obtained from IMTECH, Chandigarh, INDIA Twenty four hours old nutrient broth cultures of test bacteria were aseptically swabbed on sterile Nutrient agar plates. Wells of 6 mm diameter were made aseptically in the inoculated plates and the methanol extract (20mg/ml of 10% DMSO), Standard (Chloramphenicol, 1mg/ml) and Control (10% DMSO) were added into the respectively labeled wells. The plates were incubated at 37^{o}C for 24 hours in upright position. The experiment was carried in triplicates and the zone of inhibition was recorded.

Antifungal activity of methanol extracts by Standard blotter method

The antifungal activity of methanolic extracts in terms of inhibition of seed borne fungi was carried out using standard blotter method. A total of 400 seeds of Jowar (*Sorghum vulgare*) were soaked in extracts (20mg/ml of 10% DMSO) for one hour and placed on the moistened blotter in Petri dishes. The untreated seeds were soaked in 10% DMSO for one hour and plated on moist blotters and used as control. The extract- treated and untreated seeds were incubated in an incubator at 20°C for seven days. Seeds in blotter were examined for fungal growth and percentage seed germination after incubation [14,15]. The whole set of experiment was carried three times and the average values were recorded.

RESULTS

Preliminary phytochemical analysis showed the presence of flavonoids, alkaloids and saponins in both the extracts. In addition to these, steroids were detected in *A. precatorius* and glycosides in *A. pulchellus*. Phytoconstituents namely tannins and terpenoids were not detected in both the extracts (Table-1).

The results of antibacterial activity were recorded as presence or absence of zones of inhibition around the well. The inhibitory zone around the well indicated the absence of bacterial growth and it as reported as positive and absence of zone as negative [16]. The antibacterial activity of methanolic extract of *A. precatorius* and *A. pulchellus* indicated that the crude solvent extracts possess antibacterial activities towards the Gram-positive bacterium *S. aureus* to more extent than Gram negative bacteria namely *E. coli* and *P. aeruginosa*. Among Gram negative bacteria, *P. aeruginosa* exhibited more sensitivity to majority of extracts than *E. coli*. Among extracts, *A. pulchellus* inhibited test bacteria to more extent than *A. precatorius*. Standard drug also exhibited marked activity against Gram positive bacterium than Gram negative bacteria and the activity was greater when compared to solvent extracts (Table-2).

Table-3 shows antifungal activity, in terms of inhibition of fungal growth on seeds, of methanolic extracts of *A. pulchellus* and *A. precatorius*. The number of seeds

Table-3: Antifungal activity of extract of *A. precatorius* and *A. pulchellus*

Treatment	Total seeds	Germinated seeds	Infected seeds	% seed germination	% seed infection
A. precatorius	20	20	2	100	10
A. pulchellus	20	20	1	100	5
Control (10% DMSO)	20	19	5	95	25

Results are average of three trials

infected and germinated was counted and the percentage infection and germination was calculated. Seeds that were soaked in 10% DMSO (served as control) showed 95% germination and 1/4th of seeds were infected by fungi. In extract treated seeds, 100% germination was recorded and seed infection was considerably lesser when compared to control. The percentage of seed infection was found to be 10% in case of *A. precatorius* and 5% in case of *A. pulchellus*.

DISCUSSION

The results of antibacterial activity of methanolic extracts of *A. precatorius* and *A. pulchellus* are consistent with previous reports regarding Gram-positive bacteria. The resistance of Gram-negative bacteria to plant extracts was not unexpected as, in general, this class of bacteria is more resistant than Gram-positive bacteria. Such resistance could be due to the permeability barrier provided by the cell wall or to the membrane accumulation mechanism [17]. It appears that overall the bacteria were found to be sensitive to solvent extracts. The reasons for this could be that the components from the plant active against microorganisms are most often obtained through solvent extraction. Antimicrobial activity of tannins [18,19], flavonoids [20,21], saponins [22,23], terpenoids [24], alkaloids [25,26] have been documented. In the present study, phytoconstituents namely flavonoids, alkaloids, glycosides, steroids and saponins were detected in the extracts which may account for the activities.

The antifungal activity of methanolic extracts of *A. precatorius* and *A. pulchellus* showed the potential of extracts in controlling seed borne fungi. The percentage of seed infection was found to be less in case of extract treated seeds. An additional observation also was made in the study that the extract treated seeds showed germination which was greater than that of control. In recent years much attention has been given to nonchemical systems for seed treatment to protect them against seed-borne pathogens. Plant extracts have played significant role in the inhibition of seed-borne pathogens and in the improvement of seed quality and field emergence of plant seeds [14].

The use of higher plants and their extracts to treat infections is an age-old practice. Traditional medicinal practice has been known for centuries in many parts of the world. Ayurveda, the science of life, prevention and longevity is the oldest and most holistic medical system available on the planet today. Herbal medicines are gaining growing interest because of their cost effective and eco-friendly attributes [27]. Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes.

CONCLUSION

A marked antibacterial activity was observed in this study. The inhibition of bacteria tested by the extracts of *A. pulchellus* and *A. precatorius* may be exploited in treatment of various diseases caused by these bacteria. Inhibition of seed borne fungi in extract treated seeds reflects the possible long time storage of seeds on dressing with the extracts. Enhancement in germination of seeds by extracts may improve the better emergence of seeds in fields. Further studies on isolation of active constituents responsible for the activities and field trials using extract treated seeds are under investigation.

ACKNOWLEDGEMENT

The authors express their sincere thanks to Head of the Dept. of Microbiology and Principal, S.R.N.M.N College of Applied Sciences for providing all facilities. Authors are also grateful to NES for giving the moral support to conduct the work..

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