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ABRUS PRECATORIUS SEED COAT: PHYTOCHEMICAL SCREENING AND ANTIBACTERIAL EVALUATION

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ABSTRACT
Seed of *Abrus precatorius* Linn (*A, precatorius*), a known poisonous drug, is used extensively in numerous Ayurvedic formulations with great therapeutic impact. The medicinal properties of the plants are determined by the presence of secondary metabolites (phytochemicals) in it. In the present study the phytochemical analysis, antioxidant and antibacterial activity in the aqueous extract of seed coat of *Abrus precatorius* was carried out to evaluate the presence of phytochemicals and inhibitory effect of the seed coat against some gram +ve and gram –ve bacteria. The result of the phytochemical analysis revealed the presence of many bioactive chemical constituents such as alkaloids, saponins, glycosides, tannins, flavonoids and phenolic compounds in it. Antioxidant activity was determined by using 1, 1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging assays and the maximum percentage of scavenging activity was observed at 100µg/ml. The aqueous extract of seed coat showed significant inhibition in antibacterial studies. The compounds also showed less Cytotoxicity effects on human peripheral lymphocytes. Hence due to the presence of maximum numbers of the phytochemicals and inhibitory effects, *Abrus precatorius* seed coat might be used as antibacterial agent to control the plant and animal pathogens and also it can be concluded that, its aqueous extract could be considered for prevention and treatment of human diseases and its complications as potent antioxidant.

KEYWORDS: Seed coat**;** *Abrus precatorius*; Phytochemicals; Antioxidant; Antibacterial activity.

INTRODUCTION
Medicinal plants, from the dawn of civilization, have been a part and parcel of human society in combating diseases.[1] India is one of the major producers of herbs and herbal products. The large resources of medicinal plants have been used continuously for the treatment of several diseases.[2] The medicinal plants are the vital biological source whose parts (leaves, seeds, stem, roots, fruits, foliage etc.) extracts, decoctions, infusions, powders are used in the treatment of various diseases of humans, plants and animals.[3] Plant extracts are highly efficient against microbial infections.[4] It is estimated that over 70,000 plant species, from lichens to tall trees, have been utilized at one time or other for medicinal purposes.[5] Secondary metabolites such as alkaloids, tannins, flavonoids and phenolic compounds are rich in plants which have been found *in-vitro* to have antimicrobial properties.[6] World Health Organization (WHO) has estimated that around 80% of the world's population still relies on plant derived medicines, usually obtained from traditional healers, for its basic health care needs.[7] Herbal medicines are in great demand in the developed as well as developing countries for primary healthcare because of their wide biological and medicinal activities, greater safety margins and lesser costs.[8]

Abrus precatorius L. [*A. precatorius*] is a leguminous plant belongs to the Fabaceae family also known as Indian liquorices, Jequirity, Crab eye, saga-saga, Ratti, Glycyrrhizin glabra, among others and is indigenous to India. The plant grows wild in thickets, farms, secondary clearings, occasionally in hedges and extensively in dry climates of tropical and subtropical regions, such as India, Sri Lanka, Nigeria and West Indies. The name Abrus, meaning beautiful or graceful, is used to describe the appearance of the seed.[9] *A. precatorius* leaves, seeds and roots are used for medicinal purposes and it is a practice from ancient times.[10] The leaves and roots contain glycyrrhizin, the principal factor of licorice. These tissues prepared in several ways are used to treat coughs and a number of other ailments.[11] Abrus seeds have also the potential of good insecticide and antimicrobial activity.[12] Seeds are considered as antimicrobial, abortifacient, anodyne, diuretic, expectorant, emollient, emetic, febrifuge, hemostat, laxative, purgative, vermifuge, refrigerant, sedative,

antidote and used in various ailments to cure cough, headache, fever, conjunctivitis, convulsion, diarrhoea, gastritis, gonorrhea, jaundice, malaria, ophthalmia, rheumatism, nightblindness, diabetes and chronic nephritis. Dried Abrus seeds are taken orally as an aphrodisiac.[13,14] Abrus seeds are also taken for tuberculosis and painful swellings.[15] The seeds are descried as poisonous, and used internally in affections of the nervous system and externally, in skin diseases, ulcers, affections of the hair. The seeds reduced to a paste are recommended to be applied locally in sciatica, stiffness of the shoulder joint, paralysis and other nervous diseases. In alopesia a paste of the seed is recommended to be rubbed on the bare scale. Taken internally by women, the seed disturbs the uterine functions and prevents conception. Reduced to a paste they are used for contusion and inflammation.[16] Antifertility activity of *A. prectorius* root as well as seed extracts was found and seeds are used as an antifertility agent.[17,18]

Ayurveda recommends the administration of *A. precatorius*, in diseases like alopecia, edema, helminthes, skin diseases, itching, urinary disorders etc., after being passed through specific purificatory procedures.[19] Leaves, stem, seed, and seed oil of *A. precatorius* have been reported for their antibacterial and antifungal activities.[19-24] The antibacterial effect of *A. precatorius* seed coat hasn't yet been reported. Hence the present investigation is focused to screen the phytochemical, antioxidant activity and antibacterial activity of seed coat against clinically important bacterial strains.

MATERIALS AND METHODS

Collection of the plant material

The seeds of *A. precatorius* were collected during mid-February 2014 from Mysore, Karnataka, India. The seed coat was separated by crushing the seeds using a mortar and pestle and was stored in air tight containers until further use.

Preparation of the extract

1g of the seed coat was boiled in 20ml of water and the extract was examined for different biological activities.

Qualitative Phytochemical analysis

Phytochemical analysis of aqueous extracts of seed coat of *A. precatorius* was conducted following the standard procedures.

Test for Alkaloides

This was performed according to the method of Magadula and Tewtrakul.[25] In this test, about 1ml of the plant extract was dissolved in 5 ml of 27% HCl and warmed on steam bath. The filtrate (1ml) was mixed with drops of Dragendorff's reagent. Reddish orange precipitation was considered as indicative of the presence of alkaloids.

Test for saponins (frothing test)

This was carried out according to the method of Horbone.[26] 3ml of plant extract was taken in test tube and shaken vigorously. It was then allowed to stand for about a minute and observation was made for the formation of stable froths.

Test for cardiac glycosides

In this test, 2ml of plant extract was taken in a test tube and mixed with CCl_4, and 1ml of concentrated H_2SO_4 which was run along the side of the tube. Observation was made for brown coloration.

Test for flavonoids

To 1ml of the plant extract in a test tube, 1ml of 5% lead acetate was added and allowed to stand. Observation was made for any precipitation.

Test for tannins

To 2ml of the plant extract, three drops of 10% $FeCl_3$ was added and observation was made for the appearance of blackish-blue or blackish green coloration.

Estimation of total flavonoids by aluminum chloride method

The aluminum chloride colorimetric method was followed from the procedure described by Woisky and Salatino with slight modifications.[27] Quercetin was used to make the calibration curve; 10 mg of quercetin was dissolved in 80% ethanol and then diluted to 25, 50 and 100 µg/ml. The diluted standard solutions (0.5 mL) were mixed separately with 1.5 mL of 95% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After incubation at room temperature for 30 minutes, the absorbance of the reaction mixture was measured at 415 nm with a Shimadzu UV-160A spectrophotometer (Kyoto, Japan). The amount of 10% aluminum chloride was substituted by the same amount of distilled water in blank.

Estimation Of total phenolics by FolinCiocalteu method

The total phenolics in the extract were estimated using Folin-Ciocalteu method as described by Kujala et al.[28] 5 ml of Folin-Ciocalteu (Sigma-Aldrich) and 4 ml Sodium carbonate (7% w/v) was added to each sample solution (1.0 ml) and the standard (gallic acid) and shaken. The solution was allowed to stand for 30 minutes in the dark at room temperature, after which absorbance was measured at 765 nm using a spectrophotometer. The amount of total phenolics was expressed as gallic acid equivalent (GAE) in milligram per gram dry plant extract using the expression;

$C = c \times V/m$

Determination of antioxidant activity using DPPH radical scavenging activity

The antioxidant activity of the extracts was measured by determining the hydrogen donating or radical scavenging ability, using the stable radical, DPPH as reported

previously.[29] An aliquot (120µL) of 0.25 mM DPPH solution in methanol and 10µL of extract at increasing concentrations (10, 20, 30, 40, 50µg/mL) were mixed together vigorously and left at room temperature in the dark. The absorbance was measured at 518 nm after 30 min against various concentrations of the extracts in methanol as blanks and DPPH in methanol without extract as the control. The standard synthetic antioxidant, butylhydroxytoluene was used as the positive control.
% of inhibition is calculated using the formula,

$$\frac{\text{Absorbance of Control} - \text{absorbance of Test}}{\text{Absorbance of Control}} \times 100$$

Cytoxicity Assay

Isolation of human peripheral lymphocytes

The peripheral lymphocytes were isolated from 10 to 15 ml of freshly drawn venous blood from healthy male donors aged between 22-26 years. Blood was collected in anticoagulant Acid Citrate Dextrose (ACD, 85mM Citric acid, 71mM trisodium Citrate, 165mM D-Glucose) in the ratio of 5:1. To these four volumes of hemolysing buffer (0.85% NH_4Cl in 10mM Tris buffer, pH 7.4) was added, mixed well and incubated at 40˚C for 30 minutes.

Then the cells were centrifuged at 1200 rpm for 12 minutes, the supernatant was discarded, pellet was washed thrice with 10 ml of Hanks Balance Salt solution (HBBS, 137mM KCl, 8.5mm phosphate buffer pH 7.4, 0.8mm $MgS0_4$ and 5mM D-Glucose) and suspended in the same buffer solution. Cells were suspended in HBBS and it is stored at 40˚C.[30]

Cell viability test

The cell viability was determined by trypan dye blue exclusion method. To 10 µl of cell suspension an equal volume of 0.4% trypan blue dye was added. The cells were then transferred to haemocytometer and the cell number was counted. The dead cells being permeable to trypan blue appeared blue against white color of the viable cells. The percentage of viability was calculated as follows.

$$\text{Percentage of Viability} = \frac{\text{Number of viable cells}}{\text{Total number of cells}} \times 100$$

Antimicrobial activity

Bacterial Strains and Culture Conditions

Four strains of bacteria were used for antibacterial evaluation against the seed coat extract. The strains were E.coli, Pseudomonas fluorescence (Gram –ve bacteria) and Micrococcus luteus, Bacillus subtilis (Gram +ve bacteria) which were obtained from PG Dept. of Biotechnology, JSS College, Ooty road, Mysore. The strains were maintained on Mueller-Hinton (MH) agar and were diluted periodically with fresh broth until A_{600} reaches 0.05.

Parallely, the sample to be assayed are dissolved in their respective solvents and stock solutions (100mg/ml) were prepared followed by preparation of working standard solutions of required concentration.

Antimicrobial Activity Assay

Known concentrations of the sample (lµg, 10µg, 50µg, 100µg, 200µg, 300µg, 400µg, 500µg) were loaded to separate wells in the fresh 96 well microtiter plate and Negative Control was maintained for each pathogen where it carries no sample (0µg). All the assays were carried in triplicates for each bacterial culture. Then the volume was made up to 50µl with sterile distilled water in each well. 100µl of the each diluted bacterial cultures (A_{600}=0.05) were dispensed to their respective wells (96 well polypropylene microtiter plate) in triplicates. A blank is maintained which contains sterile Muller Hinton's broth. The plates were covered with sterile aluminum foil to avoid contamination and were incubated at 37^0C for 18 hours in a refrigerated bacteriological incubator.

The plate was read at 600nm in UV-Visible microplate using Spectrophotometer (Photometric) with 10 seconds of shaking; the values obtained for each pathogen and sample of different concentrations were averaged and are negative with the empty broth (Blank). Results were represented in graph; MIC values and comparison with STD antibiotic (Carbenicillin) were tabulated.

Statistical Analysis

The results are presented as mean±SEM (standard error of mean) of three independent experiments performed in duplicate.

RESULTS AND DISCUSSION

Qualitative Phytochemical screening

The phytochemical active compounds of seed coat extract were qualitatively analyzed and the results are presented in Table 1. The data indicate that the aqueous extract of seed coat showed the presence of phytochemical active compounds such as alkaloids, saponins, cardiac glycosides flavonoids, tannins and phenols. The aqueous extract of *A. precatorius* found to contain a noticeable amount of total flavonoids i.e., 6mg/g which plays a major role in controlling antioxidants and total phenolic content of 2mg/g.

Table. 1: Preliminary Phytochemical screening of seed coat extract of *Abrus precatorius.*

Phytochemical components	Seed coat extract
Alkaloids	+
Saponins	-
Cardiac glycosides	+
Flavonoids	+
Tannins	+
Phenols	+

"+" =Present; "-" = absent

DPPH Scavenging Assay

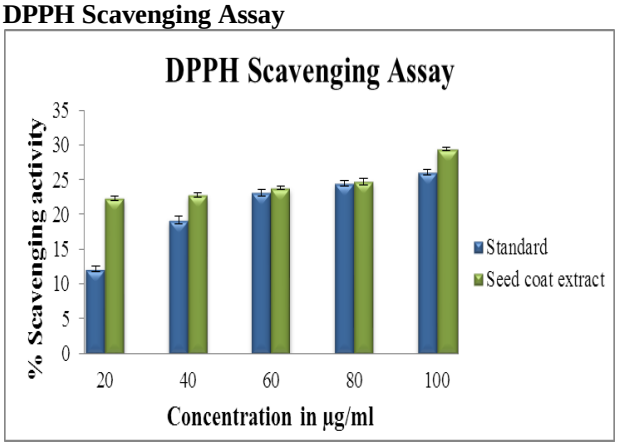

Fig. 1. DPPH Radical scavenging activity of *Abrus precatorius* seed coat.

The DPPH test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. It is evident from the graph that the percentage scavenging of DPPH radical was found to rise with increasing concentration of the samples. The results of the DPPH radical scavenging activity of seed coat extract of *A. precatorius* (Fig. 1) shows that it possesses very high percentage antioxidant activity when compared to ascorbic acid (standard). The maximum percentage of scavenging activity of the aqueous extract of seed coat was observed at100µg.

I. Cytotoxicity Assay

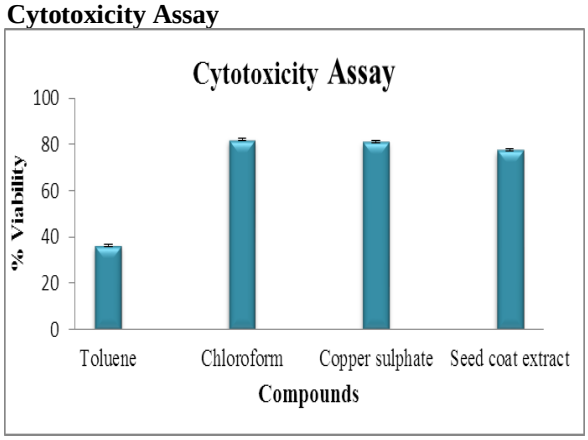

Fig. 2. Cytotoxicity Assay of *Abrus precatorius* seed coat.

Toulene, chloroform and copper sulphate were taken as standards to compare the percentage of viability against the seed coat extract (Fig. 2). The percentage of viability of the cells when treated with seed coat extract was found to be 77.63%, which is quite higher when compared to toluene that is less than chloroform and copper sulphate.

Antibacterial Activity

The anti-microbial effect of *A. precatorius* seed coat extract was evaluated against some Gram +ve and Gram –ve bacteria namely Escherichia coli, Pseudomonas fluorescens and Micrococcus luteus, Bacillus subtilis using broth dilution assay. The sample showed good anti-bacterial activity with the Minimum Inhibitory Concentrations (MIC) of 40µg, 80µg, 60µg and 80µg for *E. coli*, *P.fluorescence*, *M. luteus* and *B. subtilis* respectively (Table 2). These values are significantly high when compared to the other solvent extracts of *A. precatorius*. Extract from the stem and seed oil were potent against some of the gram-positive bacteria and Candida albicans but not against S. anginosus, E. faecalis and some gram-negative bacteria.[31] The presence of flavonoids, alkaloids and saponins in the methanolic extract may be responsible for the antibacterial and antifungal activity.[32] Antimicrobial activity of *A. precatorius* seed methanol extract was investigated against ten bacterial species. The extract revealed antibacterial activity towards almost all the bacterial microorganisms.[33]

Table. 2. Anti-bacterial activity of of *Abrus precatorius* seed coat.

Bacterial Strains	Minimum Inhibitory Concentration (MIC)	% growth inhibition compared to control
Escherichia coli	40µg	23.09
Pseudomonas fluorescens	80µg	47.92
Micrococcus luteus	60µg	32.06
Bacillus subtilis	80µg	12.49

Hence to attain the successful results in bio control of pathogens and screen the potential medicinal properties of *A. precatorius* seed coat, the present work was carried out. The investigation provides successful positive results.

CONCLUSION

A. precatorius has been explored exhaustively for its ethnomedicinal, phytochemical, pharmacological and ethnopharmacological applications. From the earlier, it is evident that *A. precatorius* has been used ethnomedicinally as a valuable therapeutic agent for a

various diseases. Results of the current study reveal that *A. precatorius seed coat* is a potential source of phytochemicals and antioxidants which are responsible for the antioxidant activity. From this study, it may be concluded that, aqueous extract of seed coat shows greater antibacterial effect. Myriads of phytocompounds found in this plant are responsible for its pharmacological activities. However several therapeutic claims have been reported as the plant is gaining widespread popularity in terms of traditional medicinal uses. Therefore more investigations are suggested to validate these claims and even identify new bioactive components with potential therapeutic benefits.

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