



Phytochemical Evaluation and *In-vitro* Anti Bacterial Activity of Dried Seeds of *Abrus precatorius*

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ABSTRACT

Abrus precatorius is a folklore medicinal plant. *Abrus precatorius* commonly known as Gunja has been used for therapeutic purpose since vaidic period. The roots, seeds and leaves are used in traditional & folklore Medicine. The study was carried out to ascertain the antibacterial properties present in different extracts of dried scale seeds of *Abrus precatorius*. The Antibacterial testing of seeds extract *Abrus precatorius* was evaluated by Agar well diffusion method using gram positive bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, gram negative bacteria like *Escherichia coli*, *Klebsiella pneumoniae*. Amongst the test extracts, the results suggested that, isopropyl alcohol and ethyl acetate extracts of seeds showed significant antibacterial activity compared with standard drug. It is considered as a valuable source of natural products for development of medicines against various diseases.

Keywords: *Abrus precatorius*, Gentamycin, Flavonoids, Anthraquinons.

INTRODUCTION

Traditionally plants are used as drugs and have genuine utility because they contain some components which have healing and pain relieving properties. For the primary health care about 80% of rural population depends on these medicinal plants. Usage of plants for the treatment of diseases is as old as human species which produces various secondary metabolites like alkaloids, terpenoids, steroids, phenols, tannins, flavonoids, and other metabolites and which have antimicrobial and antioxidant types of properties. Plants are the main source of food and rich nutrients content. Traditional societies around the world had deep knowledge of various plants and their medicinal value, though they did not possess knowledge on components present and their mode of action. Medicinal properties attributed to various herbs have paved way to the discovery of new drugs, as they are the reservoirs of potential chemical compounds. For the benefit of mankind it is necessary to prefer herbal usages to avoid chronic stress and synthetic drugs.

Herb is an immeasurable wealth of nature not only from the global environmental perspective but also from the medicinal point of view. It plays a significant role in ameliorating the disease resistant ability and combating against various unfavorable metabolic activities within the living system. Herbal medicine is the mainstay of about 75 – 80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. The chemical constituents present in them are a part of the physiological functions of living flora and hence they are believed to have better compatibility with the human body. The clinical efficacy of many existing antibiotics is

being threatened by the emergence of multidrug-resistant pathogens. There is a continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections.

Botanical description

A woody twinning plant of the leguminosae family with characteristic red and black seeds. The leaves are pinnate and glabrous, with many leaflets (12 or more) arranged in pairs. The leaflets are oblong, measuring 2.5-cm long and 1.5-cm wide. The plant bears orange-pink flowers, which occur as clusters in short racemes that are sometimes yellowish or reddish purple in color, small and typically pea like. The plant produces short and stout brownish pods, which curl back on opening to reveal pendulous red and black seeds, 4 to 6 peas in a pod.

Origin and distribution

It grows wild in thickets, farms, and secondary clearings, and sometimes in hedges. It is most common in rather dry areas at low elevation throughout the tropics and subtropics. The plant *Abrus precatorius* Linn popularly known as Rosary pea, jequirity bean belong to the family leguminosae (Fabaceae) is found throughout India in hedges and bushes in exposed areas. The seeds are deadly poisonous but it has been reported that the toxic form of abrin gets converted to mitogenic form upon long refrigerated storage. Usually seeds are of two types one is scarlet with black spot and the other variety is pure white. *Abrus precatorius* L. belonging to family Fabaceae is a leguminous climber popularly known as Rati in Hindi, Crab's eye in English, Gunja in Sanskrit. The plant has



been used in Hindu medicines from very early times, as well as in China and other ancient cultures.

Abrus precatorius is one of the important herbs commonly known as Indian licorice belonging to family Fabaceae. It is reported to have a broad range of therapeutic effects, like anti-bacterial, anti-fungal, anti-tumor, analgesic, anti-inflammatory, anti-spasmodic, anti-diabetic, anti-serotonergic, anti-migraine, including treatment of inflammation, ulcers, wounds, throat scratches and sores. It is now considered as a valuable source of unique natural products for development of medicines against various diseases and also for the development of industrial products but still additional information needs to be updated.

Plant profile:

Taxonomical classification

Kingdom	:	Plantae
Division	:	Magnoliophyta
Order	:	Fabales
Family	:	Fabaceae
Subfamily	:	Faboideae
Tribe	:	Abreae
Genus	:	<i>Abrus</i>
Species	:	<i>Abrus precatorius</i>



Figure 1: *Abrus precatorius* seeds

MATERIALS AND METHODS

Collection of plant material

The seeds of *Abrus precatorius* were collected from surrounding places of Rangareddy Dist.

Phytochemical Evaluation

The different chemical tests were performed for establishing profile of the extract for its chemical composition; the following chemical tests for various phytoconstituents in the different extracts were carried out as described below.

(A) Test for alkaloids

i) Dragendroff's Test

In a test tube containing 1ml of extract, few drops of Dragendroff's reagent was added and the color

developed was noticed. Appearance of orange color indicates the presence of alkaloids.

ii) **Wagner's Test:** To the extract, 2 ml of Wagner's reagent was added; the formation of a reddish brown precipitate indicates the presence of alkaloids.

ii) **Mayer's Test:** To the extract, 2 ml of Mayer's reagent was added, a dull white precipitate revealed the presence of alkaloids.

iv) **Hager's Test:** To the extract, 2 ml of Hager's reagent was added; the formation of yellow precipitate confirmed the presence of alkaloids.

(B) Test for terpenoids

i) **Salkowski test:** To 1 ml of extract, tin (one bit) and thionyl chloride were added. Appearance of pink color indicates the presence of terpenoids.

ii) **Hirshonn reaction:** When the substance was heated with trichloro acetic acid, red to purple colour was observed.

(C) Test for steroids

i) **Liebermann Burchard Test:** To 1ml of extract, 1ml of glacial acetic acid and 1ml of acetic anhydride and two drops of concentrated sulphuric acid were added. The solution become red, then blue and finally bluish green indicates the presence of steroids.

(D) Test for coumarins

To 1 ml of extract, 1 ml of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow color.

(E) Test for tannins

i) To few mg of extract, ferric chloride was added, formation of a dark blue or greenish black color showed the presence of tannins.

ii) The extract was mixed with basic lead acetate solution; formation of white precipitate indicated the presence of tannins.

(F) Test for saponins

To 1 ml of the extract, 5 ml of water was added and the tube was shaken vigorously. Copious lather formation indicates the presence of saponins.

(G) Test for flavones

i) **Shinoda Test:** To the extract, a few magnesium turnings and 2 drops of concentrated hydrochloric acid were added, formation of red color showed the presence of flavones.

ii) To the extract, 10% sodium hydroxide or ammonia was added; dark yellow color shows the presence of flavones.

(H) Test for quinones



To 1 ml of the extract 1 ml of concentrated sulphuric acid was added. Formation of red color shows the presence of quinones.

(I) Test for flavanones

i) To the extract, 10% sodium hydroxide was added and the colour changes from yellow to orange, which indicates the presence of flavanones.

ii) To the extract, conc. sulphuric acid was added, and the colour changes from orange to crimson red, which indicates the presence of flavanones.

(J) Test for anthocyanins

i) To the extract, 10% sodium hydroxide was added, and the blue color shows the presence of anthocyanins.

ii) To the extract, conc. sulphuric acid was added, and the yellowish orange color confirms the presence of anthocyanins.

(K) Test for anthraquinones

Boritrager's test The extract was macerated with ether and after filtration, aqueous ammonia or caustic soda was added. Pink red or violet color in the aqueous layer after shaking indicates the presence of anthraquinones.

(L) Test for phenols

Ferric chloride test: To the extract, few drops of 10 % aqueous ferric chloride were added. Appearance of blue or green color indicates the presence of phenols.

(M) Test for proteins

i) **Biuret Test:** To the extract, 1 ml of 40% sodium hydroxide solution and two drops of one percent copper sulphate solution were added. Formation of violet color indicates the presence of proteins.

ii) **Xanthoprotein Test:** To the extract, 1 ml of concentrated nitric acid was added. A white precipitate was formed, it is then boiled and cooled. Then, 20% sodium hydroxide or ammonia was added. Orange color indicates the presence of aromatic amino acids.

iii) **Tannic Acid Test:** To the extract, 10% tannic acid was added. Formation of white precipitate indicates the presence of proteins.

(N) Test for carbohydrates

i) **Molisch's Test:** To the extract, 1 ml of alpha-naphthol solution, and concentrated sulphuric acid through the sides of test tube were added. Purple or reddish violet color at the junction of the two liquids revealed the presence of carbohydrates.

ii) **Fehling's Test:** To the extract, equal quantities of fehling's solution A and B were added and on

heating, formation of a brick red precipitate indicates the presence of carbohydrates.

iii) **Benedict's Test:** To 5 ml of Benedict's reagent, extract was added and boiled for two minutes and cooled. Formation of red precipitate showed the presence of carbohydrates.

(O) Test for amino acids

Ninhydrin test: Two drops of ninhydrin solution were added to the extract, a characteristic purple color indicates the presence of amino acids.

Procedure for extraction

Dried seeds of *Abrus precatorius* were ground to coarse powder. The powder was extracted with different solvents like isopropyl alcohol and ethyl acetate by soxhlation for 6 hours for the preparation of different extracts and the obtained extracts were subjected to antibacterial screening.

Microorganisms

The test organisms included for study were gram positive bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, gram negative bacteria like *Escherichia coli*, *Klebsiella pneumoniae*. All the bacterial strains were procured from Osmania University, Hyderabad, Telangana. The bacteria were grown in the nutrient broth at 37°C and maintained on nutrient agar slants at 4°C.

Bacterial media

Muller Hinton Media was mixed with distilled water and then sterilized in autoclave at 15lb pressure for 15 minutes. The sterilized media were poured into Petri dishes and allowed for solidification. The solidified plates were bored with 5mm diameter cork borer. The plates with wells were used for the antibacterial studies.

Antibacterial activity of the plant extracts

Different seeds extracts of *Abrus precatorius* at a concentration of 500µg/ml, 750µg/ml, 1000µg/ml were tested against the gram positive bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, gram negative bacteria like *Escherichia coli*, *Klebsiella pneumoniae* by Well Diffusion Method.

Well Diffusion Method

Antibacterial activity of the plant extract was tested using Well diffusion method. The prepared culture plates were inoculated with different selected strains of bacteria using streak plate method. Wells were made on the agar surface with 6mm cork borer. The dried extracts were dissolved in 95% of ethanol for preparation of different concentration ranges of extracts. The extracts were poured into the well using sterile syringe. The plates were incubated at 37°C ± 2°C for 24 hours for bacterial activity. The plates were observed for the zone clearance around the wells. The extracts of the dried scale leaves were used for the study. The extracts were dissolved in sterile



distilled water to form dilution such as 500µg/ml, 750µg/ml and 1000µg/ml. Each concentration of the extract was tested against different bacterial pathogens. Gentamycin at a concentration of 5µg/ml and 10µg/ml was used as standard antibacterial drug. The zone of inhibition was calculated by measuring the diameter of the inhibition zone around the well (in mm) including the

well diameter. The readings were taken in three different fixed directions in all three replicates and the average values were tabulated.

RESULTS AND DISCUSSION

Table 1: Preliminary phytochemical screening of *Abrus precatorius* seeds

Constituents	Pet ether Extract	Isopropanol extract	Ethyl acetate Extract	Alcohol Extract
Terpenoids	-	-	-	-
Saponins	+	+	-	+
Steroids	-	+	+	-
Phenols	-	-	+	-
Flavonoids	+	+	+	+
Coumarins	-	-	+	+
Reducing sugars	-	+	-	-
Alkaloids	+	-	+	+
Quinones	-	+	+	+
Tannins	+	+	+	+
Proteins	-	-	-	-
Amino acids	-	-	-	-
Anthraquinones	+	+	+	+

+ Present, - Absent

Anti-bacterial assay of the isopropyl alcohol, ethyl acetate extracts of dried seeds of *Abrus precatorius* exhibited dose dependent antibacterial activity against the tested microorganisms at three different concentrations of 500, 750 and 1000µg/ml. The potential sensitivity of the extracts was obtained against all the tested microorganisms and the zone of inhibition was recorded and presented in the table given below (Table 2). From the above study the zone of inhibition obtained was dose dependent and the activity shown by the isopropyl alcohol, ethyl acetate extracts of seeds of *Abrus precatorius* at a concentration of 1000µg/ml against gram positive bacteria like *Staphylococcus aureus*, *Bacillus subtilis*, and gram negative bacteria like *Escherichia coli*, *Klebsiella pneumoniae* strains involved in present study was more in comparison to Gentamycin at a concentration of 5µg/ml. The zone of inhibition shown by the extracts were tabulated in the below given below (Table 2). The antibacterial potential exhibited by seeds

extracts may be contributed to the presence of tannins, flavonoids and anthrax quinones in preliminary phytochemical investigations. Further study is needed to characterize the active principles.

CONCLUSION

From the above study, it is concluded that the seeds of *Abrus precatorius* may represent a new source of anti-bacterial with stable, biologically active components that can establish a scientific base for the use of this in modern medicine. These local ethno medical preparations of plant sources should be scientifically evaluated and then disseminated properly. This knowledge about the medicinal plants usage can also be extended to other fields like field of pharmacology. In view of the nature of the plant, more research work can be done on humans so that a drug with multifarious effects will be available in the future market.



Table 2: Zone of inhibition shown by the Gentamycin and the isopropyl alcohol, ethyl acetate extracts of dried seeds of *Abrus precatorius*

Micro organism	Zone of inhibition (mm)			
	GENTAMYCIN		EXTRACTS (1000µg/ml)	
	5µg/ml	10µg/ml	Isopropyl alcohol extract	Ethyl acetate extract
<i>Bacillus subtilis</i>	7.5 mm	9 mm	8 mm	7 mm
<i>Escherichia coli</i>	7 mm	9 mm	6.5 mm	6 mm
<i>Klebsiellapneumoniae</i>	7 mm	9 mm	8 mm	7 mm
<i>Staphylococcus aureus</i>	7.5 mm	9 mm	8 mm	8 mm



Figure 2: Zone of inhibition shown by the isopropyl alcohol and ethyl acetate extracts of seeds of *Abrus precatorius* on *Bacillus subtilis* bacteria

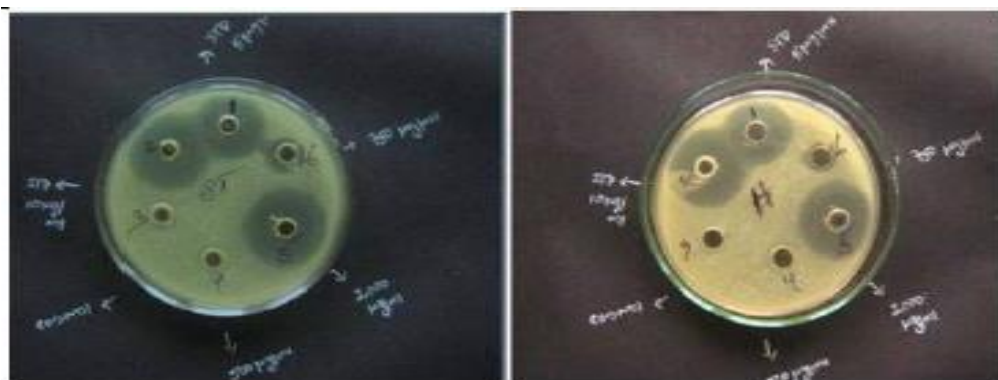


Figure 3: Zone of inhibition shown by the isopropyl alcohol and ethyl acetate extracts of seeds of *Abrus precatorius* on *klebsiella pneumonia* bacteria

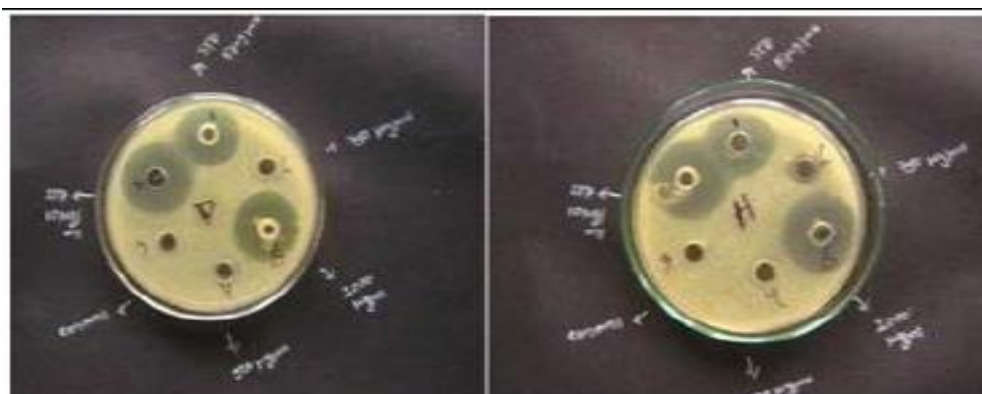


Figure 4: Zone of inhibition shown by the isopropyl alcohol and ethyl acetate extracts of seeds of *Abrus precatorius* on *Staphylococcus* bacteria

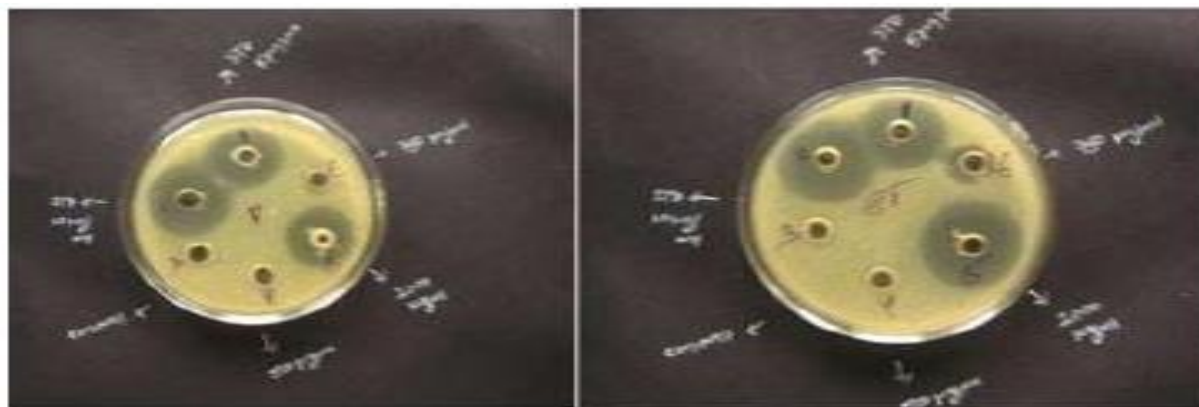


Figure 5: Zone of inhibition shown by the isopropyl alcohol and ethyl acetate extracts of seeds of *Abrus precatorius* on *E. coli* bacteria

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