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Original Research Article

Phytochemical Screening and Antibacterial Activity of Leaf Extracts of *Abrus precatorius* L.

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Abstract

Abrus precatorius, Linn commonly known as jequirity bean or rosary pea, is a herbaceous flowering plant in the bean family Fabaceae. The plant is best known for its leaves and seeds, which are used as beads and in percussion instruments, and which are toxic because of the presence of abrin. The phytochemical evaluation of the different extract of the *Abrus precatorius* leaves shows the presence of carbohydrates, alkaloids, steroids, sterols, flavanoids, tannins, phenolic compounds proteins and amino acids, fixed oil, and the absence of glycoside, saponin, and anthraquinone. This study aims to determine the antibacterial activity of extracts and fractions from leaves on the growth of *Staphylococcus*, *E.coli*, and *Pseudomonas.* The leaf powder was macerated using 96% ethanol, chloroform, petroleum ether, and aguish then fractionated using as solvent. The antibacterial activity test using the diffusion method showed that the extract of leaves had antibacterial activity against *Staphyloococcus E.coli* and *Pseudomonas*. The most active fraction was the ethanol fraction, with a concentration of 50% with an average inhibition zone diameter of 12.2 mm.

Keywords: *Abrus precatorius*, Phytochemical study, antibacterial activity, Medicinal uses.

1. INTRODUCTION

Abrus precatorius Linn is a herbaceous plant it belongs to the family Fabaceae. The plant leaves are compound pinnate turgid, oblong, obtuse, and truncated at both ends with seven to twenty four pairs of leaflets. The flowers are found in auxiliary racemes with a pink or pinkish white color the grains are found in pods that are 1.5 to 5.0 cm long turgid oblong and apprised hairy texture, and red poisonous seeds with a black mark at the base (Wan Suriyani, 2018, Wakawa *et al.*, 2017).

The plant is used in some traditional medicine and it is noted to have a wide range of therapeutic effects such as antibacterial, antifungal, antitumor, analgesic, antispasmodic, antidiabetic, antiserotonergic, antimigraine, and anti- inflammatory effects, including in the treatment of various disease like ulcers, wounds, scratches of the throat (Pavithra, *et al*., 2020). It is also considered to be a valuable source of natural products for the development of drugs against various diseases and for the development of industrial products (Sunday, *et al*., 2017). Traditional medicine has become puissant for the visionary therapeutics as Ayurveda, Chinese, Siddha, Unani, Korean, Kampo and Tribal practices are well admired. The Phytochemicals compounds phenols, flavonoids, alkaloids, steroids, glycosides, etc. and have been explored to the extreme for their medicinal properties (Ansari *et al.*, 2021). *Abrus precatorius* is considered to be a valuable source of natural products for the development of drugs against various diseases (Yusuf, 2017, Samuel, 2020).

A. precatorius against multidrug resistant wound pathogens recovered from patients from a referral hospital in Nigeria. The susceptibility of the recovered

isolates against ethanolic extracts of *Abrus precatorius* was evaluated (Sengupta, 2020). Sixty-six bacterial organisms were isolated and 3 different bacterial species were most predominately isolated *Staphylococcus aureus* followed by *Pseudomonas aeruginosa* and *Escherichia coli*. Interestingly, apart from its anti bacteria activity it also showed potent activity on multi drug resistant bacteria isolates. Finding from the present study substantiate the folkloric use *of A. precatorius* leaf extract for anti bacterial treatment (Oka Chiamaka *et al*., 2020).

2. MATERIALS AND METHODS

2.1 Sample collection

Abrus precatorius popularly known as "Kundumani one of the important medicinal plant. In the present study the plant leaves was collected in polythene bags during the November 2023 from in around Pachmalai and Tiruchy District Tamil nadu India.

2.2 Preparation of plant extract

The leaves were washed several times with distilled water to remove the traces of impurities. They were dried at room temperature and coarsely powdered. The powder was extracted with ethanol, petroleum ether, aqueous, chloroform for 48 hours. A semi solid extract was obtained after a complete elimination of solvent under reduced pressure. The leaf extract was stored in refrigerator until used.

2.3 Collection of culture

In the present investigation the following pure cultures of human pathogenic bacteria were collected from Microbial type culture collection (MTCC) Institute of Microbial Technology (IMTECH) Chandigarh, India. *Escherichia coli* (MTCC1574) *Pseudomonas* (MTCC1264) Staphylococcus aureus (MTCC0733).

1. Test for Alkaloids

i) Dragendroff's test

1 ml of the extract was added with 1ml of Dragendroff's reagent (potassium bismuth iodine solution). An orange and red precipitate indicates the presence of alkaloids.

ii) Mayer's test

1 ml of the extract was added with 1ml of Mayer's reagent (potassium mercuric iodide solution). Whitish yellow of cream coloured precipitate indicates the presence of alkaloids.

2. Test for Steroids

Two ml of acetic anhydride was added with 0.5 ml of extract of each sample with 2ml of H_2So_4. The colour changed from violet to blue or green in some samples indicates the presence of steroids.

3. Test for Terpenoids

Salkowski test

0.5 ml of the extract was added with 2ml of chloroform was added and 3ml of concentrated H_2SO_4 was also carefully added to form layer. A reddish brown coloured of the interface indicates the presence of terpenoids. This type of reaction was observed and recorded.

4. Test for Flavonoids

i) Alkaline reagent test

Few drops of dilute ammonia were added to a portion of the leaf extract and concentrated Hcl was also added. A yellow colouration indicated the presence of flavonoids. The reaction was observed and recorded.

ii) Zinc hydrochloric test

Few drops of extract were added with zinc dust and concentrated Hcl. The presence of red colouration indicates the presence of flavonoids.

iii) Aluminium test

Few drops of the extract was added with 1% aluminium solution was added. Formation of yellow coloured indicated the presence of flavonoids. The type of reaction was observed and recorded.

5. Test for Saponins

2 gram of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10ml of the filtrate was mixed with 5ml of distilled water and shaken vigorously until the formation of a stable persistent froth. The frothing was mixed with 3 drops of olive oil and again shaken vigorously, and observed for the formation of emulsion.

6. Test for Tannins

i) Lead acetate test:

A little quantity of the test solution was mixed with basic lead acetate solution Formation of white precipitate indicates the presence of tannins.

ii) 1ml of the extract was added with ferric chloride solution. Formation of a blue black or brownish green colour product shows the presence of tannins.
iii) A little quantity of the extract was tested with treated with aqueous ammonia is solution. A deep green colour indicates the presence of tannins and the type of reaction was observed and recorded.

7. Test for phlobatannins

The aqueous extract of each plant sample was boiled with 1% aqueous hydrochloric acid. Deposition of a red precipitate indicates the presence of phlobatannins.

8. Test for cardiac glycosides

Keller-killiani test

0.5ml g of extract was diluted with 5ml water and 2ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was under layed with 1ml of concentrated sulphuric acid. This was under layer with 1ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of a deoxy sugar, characteristic of cardenolides. A violet ring appeared below the brown ring, while in the acetic acid layer a greenish ring formed just above the brown ring and gradually spread throughout this layer.

9. Test for Proteins

Xanthoprotein test -One ml of the extract was added with 1ml of concentrated nitric acid. A white precipitate is formed and it is boiled and cooled. To that 20% of sodium hydroxide or ammonia was added. Orange colour indicates the presence of aromatic amino acids.

10. Test for Carbohydrates

2 ml of the extract was added with 1ml of Barfoed's reagent and boiled. Reddish brown precipitates indicate the presence of carbohytrates.

11. Test for Aminoacids

Ninhydrin test

3 ml of test solution was added with 3 drops of 5% ninhydrin solution in a tube and heated in boiling water bath for 10 minutes. Formation of purple or bluish colour indicates the presence of amino acids.

12. Test for reducing sugars

i) Fehling's test

1 ml of the extract was added with equal quantities of Fehlings solution A and B and upon heating, formation of red precipitate indicates the presence of sugars.

ii) Benedict's test

5 ml of Benedict's reagent was added with 1ml of extract solution and boiled for 2 minutes and cooled. Formation of red precipitate was showed the presence of sugars.

A few ml of extract was treated with saffranine solution. Pink colour formation indicates the presence of lignin.

13. Test for inulin

A few ml of the extract was added with 1ml solution of naphthol and 0.5 ml sulphuric acid. Formation of brownish red colour indicates the presence of inulin.

2.4 Culture medium

Nutrient agar medium is one of the most commonly used bacterial medium for several bacteriological strains. The medium components are peptone 5.0 g, beef extract 3.0g agar 15.0g sodium chloride 5.0g distilled water 1000ml and the pH was adjusted to 7.0. After mixing the ingredients in to the distilled water it was melted in the water bath and sterilized by autoclaving at 15 lbs pressure of 121°C for 15 minutes.

2.5 Antibacterial tests

Antibacterial activity was tested using a modification of the disc diffusion method originally described. A loop of bacteria from the agar slants was culture nutrient broth over night and spread with a sterile cotton swap into petriplates containing 10 ml of nutrient

agar. Sterile Whatman No.1 filter paper discs were the plant extract and placed on the culture plates and incubated at 25 or 37°C, depending on the bacteria. The solvent without extracts served as negative control. After 24 h of incubation, the diameter in mm of the inhibitory or clear zones MIC around the disc was recorded. Standard antibiotic tetracycline 30 mg Span Diagnostics Limited, Surat, India was used as reference or positive control.

3. RESULTS AND DISCUSSION

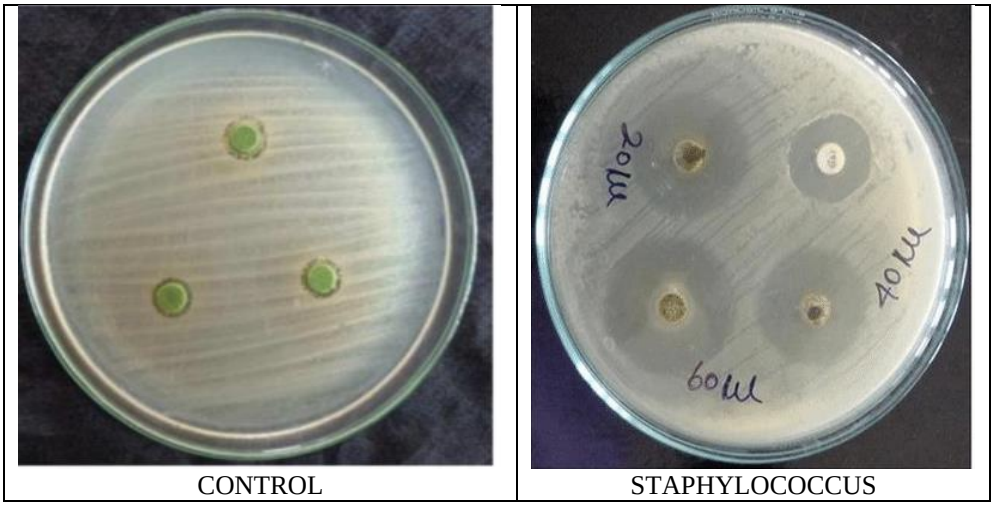
Abrus precarious deals with a of phytochemical constituents including alkaloids, carbohydrates, phytosterols, saponins, phenols, glycosides, tannins, flavonoids, terpenoids, phlobatannins, protein and free amino acids are present in the leaves (Table-1).

Table 1: Preliminary phytochemical analysis of plant extracts (Harborne *et al*., 1998)

S. No	Name of the Test	Phytochemical constituents	Ethanol	Petroleum ether	Chloroform
1	Alkaloid	Mayer's test Dragendroff's test Wagner Test	+ + +	+ + +	+ + +
2	Carbohydrate	Molish Test Fehling Test Benedicts Test	- + +	- - -	- + +
3	Steroidal Glycosides	Libermann's test Salkowaski test	+ +	+ +	+ +
4	Saponin	Foam Test	-	-	-
5	Tannin	Lead Acetate	+	+	+
6	Phenol	Phenol reagent	+	+	-
7	Terpenoid	H_2So_4 test	+	+	+
8	Flavonoids	Ammonia test	+	-	+
9	Coumarin	Sodium chloride test	-	-	-
10	Anthocyanin	NaOH	-	-	-

Antibacterial activity

Antibacterial activity of different solvent extracts of *A. precarious* was tested against three human pathogenic multi drug resistant bacteria *Escherchia coli*, *Pseudomonas* and *Staphylococcus aureus*. In the present study, extracts were derived from all the two selected populations, and their efficacy to inhibit the growth of pathogenic bacteria was studied. The antibacterial activities of the different solvent extracts obtained from the plants were studied by disc diffusion method shown in the (Table–2). The obtained results showed that the ethanol extracts inhibited significantly the growth of most of the organisms tested. Ethanol extracts of *A. precarious* showed maximum zone of inhibition against multidrug resistant strains of three common human pathogenic bacteria viz., *Escherichia coli Pseudomonas* and *Staphylococcus aureus.*



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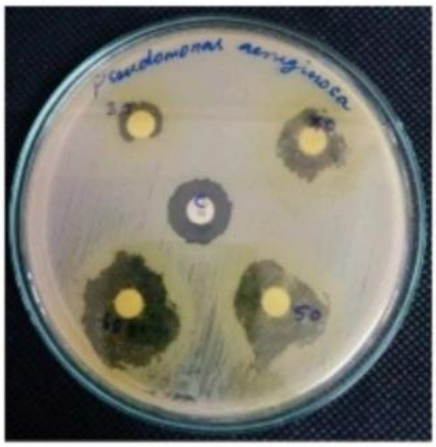

E.COLI

PSEUDOMONAS

Table-2: Antibacterial activity of the four different extracts of *A.precarious* in against two human pathogenic Bacteria

Plant Samples	Solvent	% of Sample	E. coli (Zone of Inhibition mm)	Pseudomonas (Zone of Inhibition mm)	Staphylococcus aureus. (Zone of Inhibition mm)	Control
Leaf	M	25	1.1	1.3	1.4	Nil
	E	50	2.3	2.5	1.9	Nil
	A	75	5.2	5.5	4.5	Nil
	C	100	6.3	7.0	6.5	Nil

Different populations of plant sample tested, accession from showed more activity than the other plant samples. Antibacterial activity of plant extracts and their efficiency was quantitatively assessed by the disc diffusion method by measuring the diameter of growth in inhibitory zones (Fig-1).

DISCUSSION

In the previous study he screened for phytochemical compounds present in *A. precatorius* and showed the presence of various secondary metabolites such as alkaloids, flavonoids, phenols, tannins and steroids (Kalaipriya, 2021 Abdul Kabir *et al*., 2016). The chemical constituent of plants is desirable because such information will be value for synthesis of complex chemical substances (Rupa, 2020). A. precatorius is recommended as a plant of phyto pharmaceutical importance on account of the abundance level of major phyto compounds that may be utilized by drug designer's following appropriate isolation and characterization procedures for the active compound present in the plant (Ihsan, 2000, Hussain, 2014).

The current result in below show antibactericidal activity of the leaf extract of *A. precatorius* was observed against the growth of the test organisms with mean values of zone of growth inhibition ranging from10 mm to 11mm against *E-coli*, 9 mm-12 mm against *Pseudomonas* and10 mm to12 mm against *S. aureus*, as compared to the standard drug chloramphenicol at 500 µg /ml. An exception was observed as not to exhibit significant antibacterial activity in ethanol fraction of the leaf extract against the bacterial strain *E-coli* at 500 µg /ml. Prashanth, *et al*., 2006 (Table-2). In previous study A. precatorius also exhibit antibacterial activity against the test organisms with mean values of zone of growth inhibit from10 mm to11 mm against *E.coli*, 10 mm1mm against *S. aureus* as compared to the standard drug chloramphenicol at 500 µg / ml (Boyan *et al*., 2016).

4. CONCLUSION

The plant commonly used for various kinds of diseases .*A. precarious* is regarded as a plant of high medicinal value because of its leaves and seed. *A. precarious* suggested various therapeutic use of plant reported such as antidepressant, anticancer, anti-inflammation. *Abrus precarious* deals with a of phytochemical constituents including alkaloids, carbohydrates, phytosterols, saponins, phenols, tannins, flavonoids, terpenoids, phlobatannins, protein and free amino acids are present in the leaf material. Ethanol extracts of *A. precarious* showed maximum zone of inhibition against multidrug resistant strains of three common human pathogenic bacteria viz., *Escherichia coli Pseudomonas. Staphylococcus aureus*. The plant can be extended for future investigation into the field of phytochemistry, ethnobotany, pharmacology and other biological action for drugs are present.

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