Phytochemical Screening and Estimation of Total Phenolic and Flavonoid Content in the Ethanolic Leaf Extract of Abrus precatorius L. Using UV Spectroscopy

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***Abstract*—Abrus precatorius L. (family Fabaceae), commonly known as rosary pea or Indian licorice, is a climbing medicinal plant widely used in traditional medicine for cough, fever, skin disorders and inflammatory conditions, although its seeds contain the toxic protein abrin. The present study was undertaken to investigate the qualitative phytochemical composition and to quantify the total phenolic content (TPC) and total flavonoid content (TFC) of the ethanolic leaf extract of A. precatorius using UV-Visible spectroscopy. The leaves were shade-dried, powdered and extracted by continuous hot Soxhlet extraction using ethanol-water (75:20). Preliminary phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins and glycosides. TPC was estimated by the Folin-Ciocalteu colorimetric method using gallic acid as standard (calibration equation y = 0.0610x + 1.221, R^2 = 0.9915, measured at 765 nm) and ranged from 0.00 to 12.30 mg gallic acid equivalent (GAE)/g of extract across the tested sample concentrations (AP1-AP5). TFC was estimated by the aluminium chloride colorimetric method using quercetin as standard (calibration equation y = 0.0773x + 0.823, R^2 = 0.9781, measured at 415 nm) and ranged from 0.862 to 3.763 mg quercetin equivalent (QE)/g of extract. The results confirm that the ethanolic leaf extract of A. precatorius is a promising natural source of phenolic and flavonoid antioxidants, supporting its traditional medicinal use.**

***Index Terms*—Abrus precatorius, phytochemical screening, total phenolic content, total flavonoid content, UV-Visible spectroscopy, Folin-Ciocalteu method**

I. INTRODUCTION

Medicinal plants remain one of the most important sources of bioactive phyto constituents used in the discovery and development of new therapeutic agents. Natural antioxidants present in plant materials have attracted considerable scientific interest because of their presumed safety and potential nutritional and therapeutic benefits, leading to a search for natural alternatives to synthetic antioxidants [1], [2].

Abrus precatorius L., commonly known as rosary pea, jequirity bean or Indian licorice (Gunja, Kundumani), is a perennial twining climber of the family Fabaceae, native to tropical and subtropical Asia and now widely distributed across Africa, the Americas and other warm regions of the world [3]. The plant has a long history of use in Ayurveda, Siddha and traditional Chinese medicine; the leaves and roots have traditionally been used to treat cough, sore throat, fever, skin diseases, wounds and inflammatory disorders [4]. The seeds, however, contain abrin, a highly toxic toxalbumin that inhibits protein synthesis and can cause severe poisoning; hence the plant must be handled only after appropriate processing and under expert supervision [5].

Phytochemical studies have shown that A. precatorius contains a wide range of secondary metabolites, including alkaloids, flavonoids, glycosides, saponins, triterpenoids, steroids and phenolic compounds, which are responsible for its reported antioxidant, anti-inflammatory, antimicrobial, analgesic, antidiabetic and hepatoprotective activities [6], [7]. Phenolic and flavonoid compounds are of particular interest because

of their well-documented free-radical scavenging and antioxidant properties, which contribute to the therapeutic potential of many plant-derived drugs [8]. UV-Visible spectrophotometry is a simple, rapid and widely used analytical technique for the quantitative estimation of such compounds, based on the Beer-Lambert relationship between absorbance and concentration [9]. The Folin-Ciocalteu colorimetric method (for total phenolic content) and the aluminium chloride colorimetric method (for total flavonoid content) are the most extensively employed UV-Visible-based assays for this purpose in medicinal plant research [10], [11].

The present study was therefore undertaken with the aim of investigating the pharmacognostical and phytochemical characteristics of the ethanolic leaf extract of A. precatorius, with the specific objectives of (i) performing preliminary qualitative phytochemical screening, (ii) evaluating key physicochemical parameters, and (iii) quantitatively estimating the total phenolic and total flavonoid content of the extract by UV-Visible spectroscopy, in order to provide scientific evidence supporting its traditional medicinal use and its potential as a natural source of antioxidant phytoconstituents.

II. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Fresh, healthy leaves of Abrus precatorius were collected from Ancheety (PIN 635104), Tamil Nadu, India. The collected material was cleaned to remove soil, dust and other unwanted matter, and the species was authenticated using standard botanical references.

2.2 Preparation of the Ethanolic Leaf Extract

The leaves were washed thoroughly under running water and shade-dried at room temperature for 2-3 weeks. The dried leaves were then ground to a fine powder using a mechanical grinder and passed through sieve no. 80. About 100 g of the dried powder was subjected to continuous hot extraction in a Soxhlet apparatus using ethanol-water (300 mL : 100 mL, approximately 75:20 v/v) at 50-80 °C for 12 hours. The extract was filtered through Whatman filter paper and concentrated using a heating mantle at 30-50 °C. The dried extract obtained was greenish in colour with a pungent odour and gummy consistency, and was stored in a clean container at approximately 30 °C. The percentage yield of the dried ethanolic extract was calculated to be 1.54% w/w on a dry-weight basis.

Fig. 1. Soxhlet extraction of Abrus precatorius leaves.

2.3 Preliminary Phytochemical Screening

The ethanolic leaf extract (20 mg/mL working solution in ethanol) was subjected to standard qualitative chemical tests for the detection of major phytoconstituents, with appropriate positive and negative controls, as summarised in Table I.

TABLE I. Reagents and Reactions Used for Preliminary Phytochemical Screening

Constituent	Test Performed	Observation Indicating Presence
Alkaloids	Mayer's test / Dragendorff's test	Cream/white or orange-red precipitate
Saponins	Froth test	Persistent froth (≥1 cm) for 10 min
Tannins & phenolics	Ferric chloride test / lead acetate test	Blue-black/greenish colour; white precipitate
Flavonoids	Shinoda test	Pink, red or orange colouration
Glycosides	Keller-Killiani test	Bluish-green/brown ring at the interface

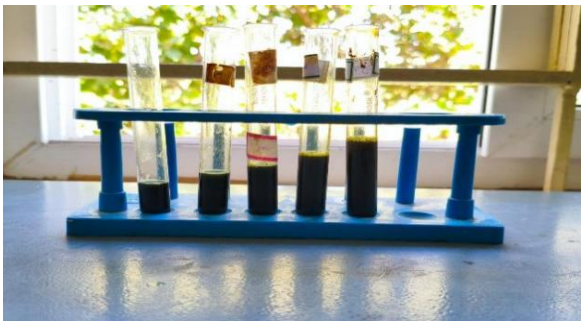

Fig. 2. Qualitative phytochemical test tubes of the ethanolic leaf extract.

2.4 Physicochemical Analysis

Physicochemical parameters, including pH, temperature-related decomposition behaviour and solubility, were evaluated to assess the identity, purity and quality of the extract. A 1% w/v aqueous solution of the powdered leaf material was prepared, filtered, and its pH was measured using a calibrated pH meter. Solubility of the extract was assessed in water, alcohols (ethanol, methanol) and organic solvents (chloroform, hexane, ether).

2.5 Estimation of Total Phenolic Content (TPC)

TPC was determined by the Folin-Ciocalteu colorimetric method, using gallic acid as the reference standard [10]. A gallic acid stock solution (1 mg/mL) was serially diluted to obtain standard concentrations of 10, 20, 30, 40 and 50 µg/mL. The ethanolic leaf extract (1 g in 100 mL ethanol) was similarly prepared, and five sample concentrations (AP1-AP5) were analysed. To 1 mL of standard or sample solution, 5 mL of diluted Folin-Ciocalteu reagent was added; the mixture was allowed to stand for 5 minutes, after which 4 mL of 7.5% sodium carbonate solution was added, mixed and incubated for 30 minutes at room temperature. Absorbance was measured at 765 nm against a reagent blank on a UV-Visible spectrophotometer. TPC was calculated using the equation TPC = (C × V)/M, where C is the concentration obtained from the calibration curve (mg/mL), V is the volume of extract (mL) and M is the weight of extract (g), and expressed as mg gallic acid equivalent (GAE) per gram of extract.

2.6 Estimation of Total Flavonoid Content (TFC)

TFC was estimated by the aluminium chloride colorimetric method, using quercetin as the reference standard [11]. A quercetin stock solution (1 mg/mL) was diluted to obtain standard concentrations of 10, 20, 30, 40 and 50 µg/mL. To 1 mL of standard or sample solution, 1 mL of 2% aluminium chloride solution and 3 mL of methanol were added, mixed thoroughly and incubated for 15-30 minutes at room temperature. Absorbance was measured at 415 nm against a reagent blank. TFC was calculated as TFC = (C × V)/M and expressed as mg quercetin equivalent (QE) per gram of extract. All measurements were carried out in triplicate (n = 3).

III. RESULTS AND DISCUSSION

3.1 Extraction Yield

Soxhlet extraction of 100 g of dried, powdered A. precatorius leaves with ethanol-water yielded a greenish, gummy crude extract with a percentage yield of 1.54% w/w, consistent with the moderate polarity of ethanol-water mixtures for extracting phenolic and flavonoid phytoconstituents.

3.2 Qualitative Phytochemical Screening

Preliminary phytochemical screening of the ethanolic leaf extract confirmed the presence of alkaloids, flavonoids, phenolic compounds, tannins, saponins and glycosides (Table I, Fig. 2), indicating that the leaves of A. precatorius are a rich source of biologically active secondary metabolites. This qualitative profile is consistent with previous reports on other parts of the plant and supports its traditional use in the treatment of inflammatory, infective and dermatological conditions [6], [7].

3.3 Physicochemical Parameters

The aqueous extract of the leaf powder showed a pH in the mildly acidic-to-neutral range, consistent with the presence of phenolic acids and other weakly acidic phytoconstituents. The extract showed high solubility in ethanol and methanol, moderate/cloudy solubility in water due to mucilage content, and limited solubility in non-polar solvents such as hexane, confirming its predominantly polar-to-moderately-polar phytochemical composition. Thermal analysis indicated softening/charring in the range of 150-200 °C, major decomposition between 200-250 °C, and a stable residual ash above 450-600 °C.

3.4 Total Phenolic Content

The calibration curve constructed with gallic acid standards (10-50 µg/mL) showed excellent linearity (y

= 0.0610x + 1.221, R^2 = 0.9915) when absorbance was measured at 765 nm (Fig. 3), confirming the suitability of the Folin-Ciocalteu method for quantitative estimation of phenolics in the extract. The TPC of the five tested sample concentrations (AP1-AP5) of the ethanolic leaf extract, calculated using this calibration equation, is presented in Table II and Fig. 4.

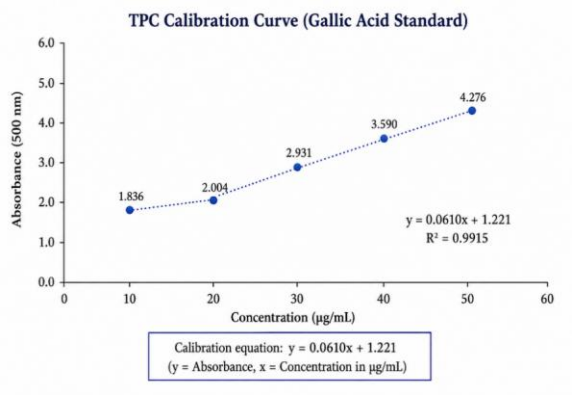

Fig. 3. Calibration curve of gallic acid for determination of total phenolic content (765 nm).

TABLE II. Total Phenolic Content of the Ethanolic Leaf Extract of A. precatorius

Sample	TPC (mg GAE/g of extract)
AP1	0.000
AP2	0.000
AP3	1.157
AP4	5.479
AP5	12.300

TPC increased progressively from AP1 to AP5 with increasing extract concentration, reaching a maximum of 12.30 mg GAE/g of extract at the highest concentration tested. Phenolic compounds are known to neutralise free radicals by donating hydrogen atoms or electrons, thereby reducing oxidative stress; the concentration-dependent rise in TPC observed here indicates an appreciable phenolic content in the ethanolic leaf extract of A. precatorius and supports its traditional use and reported antioxidant potential [8], [12].

3.5 Total Flavonoid Content

The calibration curve constructed with quercetin standards (10-50 µg/mL) at 415 nm showed a good linear relationship (y = 0.0773x + 0.823, R^2 = 0.9781; Fig. 5), validating the aluminium chloride method for flavonoid quantification in the extract. The corresponding TFC values for samples AP1-AP5 are presented in Table III and Fig. 6.

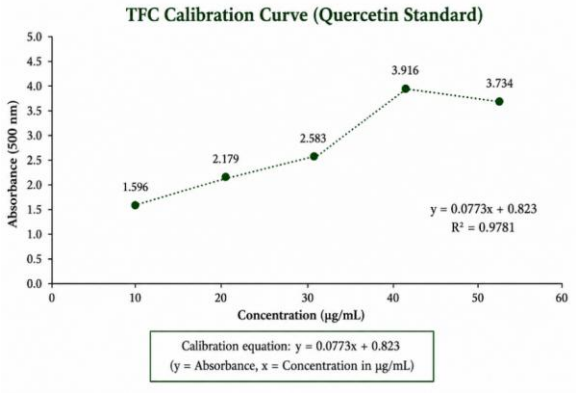

Fig. 5. Calibration curve of quercetin for determination of total flavonoid content (415 nm).

TABLE III. Total Flavonoid Content of the Ethanolic Leaf Extract of A. precatorius

Sample	TFC (mg QE/g of extract)
AP1	0.862
AP2	1.933
AP3	2.753
AP4	2.529
AP5	3.763

TFC values ranged from 0.862 to 3.763 mg QE/g of extract, with an overall increasing trend across the sample concentrations. Flavonoids are known to scavenge reactive oxygen species, chelate transition-metal ions and inhibit lipid peroxidation, thereby contributing to the antioxidant and anti-inflammatory potential of plant extracts [11], [13]. The presence of appreciable flavonoid content, together with the confirmed phenolic content, suggests that the ethanolic leaf extract of A. precatorius may serve as a valuable natural source of antioxidant phytoconstituents.

IV. CONCLUSION

The present study demonstrates that the ethanolic leaf extract of Abrus precatorius L. contains a diverse range of phytoconstituents, including alkaloids, flavonoids, phenolic compounds, tannins, saponins and glycosides. UV-Visible spectrophotometric estimation confirmed the presence of measurable total phenolic content (up to 12.30 mg GAE/g of extract, Folin-Ciocalteu method) and total flavonoid content

(0.862-3.763 mg QE/g of extract, aluminium chloride method) in the extract. These findings provide scientific support for the traditional medicinal use of A. precatorius leaves and indicate their potential as a natural source of antioxidant agents with possible pharmaceutical applications. Since the seeds of A. precatorius contain the highly toxic protein abrin, careful identification of plant parts and appropriate safety precautions remain essential during research and any therapeutic use. Further in vitro and in vivo studies, including free-radical scavenging assays and toxicological evaluation, are recommended to fully establish the safety, efficacy and therapeutic potential of the leaf extract prior to its use in herbal formulations.

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