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Research Article

SPECTROPHOTOMETRIC QUANTIFICATION OF TOTAL PHENOLIC, FLAVONOID, AND ALKALOID CONTENTS OF *ABRUS PRECATORIUS* L. SEEDS

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ABSTRACT

Objective: The aim of the present work was to assess the total phenolic content (TPC), total flavonoid content (TFC), and total alkaloid content (TAC) of crude hydro-methanolic seed extract of *Abrus precatorius* L. which is one of the beautiful seed bearing plant belonging to family Fabaceae and used extensively in many Ayurvedic preparations with significant therapeutic significance.

Methods: The amount of total phenols was analyzed using Folin–Ciocalteu assay, the amount of total flavonoids by aluminum chloride assay and total alkaloids by bromocresol green-complex assay.

Results: The TPC, TFC, and TAC of the hydro-methanolic seed extract were 219.966±4.714 mg gallic acid equivalent per gram, 73.333±2.357 rutin equivalent per gram, and 41.666±4.784 atropine equivalent per gram, respectively.

Conclusion: The result of the study highlighted a potent phenol, flavonoid, and alkaloid contents in the crude extract and thus can be used to explore new drugs. The study also justifies some of the medicinal properties of the plant.

Keywords: *Abrus precatorius*, Spectrophotometry, Total phenolic content, Total flavonoid content, Total alkaloid content.

INTRODUCTION

Plants are an important part of the universe. Human beings have been utilizing plants as medicine since time immemorial. These medicinal properties of plants are due to the presence of bioactive constituents in them and plants represent a main source of biologically active molecules. Bioactive constituents derived from plant extracts have been reported scientifically for biological activities. Plants produce these chemicals to protect themselves, but recent researches indicate that most of them can also be used against various human diseases and disorders. The most important of these biologically active ingredients are alkaloids, flavonoids, steroids, glycosides, terpenes, and tannins. These components can be extracted and used in the preparation of useful drugs. The importance of biological, chemical and pharmacological evaluation of plant-derived bioactive compounds used to cure numerous human ailments has been increasingly recognized in the last few decades, but still there are innumerable potentially useful medicinal plants and herbs waiting to be evaluated and exploited for their effective therapeutic application [1-4]. Plant biochemical components are also of great interest because of their extensive use as flavoring agents, fibers, oils, glues, waxes, perfumes, herbicides, and insecticides [5,6]. Based on the strong evidence of biological and pharmacological activities of these bioactive components, the present study was conducted to determine the total phenolic, flavonoid and alkaloid contents of a hydro-methanolic extract of *Abrus precatorius* L. seeds.

Phenolics comprise a large group of plant biologically active ingredients. Different parts of medicinal plants are rich in various phenolic substances such as tannins, phenolic acids, coumarins, stilbenes, lignans and lignins [7]. The occurrences of phenolic compounds in medicinal plants are responsible for multiple biological activities such as anti-inflammatory, antioxidant, anticarcinogenic, antimicrobial, antihypertensive, and antimutagenic as well as ability to modify the gene expression, etc. [8-10]. These are famous as reducing agents, metal chelators, radical scavengers, hydrogen donors, and singlet oxygen quenchers due to their strong antioxidant activity [11]. Flavonoids are the major and one of the most ubiquitous group of all naturally occurring plant phenolic compounds occurring in free-state and as glycosides in different plant parts [12]. These are widely distributed in flowers, seeds, leaves and bark and are responsible for the characteristic blue and red colors of berries, wines and certain vegetables [13]. These are also known as nutraceuticals on account of a broad sprectum of pharmacological activities in human body [14]. The reported activities of flavonoids include antiulcer, anticancer, antimicrobial, antiangiogenic, antiarthritic, antiallergic, antithrombotic, anti-inflammatory, mitochondrial adhesion inhibition, and protein kinase inhibition, etc. Moreover, these act against cardiovascular diseases and are also recognized to be hepatoprotective [12,15,16]. Alkaloids are naturally occurring organic bases found in about 20% of plant species. These are viewed upon as a more genera- and species-specific. The strong biological activities of many alkaloids have also directed their utilization as stimulants, narcotics, pharmaceuticals and poisons. At present, the alkaloids of the plant origin in clinical use include the anti-malarial quinine, the anesthetic cocaine, the stimulant caffeine and nicotine, the analgesics morphine and codeine, the gout suppressant colchicine, the antibiotic sanguinarine, the antiarrhythmic ajmaline, the anticancer vinblastine and taxol, and sedative scopolamine. These are even used as antiseptics in medicine [17,18].

A. precatorius L. is a beautiful seed bearing woody vine belonging to family Fabaceae. It is native to India, but now found in all tropical parts throughout the world. The plant is locally known as Rati and has been used for the therapeutic purposes in India, china and other cultures from ancient times [19]. The main phytochemicals of the seeds are proteins, carbohydrates, triterpenoids, steroids, amino acids, alkaloids, flavonoids and isoflavonoids [20-22]. The major reported activities of the seed are anticancer [23], antifertility [24], antispermatogenic, antibacterial [25,26], antidiabetic [27], antioxidant [19], nephroprotective [28], anti-arthritic [29], antimicrobial [30], antimalarial [31], etc. Thus, considering its wide medicinal importance and use in traditional medicine, *A. precatorius* L. was selected for the present study.

METHODS

Chemicals and reagents

All the chemicals and reagents used in the present study were of analytical grade and were obtained from Himedia, Sigma, Merk and SD Fine.

Plant collection and authentication

The mature seeds of *A. precatorius* L. were purchased from the local market of Bhopal, Madhya Pradesh and the identified and authenticated was done by Dr. Zia-ul-Hassan, Head of the Department of Botany, Safia Science College, Bhopal, Madhya Pradesh, India. A sample voucher specimen No. 520/Bot/Saifia/2015 was deposited there for further reference.

Extract preparation

The seeds were washed repeatedly with distilled water to remove residual material, shade dried, crushed into a coarse powder using an electrical grinder and stored in air tight containers. A weighed amount of the seed powder was subjected to successive extraction with solvents such as petroleum ether and 70% methanol in increasing polarity for 7 days, respectively, through cold maceration process. Filtrates obtained from both the solvents were evaporated in rotary evaporator under reduced pressure and were vacuum dried. The dried extracts were packed in airtight containers, labeled and stored in a refrigerator (2-4°C) until needed for the experimental purpose. The crude hydro-methanolic extract was used for the present study. After the confirmation of different phyto-constituents by preliminary phytochemical analysis, the extract was taken for quantitative estimation.

Spectrophotometric measurements

UV/Vis double beam spectrophotometer (Systronix, Model 2202) and standard quartz cuvetts were used for all the absorbance measurements.

Preparation of standard solution

About 10 mg each of gallic acid and rutin were accurately weighed into clean and dry volumetric flasks, dissolved in methanol and the volume was made up to 10 ml using the same solvent so as to make the concentration of the solution as 1 mg/ml. Atropine standard solution was taken by dissolving 1 mg pure atropine in 10 ml distilled water.

Preparation of test sample

A stock solution of the test substance was prepared by dissolving 10 mg of dried hydro-methanolic extract in 10 ml methanol to give concentration of 1 mg/ml.

Determination of total phenolic content (TPC) [32,33]

Spectrophotometric methods are most commonly used for the quantification of phenolic content. Estimation of total phenol content in the selected plant seed extract was measured spectrophotometrically by Folin–Ciocalteu colorimetric method, using Gallic acid as the standard and expressing results as gallic acid equivalent (GAE) per gram of sample. Different concentrations (0.01-0.1 mg/ml) of gallic acid were prepared in methanol. Aliquots of 0.5 ml of the test sample and each sample of the standard solution were taken, mixed with 2 ml of Folin–Ciocalteu reagent (1:10 in deionized water) and 4 ml of saturated solution of sodium carbonate (7.5% w/v). The tubes were covered with silver foils and incubate at room temperature for 30 minutes with intermittent shaking. The absorbance was taken at 765 nm using methanol as blank. All the samples were analyzed in three replications. The total phenol was determined with the help of standard cure prepared from pure phenolic standard (gallic acid).

Folin–Ciocalteu is a very sensitive reagent containing phosphomolybdate and phosphotungstate that form blue-complex in alkaline solution by the reduction of phenols. This blue color was measured spectrophotometrically.

Determination of total flavonoid content (TFC) [34]

The TFC of the seed extract was determined by aluminum chloride colorimetric assay. Briefly, 0.5 ml aliquots of the extract and standard solution (0.01-1.0 mg/ml) of rutin were added with 2 ml of distilled water and subsequently with 0.15 ml of sodium nitrite (5% $NaNO_2$, w/v) solution and mixed. After 6 minutes, 0.15 ml of (10% $AlCl_3$ w/v) solution was added. The solutions were allowed to stand for further 6 min and after that 2 ml of sodium hydroxide (4% NaOH, w/v) solution was added to the mixture. The final volume was adjusted to 5 ml with immediate addition of distilled water, mixed thoroughly and allowed to stand for another 15 min. The absorbance of each mixture was determined at 510 nm against the same mixture but without seed extract as a blank. TFC was determined as mg rutin equivalent per gram of sample with the help of calibration curve of rutin. All determinations were performed in triplicate (n=3).

Determination of total alkaloid content (TAC) [35,36]

TAC was also quantified by spectrophotometric method. This method is based on the reaction between alkaloid and bromocresol green (BCG). The plant extract (1 mg/ml) was dissolved in 2 N HCl and then filtered. The pH of phosphate buffer solution was adjusted to neutral with 0.1 N NaOH. 1 ml of this solution was transferred to a separating funnel, and then 5 ml of BCG solution along with 5 ml of phosphate buffer were added. The mixture was shaken and the complex formed was extracted with chloroform by vigorous shaking. The extract was collected in a 10 ml volumetric flask and diluted to volume with chloroform. The absorbance of the complex in chloroform was measured at 470 nm. The whole experiment was conducted in three replicates.

Preparation of atropine standard curve

Accurately measured aliquots (0.5, 1, 1.5, 2, and 2.5 ml) of atropine standard solution were taken and transfer each to different separating funnels. To each solution, 5 ml of phosphate buffer (pH 4.7) along with 5 ml of BCG solution were added and shaken vigorously with 4 ml of chloroform. The extracts were collected in 10 ml volumetric flasks and then diluted to adjust volume up to the mark with chloroform. Now, the absorbance of the complex in chloroform was measured at 470 nm against the blank prepared as above but without atropine. Line of regression from atropine was used for estimation of unknown alkaloid content.

Statistical analysis

All the determinations were replicated in three independent assays, and the results were reported as a mean±standard deviation.

RESULTS AND DISCUSSION

Plant bioactive compounds have played a vital role worldwide in preventing and curing numerous human ailments. It is because of their broad spectrum of chemical and biological activities. All medicinal plants require a detailed investigation before their exploitation as medicine because the therapeutic potential entirely depends on the quality of plant material used and the study of any crude sample material of natural origin is beneficial only if it contains the active constituents which have to be recognized to validate its real value [37]. Moreover, information about different phyto-constituents of plants is a very important and advantageous as it is much valuable in the production of complex chemical compounds as well as screening of their biological activities.

The present study has been carried out for quantification of the total phenolic, flavonoid, and alkaloid contents of hydro-methanolic extract of *A. precatorius* L. seeds. The content of the phenolic compounds in the crude extract, determined from regression equation of calibration curve ($y=0.003x+0.002$, $R^2=0.981$) and expressed in gallic acid equivalent was 219.966±4.714. The concentration of flavonoids (mg/g) in rutin equivalent determined from regression equation of calibration curve ($y=0.002x+0.053$, $R^2=0.991$) was 73.333±2.357; and the total amount of alkaloid as determined using the regression equation of calibration curve ($y=0.001x+0.102$, $R^2=0.981$) and expressed in atropine equivalent was 41.666±4.784 mg/g of plant extract. The standard calibration curves of gallic acid, rutin and atropine are shown in Figs.1-3 respectively. The results are shown in Table 1.

Table 1: Total phenol, flavonoid and alkaloid contents of *Abrus precatorius* L. seeds

Name of the plant	Family	Part used	Extract investigated	Total phenolic content, mg/g plant extract (GAE)	Total flavonoid content mg/g, plant extract (RE)	Total alkaloid content mg/g, plant extract (AE)
Abrus precatorius	Fabaceae	Seeds	Hydro-methanolic	219.966±4.714	73.333±2.357	41.666±4.784

Values are presented as mean±SD (n=3). GAE: Gallic acid equivalent, SD: Standard deviation, RE: Rutin equivalent, AE: Atropine equivalent

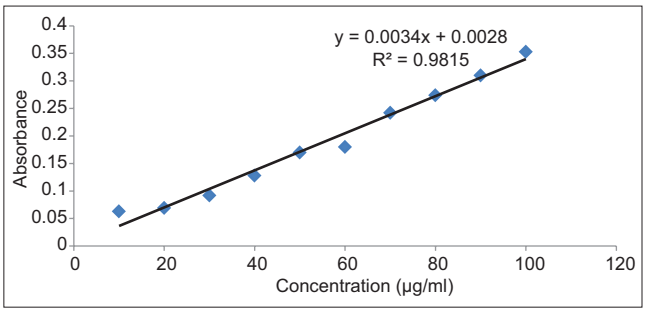

Fig. 1: Standard calibration curve for total phenolic content for standard gallic acid

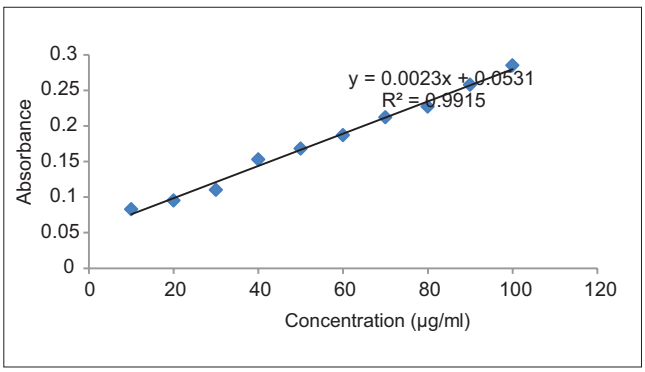

Fig. 2: Standard calibration curve for total flavonoid content for standard rutin

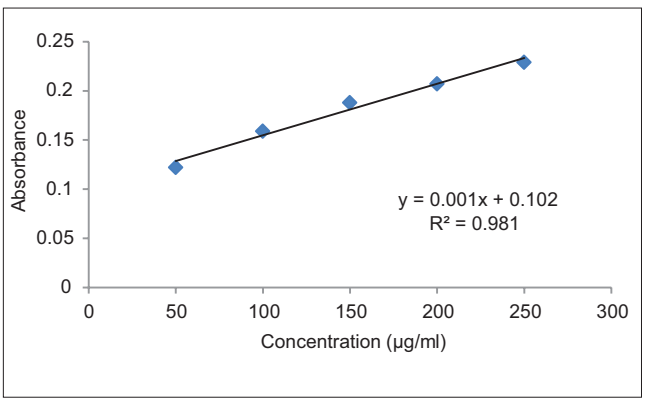

Fig. 3: Standard calibration curve for total alkaloid content for standard atropine

The results revealed that the test extract contains the potent amounts of phenolic, flavonoid and alkaloid compounds**.** Many of the curative properties of this plant may depend on these bioactive components. These findings supported the uses of *A. precatorius* L. as anti-microbial, anti-inflammatory, anti-diabetic, antitumor, antioxidant, free radical scavenging agent, etc. Further, more progress in the detailed examination of the composition of these bioactive chemicals in plant extract is required for the complete evaluation of the individual compounds exhibiting the different biochemical properties.

CONCLUSION

In the present study, we have found that the plant is rich in phenolic, flavonoid and alkaloid compounds, and therefore, has provided some biochemical basis for the ethnomedicinal use of the sample extract from *A. precatorius* L. As a promising source of bioactive compounds, it can be an excellent source of useful drugs. Moreover, it can also be concluded that hydro-methanolic seed of *A. precatorius* L. extract can also serve as much potent anti-oxidant agent than the ethanolic seed extract that was reported earlier to have antioxidant potential. It will obviously be due to high contents of the photochemicals in the hyro-methanolic extract.

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