Research Article

Analysis of Phytochemical Constituents, Antioxidant Potential of *Abrus precatorius l* Seeds

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ABSTRACT

Plants are endowed with phyto-constituents such as carotenoids, flavonoids, phenolics which can be used for scavenging free radicals in the human body towards the cure of diseases. The present study was evaluated both qualitatively and quantitatively in aqueous extract of powdered *Abrus precatorius* seeds. The estimations were performed by adopting standard procedures. From the qualitative results, it is evident that, it contains alkaloid, flavonoid, carbohydrate, protein, amino acid and steroid. This was further assessed by studying secondary metabolites as well as antioxidant activities. The results showed that aqueous extract of *Abrus precatorius* seed was found to contain high amount of flavonoid (39.0±3.60mg/g) and moderate amount of phenolic content (31.0±1.0mg/g), inducing antioxidant property to it. All assays showed very good results with 100mg concentration. Among the antioxidant activities studied, total antioxidant activity (44.0±3.0mg/g) as well as reducing power activity (37.0±2.0mg/g) showed prominent results, whereas, nitric oxide scavenging (29.0±3.0mg/g) and metal chelating activity (14.33±2.08mg/g) was lower when compared to other antioxidant activities.

Keywords: *Abrus precatorius*, Antioxidants, Free radicals, Secondary metabolites, Seeds.

INTRODUCTION

Natural products in general and medicinal plants in particular are believed to be an important source of new chemical substances with potential therapeutic effects.[1] Plant extracts as well their primary and secondary metabolites have important therapeutic role in the treatment of many human diseases.[2] Natural products mainly from plant kingdom offer a wide range of biologically active compounds that act as natural antioxidants with recognized potential in drug discovery and development.[3] seeds are used for the treatment of diabetes and chronic nephritis.[4] In Siddha medicine, the white variety is used to prepare oil that is claimed to be an aphrodisiac.[5] Biologically active ingredients from the herbal medicines are used essentially for treating mild or chronic ailments. Hot water extract of seeds is taken orally as an Antifertility agent,[6] as an abortifacient,[7] and to prevent conception.[8] Decoction of dried seeds are taken orally to induce abortion.[9] Hence, the present study was aimed to study qualitatively and quantitatively. Qualitative test was done to know the presence of phytoconstituents. Secondary metabolites and antioxidant activities of aqueous extract of non edible *Abrus precatorius* seeds was assessed quantitatively.

MATERIALS AND METHODS

Sample collection

The sample *Abrus preacatorius* seeds were purchased from shops at Krishnagiri, Krishnagiri District, Tamil Nadu, India. The purchased seeds were cleaned thoroughly and ground to powder using blender for further use.

Phytochemical Analysis

Qualitative analysis was done for the presence of alkaloids, flavonoids, carbohydrates, proteins-amino acids and steroids.[10,11]

Test for alkaloids

Hager's test

This test was performed by adding few drops of saturated solution of picric acid to a drop of extract. Positive test shows the presence of yellow colour precipitation.

Test for flavonoids

Shinoda test

To a drop of extract add magnesium turnings followed by 1/2 drops of concentrated hydro chloric acid. Positive result gives red color.

Test for Carbohydrate

Benedict's test

To a drop of Benedict's reagent add extract and this mixture was heated in a boiling water bath. Red colored precipitation confirms the presence of carbohydrate.

Test for protein and amino acid

Biuret test

To a drop of extract add 4 % sodium hydroxide followed by few drops of 15 % copper sulphate. Presence of pink color shows positive result.

Test for steroids and sterols

Lieberman-Burchard test

A drop of extract is added to 2 ml chloroform in a dried test tube, to this 10 drops acetic anhydride and 2 drops concentrated sulphuric acid was added. Changes in colour were observed from red to blue and blue to bluish green.

Determination of Secondary Metabolites

The phenol and flavonoid content of aqueous extract was analyzed.

Aqueous extract preparation

Aqueous extract was prepared by taking 75 and 100mg of *Abrus precatorius* seed powder. Each concentration was dissolved in 10ml water mixing with a magnetic stirrer at 4°C for 4h. The mixture was filtered through nylon cloth and centrifuged at 20,000g for 30min. 0.1ml of supernatant was used for the analysis. Each experiment was performed three times.

Determination of total phenol content

Total phenolic content were determined by Folil-ciocalteau method. The extract (0.1ml) was mixed with folinciocalteau reagent (5ml, 1:10 diluted with distilled water) for 5min and added aqueous $NaCo_3$ (4ml, 1M). The mixture was allowed to stand for 15min and the phenols were determined by colorimetric method at 765nm. The standard curve was prepared. Total phenol values are expressed in terms of gallic acid equivalent (mg/g of dry mass), which is a common reference compound. [12,13]

Estimation of flavonoids

The aluminium chloride method was used for the determination of the total flavonoid content. Extract solution were taken and to this 0.1ml of 1M potassium acetate, 0.1ml of $AlCl_3$ (10%), 2.8ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance at 415 nm was recorded after 30min of incubation. A standard calibration plot was generated using known concentration of quercetin. The concentration of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent/g of sample.[14]

Determination of Antioxidant Activities

Nitric oxide scavenging assay, Reducing power assay, Total antioxidant assay, Metal chelating activities were performed.

Reducing power assay

Aqueous extract was mixed with phosphate buffer (2.5ml, 0.2M, P^H 6.6) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°c for 20min. 1.0 ml of Trichloro acetic acid (10%) was added to stop the reaction, which was then centrifuged at 3000rpm for 10min. The upper layer of solution (1.5ml) was mixed with distilled water (1.5ml) and $FeCl_3$ (0.1ml, 0.1%) after mixing, the contents were incubated for 10min and the absorbance was measured at 700nm. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a positive control.[15]

Total antioxidant capacity

Total antioxidant capacity by phosphomolybdenum method assay is based on the reduction of Mo (V1) to Mo (V) by the sample analyte and the subsequent formation of green phosphate/Mo (V) complex at acidic pH by adding 4ml reagent solution containing 0.6M Sulphuric acid, 28mM Sodium phosphate, 4mM Ammonium molybdate. The tubes were incubated in water bath at 95°C for 90 minutes. After the samples had been cooled to RT, the absorbance of mixture was measured at 695nm against blank. The phosphomolybdenum method is quantitative, since, the total antioxidant activity is expressed as the number of equivalents of ascorbic acid.[16]

Nitric oxide scavenging activity

This procedure is based on the principle that, sodium nitroprusside in aqueous solution, at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide compete with oxygen, leading to reduced production of nitrite ions. For the experiment, sodium nitroprusside (10mM), in phosphate buffered saline, was mixed with extract and incubated at room temperature for 150min. After the incubation period, 0.5ml of Griess reagent was added. The absorbance of the chromophore formed was read at 546nm. Ascorbic acid was used as a positive control. [17]

Metal chelating activity

Add extract (0.1ml) to a solution of 2mM $FeCl_2$ (0.05ml). The reaction was initiated by the addition of 5mM Ferrozine (160µl), the mixture was shaken vigorously and left standing at room temperature for 10min. Absorbance of the solution was then measured spectrophotometrically at 562nm. Standard curve was plotted using ascorbic acid. Distilled water (1.6ml) instead of sample solution was used as a control. Distilled water (160µl) instead of ferrozine was used as a blank, which is used for error correction because of unequal color of sample solution. [18]

For all estimations, readings were taken using UV- Visible spectrophotometer-Shimadzu, Japan make. Model UV 1800. Standard graph were plotted for all experiments using their respective standards and samples were plotted against standard by taking concentration in X axis and OD in Y axis.

Statistical Tool

Each experiment was carried out in triplicate and the results are given as the mean ± standard deviation. The Mean and Standard deviation (S) was calculated by using the following formula: Mean = Sum of x values / n (Number of values), $s = \frac{\sqrt{\sum(X-M)^2}}{n-1}$

RESULTS AND DISCUSSION

Table 1 shows the results of phytochemical constituents present in the aqueous extract of *Abrus precatorius* seeds.

Table 1: Phytochemicals in aqueous extract of *Abrus precatorius* seeds

Phytochemicals	Present(+) /Absent (-)
Alkaloid	+
Flavonoid	+
Carbohydrate	+
Protein, Amino acid	+
Steroid	+

Table 1 show that aqueous extract contains phytochemicals such as alkaloid, flavonoid, carbohydrate, protein, amino acid and steroid. (Table 1)

Secondary metabolites

The results of total flavonoids and total phenolics are given in Table 2.

Table 2: Secondary metabolites in aqueous extract of *Abrus precatorius* seeds

Abrus precatorius seed powder(mg)	Total Flavonoids (mg/g)	Total Phenolics (mg/g)
100	39.0±3.60	31.0±1.0
75	33.76±2.04	18.2±2.02

Values are Mean ± SD for three experiments

The total flavonoid content observed was 39.0±3.60, 33.76±2.04mg/g when assessed with aqueous extract of *Abrus precatorius* seed powder. Similarly, the total phenolics observed were 31.0±1.0, 18.2±2.02mg/g when tested with aqueous extract of *Abrus precatorius* seed powder. The flavonoid content was higher in 100mg, whereas phenol content was less at 100mg concentration. (Table 2) Flavonoids are widely distributed in the plant kingdom and considered as a natural antioxidant. Phenolic compounds are a class of antioxidant agents which act as free radical terminators.[19] The antioxidant activity of phenolics is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donars and singlet oxygen quenchers.[20]

Table 3: Antioxidant activities in aqueous extract of *Abrus precatorius* seeds

Abrus precatorius seed powder (mg)	Reducing power activity (mg/g)	Total antioxidant activity(mg/g)	Nitric oxide scavenging activity (mg/g)	Metal chelating activity (mg/g)
100	37.0±2.0	44.0±3.0	29.0±3.0	14.33±2.08
75	34.22±5.30	29.76±2.04	23.55±2.03	19.76±1.66

Values are Mean ± SD for three experiments

Antioxidant activities

The results of antioxidant activities are depicted in Table 3.

Among the various antioxidant activities assessed, the reducing power as well as total antioxidant activity was higher when compared to nitric oxide scavenging and metal chelating activity. All antioxidant activities were higher at 100mg concentration. (Table 3) Apart from their role of health benefactors, antioxidants are added in foods to prevent or delay the oxidation of food, initiated by free radicals formed during their exposure to environmental factors such as air, light and temperature.[21]

CONCLUSION

In conclusion, this study shows that, *Abrus precatorius* seeds can be used as an antioxidant as it contains flavonoids and moderate amount of phenolics induced antioxidant activities. Even though, the seeds are not used as an edible seed, according to reports, *Abrus precatorius* seeds find its application in medicine largely.

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