Phytochemical Analysis & Evaluation of Antioxidant Activity of *Abrus precatorius*

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Abstract— Abrus precatorius belonging to the Fabaceae family, is an effective medicinal herb. The present study was designed to evaluate the antioxidant activity of methanolic extract of Abrus precatorius plants. We also did the phytochemical investigation of the plant extract. The methanolic extract of plant contains many bioactive chemical constituents like alkaloids, glycosides, tannins, carbohydrate and gums etc. Antioxidant activity was determined by using 1, 1-diphenyl-2- picrylhydrazyl (DPPH) radical scavenging assays. The IC50value for Abrus precatorius was found to be 14.87µg/ml. The result indicates a strong antioxidant activity.

Index Terms— *Abrus precatorius*, alkaloids, tannins, methanolic extract, DPPH, spectrophotometry, IC_{50}.

1 Introduction

Abrus precatorius is a plant that originates from Southeast Asia and now can be found in tropical and subtropical regions [1]. It grows in tropical climates such as India, Sri Lanka, Thailand, the Philippine Islands, South China, tropical Africa and the West Indies. It also grows in all tropical or subtropical areas [2]. Herbal medicines have become more popular in the treatment of many diseases due to popular belief that green medicine is safe, easily available and less side effects [3].

Plant extracts as well their primary and secondary metabolites have important therapeutic role in the treatment of many human diseases [4]. The World Health Organization (W.H.O.) reported that over 85% of the populations in Sub-Sahara Africa, including Nigeria still depend on herbal traditional medicine for their healthcare needs. The plant parts are purgative, emetic, toxic, anti-phlogistic, aphrodisiac, anticancer, anthelmintic, abortive, antidiarrheal, antimicrobial, diuretic, laxative, antipyretic and anti-ophthalmic [5]. In India hot water extract of dried leaves and roots are applied to the eye to treat eye diseases (4). In Brazil, water extract of dried leaves and roots taken orally as a nerve tonic [6]. Seeds are said to be emetic, tonic, purgative, aphrodisiac, anti-ophthalmic and antiphlogistic [7]. In Siddha medicine, the white variety is used to prepare oil that is claimed to be an aphrodisiac [7]. Hence, the present study was aimed to study qualitatively and quantitatively. Qualitative test was done to know the presence of phytoconstituents [8].

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2 Materials & Methods

2.1 Collection and Identification

The plant leaves was collected from BADC, Kashimpur under the district of Gazipur in the month of November, 2016. During collection the plant leaves was not washed or cleaned by water due to chance of hydrolysis, oxidation and other types of chemical degradation. The plant leaves was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka, Bangladesh.

2.2 Extraction preparation

A glass made jar with plastic cover was taken and the jar was rinsed with methanol and dried .Then 370 gram of the dried plants was taken in the jarand methanol (1500ml) was poured into the jar up to 1 inch height above the sample surface[5, 12].The plastic cover with aluminum foil was closed properly to resist the entrance of air into the jar. This process was performed for 7 days. The jar was shaken several times during the process to get better extraction [6,8]. Then filtered and evaporated at room temperature [11].

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2.3 Phytochemical Analysis

Qualitative analysis was done for the presence of alkaloids, glycosides, tannins, gums and carbohydrate.

Test for alkaloids

Mayer's test

2ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Mayer's reagent was added. Yellow color precipitate was formed and that was indicated as the presence of alkaloids [9].

Dragendroff's test

2 ml solution of the extract and 0.2 ml of dilute hydrochloric acid were taken in a test tube. Then 1 ml of Dragendroff's reagent was added. Orange brown precipitate was formed and that was indicated as the presence of alkaloids [10].

Tests for glycosides

The plant extract 5ml is mixed with glacial acetic acid, few drops of ferric chloride and concentrated sulphuric acid are added and observed for reddish brown colouration at the

junction of two layers and the bluish green colour in the upper layer was formed. It indicates the presence of glycosides [11].

Tests for tannins

Test for Tannins 0.5 ml of extract solution 1 ml of distilled water and 1-2 drops of ferric chloride solution was added. Blue colour was observed for Gallic tannins and green black for catecholic tannins [12].

Test for gums and carbohydrate

Molisch's test

5 ml solution of the extract was taken and then molish reagent and sulphuric acid were added. Red violet ring produced at the junction of two liquids indicate the presence of gums and carbohydrate [9].

2.4 DPPH Free Radical Scavenging Activity

The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) has been widely used to evaluate the free radical scavenging capacity of antioxidants. DPPH free radical is reduced to the corresponding hydrazine when it reacts with hydrogen donors. DPPH can make stable free radicals in aqueous or methanol solution. With this method it was possible to determine the antiradical power of an antioxidant activity by measurement of the decrease in the absorbance of DPPH at 517 nm. Resulting from a color change from purple to yellow the absorbance decreased when the DPPH was scavenged by an antioxidant, through donation of hydrogen to form a stable DPPH molecule. In the radical form this molecule had an absorbance at 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant compound to become a stable diamagnetic molecule [14].

Preparation of Solution

At first 10 mg extract of Abrus precatorius was measured by electronic balance and mixed with 10 ml of methanol (99-100%) to prepare 1000 µg/ml solution of extract as stock solution. Another ten different concentrations of solutions were prepared by proper dilution method. These concentrations were 500 µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml, 15.62 µg/ml, 7.81 µg/ml, 3.90 µg/ml, 1.95 µg/ml, 0.97 µg/ml. The following technique was followed to prepare different concentrations of solutions from stock solution:

Volume which we have to take from stock solution=

$$\frac{\text{Desired concentration}}{\text{Supplied concentration}} \times \text{Desired volume}$$

In the same way, various concentrations (500 µg/ml – 0.97 µg/ml) of ascorbic acid solutions were prepared. 2 mg DPPH powder was measured by electronic balance and mixed with 100 ml of methanol (99-100%) to prepare 20 µg/ml DPPH solution. It should be kept in cool, dry and dark place.

Assay of Free Radical Scavenging Activity

2.0 ml of a methanol solution of the sample (Control / extractives) at different concentration from 500.0 to 0.977µg/ml were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 minutes reaction period at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer. Inhibition of free radical DPPH in percent (I %) was calculated as follows-

$$\{(A_o - A_1)/A_o\} \times 100$$

Where, A_0 is the absorbance of the control reaction (containing all reagents except the test material), and A_1 is the absorbance of the extract/standard Extract/standard concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration.

3 Results & Discussion

Phytochemical Investigation

After completing wide range of chemical test for the identification of major classes of therapeutically important compounds, alkaloid, tannins, glycosides and gum were found in plant. The following table will give us a broad idea about phytochemicals present in these plants.

Group name	Name of the test	*Abrus precatorius*
Tannins	5% Ferric Chloride	+
Alkaloids	Mayer's Test	++
	Dragendroff's Test	++
Saponins	Test for Saponins	_
Flavonoids	Test for Flavonoids	_
Glycosides	Test for glycosides	+
Gum	Test for gum	+

Table 3.1: List of phytochemicals found in *Abrus precatorius.*

Amount	Moderate amount	Trace amount	absent
Symbol	++	+	_

DPPH Free Radical Scavenging Activity

The DPPH test is based on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. It is evident from the table that the % scavenging of DPPH radical was found to rise with increasing concentration of the samples. The IC_{50} value of positive control ascorbic acid is 7.61µg/ml. On the other hand, the methanol extract showed promising DPPH free radical scavenging activity with IC50 value of 14.87µg/ml (13).

Abs of con-trol	Conc. (µg/ml)	Abs of Ascorbic acid	Inhibition (%)	IC_{50} (µg/ml)
0.485	500	0.022	95.46	**7.61**
	250	0.023	95.26	
	125	0.025	94.85	
	62.5	0.026	94.64	
	31.25	0.028	94.23	
	15.63	0.038	92.17	
	7.81	0.258	46.80	
	3.9	0.332	31.55	
	1.95	0.391	19.38	
	0.98	0.466	3.92	

Table: IC_{50} value of ascorbic acid

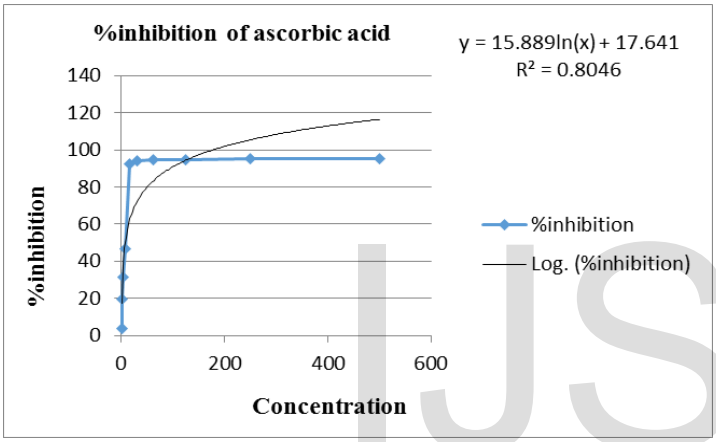
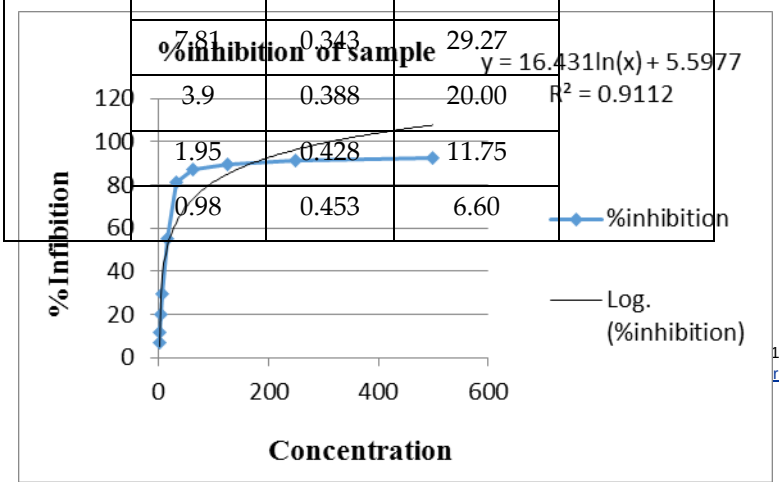


Figure: free radical scavenging activity of ascorbic acid

Abs of control	Conc. (µg/ml)	Abs of sample	Inhibition (%)	IC50 (µg/ml)
0.485	500	0.036	92.58	**14.87**
	250	0.041	91.44	
	125	0.052	89.25	
	62.5	0.061	87.42	
	31.25	0.090	81.44	
	15.63	0.219	54.85	
	7.81	0.343	29.27	
	3.9	0.388	20.00	
	1.95	0.428	11.75	
	0.98	0.453	6.60	



IC_{50} value of plant extract of *Abrus precatorius*

Figure: DPPH free radical scavenging activity of plant extract of Abrus precatorius at different concentration.

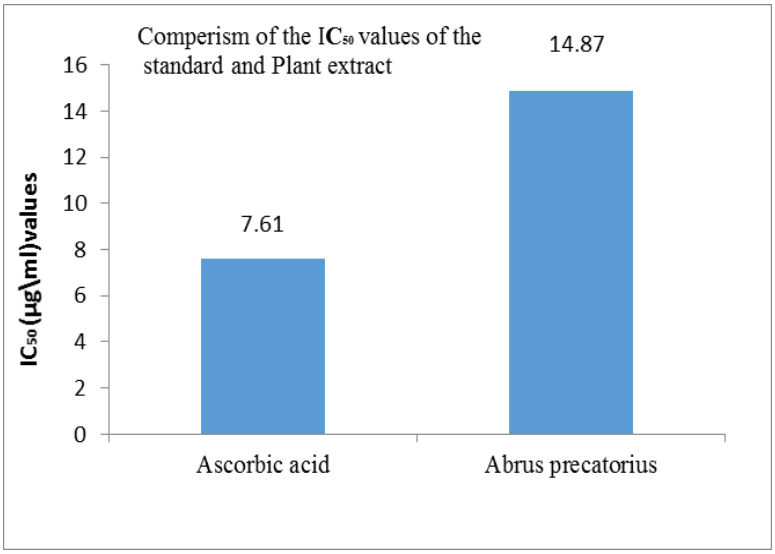


Figure: Comparative DPPH free radical scavenging activity between ascorbic acid and methanolic plant extract of Abrus precatorius.

4 Conclusion

The project was intended and aimed to find out the first steps of discovery of phytochemicals with promising pharmacological activity. The methanolic extract of plant contains many bioactive chemical constituents' alkaloids, flavonoids, phenols, saponins, glycosides, tannins, carbohydrate and terpenoids etc. Phytochemical analysis of methanolic extract of the plant showed the presence of alkaloids, tannins, gum and glycosides and the absence of flavonoid and saponins. *Abrus precatorius* possesses high antioxidant activity.

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