Research Article

Phytochemical Analysis and *in–vitro* Antioxidant Activity of Ethanolic Extract of Iraqi *Abrus precatorius* Linn. of Leguminosae Family

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ABSTRACT

Oxidative stress and impaired antioxidant system have been implicated in the pathophysiology of diverse disease states. The phytochemical screening and antioxidant property of ethanolic extract of *Abrus precatorius* Linn. , were studied. The results of phytochemical analysis were showed the presence of alkaloids, flavonoids, steroids (phytosterols) and tannins. The antioxidant activity was done by DPPH (2, 2-diphenyl-1-picryl-hydrazyl) free radical scavenging assay. The in-vitro antioxidant assays showed the extract of AP possess potent antioxidant activity when compared with reference compound ascorbic acid (Vitamin C). *Abrus precatorius* can be considered a plant for future drugs when used in the preparation of neutraceuticals as potent antioxidant to treat various human diseases and its complications.

Keywords: *Abrus precatorius*, phytochemical anlysis, antioxidant, DPPH.

INTRODUCTION

The medicinal plants refer to the plants extracts of different parts (leaves, seeds, roots, fruits etc.) which are used in the treatment of various diseases of humans, animals and plants. [1] There are increasing evidences that free radicals produced molecular alterations that are associated with various degenerative human diseases such as arteriosclerosis, cancers, Alzheimer's disease, Parkinson's disease, diabetes, asthma, arthritis, immune deficiency diseases and aging .Antioxidants are substances that mop up free radicals and prevent them from causing cell damage .Plants contain antioxidant compounds that function as free radical scavengers, reducing agents and quenchers of singlet oxygen formation [2] *Abrus precatorius* Linn. is known as crab's eyes , rosary pea , a woody twinning plant of the Leguminosae (Fabaceae) family, with characteristic red and black seeds. The leaves are pinnate and glabrous, with many leaflets (12 or more) arranged in pairs. The leaflets are oblong, measuring 2.5-cm long and 1.5-cm wide. The plant bears orange-pink flowers, which occur as clusters in short racemes that are sometimes yellowish or reddish purple in color, small and typically pea like. The plant produces short and stout brownish pods, which curl back on opening to reveal pendulous red and black seeds, 4 to 6 peas in a pod. This plant grows wild in thickets, farms, and secondary clearings, and sometimes in hedges. It is most common in rather dry areas at low elevation throughout the tropics and subtropics. [3] For a long time, several experimental and epidemiological studies have shown that a wide diversity of phytochemicals such as phenolics, flavonoids, isoflavone, flavones, anthocyanins, catechin, isocatechin and carotenoids have a potent antioxidant activities. [4] These phytochemicals and others are found in the leaves and seeds of *A. precatorius* which make this plant very signify and versatile for its wide number of medicinal properties. [5,6] .

Traditional use

Abrus precatorius is traditionally used in treatment tetanus, scratches and sores and wounds caused by animals. And to prevention rabies. [7] Each part of plant have a specific applications with different preparation in folkloric and traditional medicine. Hot water extract of dried leaves and roots are applied to the eye for eye diseases[8]. A fermented drink of root is taken orally to produce abortion[9, 10]. In West Indies, Seeds are taken orally as an emetic, purgative, and anthelmintic [11]. Various African tribes use powdered seeds as oral contraceptives and taken for tuberculosis and painful swellings[7]. In Thailand, they use the leaves crushed with oil as a poultice as an anti-inflammatory.[12] Seeds of this plant are very beautiful; and they have uniform weight of 1/10 of a gram, so it used as weighing unit .these seed are used to make necklaces and other ornaments. In China the herb of *A.precatorius* is used as a folk-medicine for the treatment of bronchitis, laryngitis and hepatitis. Because of their platelet inhibiting activity abruquinones are supposed to be the active substances [13].

Pharmacological Activities and Clinical Studies

Abrus precatorius (Linn.) is known for its medicinal properties to cure various ailments. The seeds, roots, leaves and even the whole plant are used for different medical purpose.[14] Water extract and ethanol (95%) extract of dried seeds, administered to pregnant rats at a dose of 125.0 mg/kg and 200.0 mg/kg respectively, they were active as abortifacient. Water extract was administered intragastrically while ethanolic extract was

gave orally[15, 16]. Some studies have shown that the extracts of *A.precatorius* at different concentrations (512 µg/ml - 4 µg/ml) have antimicrobial activities. *Staphylococcus aureus* was the most sensitive organism with an MIC of 8 ηg/ml for the leaf extract while the extract from the stem and seed oil were potent against some of the gram-positive bacteria and *Candida albicans* but not against *S.anginosus*, *E. faecalis* and gram-negative bacteria tested.[17] *Abrus precatorius* have antidiabetic activity; the ethanolic extract of this plant in a dose (100 and 200 mg /kg) was administered to induced diabetic rats with Streptozotocin & Nictonamide. The fast blood glucose level was reduced to normal level.[18] Seeds showed highest phenolic content and DPPH (2,2-diphenyl-1-picrylhidrazyl) radical scavenging activity in methanolic extracts. The antioxidant potential was found to be the highest in seeds followed by root, leaves and stem. Methanol proved to be the best solvent for extraction of phenolics, flavonoid and antioxidants. [19]

MATERIALS AND METHODS

Plant collection

The plant seeds and aerial parts were collected during the month of October (2016) from Mousul city which is located at the north of Iraq then dried at room temperature in the shade then pulverized by mechanical mills and weighed.

Preparation of extract

Shade-dried coarsely powdered seeds, aerial parts (200gm) were defatted with hexane for 24 hours then allowed to dry at room temperature. The defatted plant material was extracted with 80% ethanol (1 L) in soxhlet apparatus until complete exhaustion. [20]

Phytochemical analysis [20-22]

Chemical tests were carried out using the ethanolic extracts from plants and or the powdered specimens, using standard procedures to identify the active constituents.

Test for alkaloids

Alcoholic extract (10 ml) was stirred with 5 ml of 1% HCL on a steam bath. Mayer's and Dragendorff's reagents were added, white and orange color precipitate respectively, were taken as evidence for the presence of alkaloids.

Test for flavonoids

NaOH test: The extract (5 ml) was treated with aqueous NaOH and HCl, and looking for the formation of a yellow orange color that was indicating to the presence of flavonoids.

Tests for steroids

Liebermann-Burchard test: Extract (3ml) was treated with chloroform, acetic anhydride and drops of sulphuric acid was added. The formation of dark pink or red color indicates the presence of steroids.

Test for tannins

Ethanolic extract (10mg) in 10ml distilled water was filtered, and then the filtrate (3ml) + 3ml of FeCl3 solution (5%w/v) were mixed. The formation of a dark green or blue black precipitate was considered an indication for the presence of tannins.

Tests for anthraquinones

***Borntrager's test*:** 3ml of alcoholic extract was shaken with 3 ml of benzene, filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the development of a pink, red or violet color in the ammonical (lower) phase indicates the presence of free anthraquinones.

Test for terpenoids

Alcoholic extract (2ml) was dissolved in chloroform (2ml) and evaporated to dryness. Concentrated sulphuric acid (2ml) was then added and heated for about 2 min. A grayish color was considered an indication for the presence of terpenoids.

Test for cardiac glycoside

Keller-kiliani test: Alcoholic extract (2ml) +1ml glacial acetic acid+ $FeCl_3$+con.H_2SO_4. The formation of green-blue color indicates the presence of cardiac glycoside.

DPPH Radical Scavenging Activity: [23]

The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl 2-picrylhyorazyl (DPPH) free radical .Two ml of 0.1mM DPPH solution in ethanol was mixed with 1ml of plant extract solution of *Abrus precatorius* with varying concentrations (0.5,0.25, 0.125, 0.062, 0.031, 0.015 and 0.0078 mg/ml). The reaction was carried out in triplicate and the decrease in absorbance was measured at 517nm after 30 minutes in dark. The inhibition % was calculated using the following formula.

Inhibition % = (Ac-As)/Ac×100

Where Ac is the absorbance of the control. As is the absorbance of the sample.

RESULTS AND DISCUSSION

A- The results of phytochemical analysis are given in table (1.2):

Alkaloids	Flavonoids	Steroids	Tannins	Anthraquinoin	Terpenoids	Cardiac glycoside
+	+	+	+	-	+	-

(+), (-) represent presence and absence of phytoconstituents respectively; **(+ < ++ < +++)** measure the intensity of characterstic colour.

B-Assay of DPPH Radical Scavenging Activity

DPPH assay provides basic information on antiradical activity of extracts and its results can indicate the presence of phenolic and flavonoid compounds in plant extracts. Very significant antioxidant activities were found in ethanolic extract and positive control (Vitamin C), which increased with increasing concentration **(figure 1.1)**

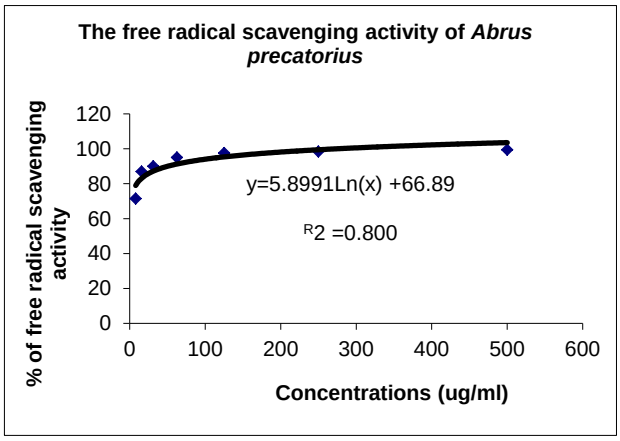

Figure (1.1): DPPH radical-scavenging activities of *A. precatorius* extracts at different concentrations

IC_{50} of ethanolic extract of *Abrus precatorius* plant, (which is the concetration of the sample required to scavenge 50% of the free radicals present in the system), was calculated by the linear regression equation as the following:

$$y=5.8991Ln(x) + 66.89$$

y= percentage of DPPH scavenging activity and represented by 50%.

X= concentration.

The IC_{50} DPPH scavenging activity for ethanolic extract 57.08 mg /ml.

Table (1.1) shows the percentage of DPPH scavenging activity of serial concentrations of the *Abrus precatorius* ethanolic extract and the positive control. Data are expressed as mean ±SD.

The results of preliminary phytochemical analysis of plant extract showed the presence of alkaloids, flavonoids, steroids, and terpenoids and tannins, and the absence of, anthraquinoin and cardiac glycosides.

Several studies have pronounced a positive correlation between phenolic content and antioxidant activity using similar assay system, but in present study could not establish correlation in similar manner.

It could be due to the existing of other reducing compounds have been established in the ethanolic extract of *Abrus precatorius* by phytochemical analysis, these compounds, nonphenolic in its nature, like terpenoids and alkaloids with antioxidant effects**.** the different compositions of extract which one of each have different antioxidant potency that make some of them having synergistic effect or they react quickly with DPPH, while the other compounds have a slower reaction mechanism and require to high concentrations to have significant effect. [24]

Table (1.1): Percentage of DPPH scavenging activity of the *Abrus precatorius* ethanolic extract at different concentrations and the positive control.

Conc. Of extract (µg/ml)	% of DPPH scavenging activity	Conc. of vit.C positive control(µg/ml)	% of DPPH scavenging activity
500	97± 0.012662	500	99.47± 0.073528
250	90 ±0.002	250	98.42± 0.160594
125	90 ±0.029501	125	97.63 ±0.143598
62.5	95 ± 0.0601	62.5	95 ±
31.25	97.63 ± 0.024906	31.25	90±
15.6	98.42 ± 0.047983	15.6	87 ±
7.8	99.47 ± 0.047127	7.8	71.45 ±

Indole alkaloids are found in the *Abrus precatorius plant* [25]. There are several studies have been performed, to investigate how indole derivatives (included the indole alkaloids) act as antioxidant and scavenge a variety of reactive oxygen species (ROS). In sequence, the ROS scavenging activities of indole derivatives have been attributed to**:** [26]

1. Exceptional redox properties of the indole ring, in particular conferred by the indolic nitrogen, which is described as the active redox center of indoles.
2. The electron-rich aromatic ring system, allowing the indoleamine to easily function as an electron donor, to form the cation radical, or through an electrophilic radical addition at the C-3 position of indole.
3. The mechanism of oxidation of indoles by DPPH could be explained as an initial hydrogen or electron abstraction of the indole ring. So the inoles have radical trapping activity.

The research was demonstrated that the (99.9%) of ethanolic extract of *A.precatorius* seeds contained the higher level of total phenol and flavonoids [27]. Flavonoids are also called as free-radical scavengers. The antioxidant activity was mainly due to their ability to donate hydrogen. Free radical scavenging capacity is primarily attributed to high reactivities of hydroxyl substituents that participate in the reaction**.** [28] Radicals are made inactive according to the following equation as shown in the figure (1.2), where R• is a free radical and Fl-O• is a flavonoid phenoxyl radical. [29]

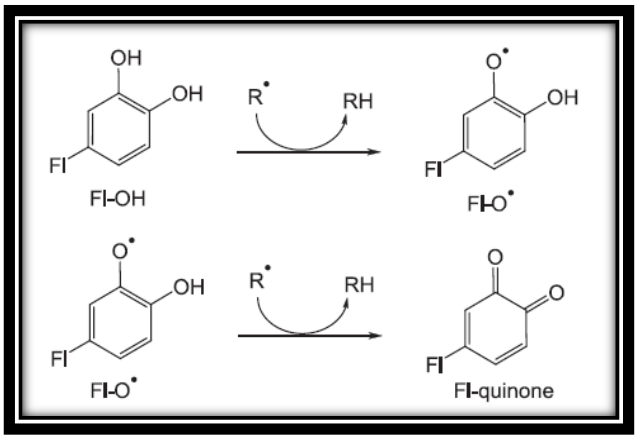

Figure (1.2): free radical scavenging activity of flavonoids.The free radical Fl-O• may react with a second radical, acquiring a stable quinone structure.

The *Abrus precatorius* plant is rich with terpenoids but the most important terpenoid have been isolated from it is the β-sitosterol which possesses antioxidant activity estimated through the scavenging of free radicals such as DPPH .[30,31]

CONCLUSION

This study has gathered experimental evidence that ethanolic extract of Iraqi plant *Abrus precatorius* contained diverse phytochemicals especially the alkaloids, flavonoids, terpenoids and others that exhibited significant antioxidant activity by scavenging DPPH free radical. Therefore, it can be used as a source of natural antioxidants and used in drug formulations for treatment of diseases resulting from oxidative stress.

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