Antioxidant Activity of Rosary Pea Plant: A Review Study

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ABSTRACT

Abrus precatorius is commonly called rosary pea, Indian licorice, Saga-Saga, Crab’s eye, jequerti, Ratti, Glycyrrhizin glabra, and Gunja. Among the antioxidant activities examined, total antioxidant activity (44.0±3.0mg/g) as well as reducing power activity (37.0±2.0mg/g) revealed prominent results, whereas, nitric oxide scavenging (29.0±3.0mg/g) and metal chelating activity (14.3±3.0mg/g) was lesser when compared to other antioxidant activities in aqueous extract of Abrus precatorius seeds. The antioxidant activity was performed by DPPH (2, 2-diphenyl-1-picryl-hydrazyl) free radical scavenging assay. The in-vitro antioxidant assays indicated the extract of AP possesses potent antioxidant action when compared with the reference substance ascorbic acid (Vitamin C). Abrus precatorius can be observed as a plant for future drugs when utilized in the preparation of nutraceuticals as a potent antioxidant to cure human ailments and its complication. The current research was designed to investigate the antioxidant activity of the methanolic extract of Abrus precatorius plants. The methanolic extract of the plant consists of many bioactive chemical compounds like alkaloids, glycosides, tannins, carbohydrates, gums, etc. Antioxidant activity was done by utilizing 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays and the maximum % of scavenging activity was noticed at 106.22 mcg/ml. The capacity of deactivating free radicals was extensively determined with in vitro biochemical techniques like DPPH free radical, OH free radical, NO, SO; scavenging assays, and inhibition ability of Fe (II)-induced lipid peroxidation. Abrus precatorius extracts exhibited strong antiradical features, the capacity to chelate Fe2+, and contain good inhibition capacity of lipid peroxidation. Seeds manifested DPPH radical scavenging activity (88.34±0.08 %) in methanolic extracts. The antioxidant potential was examined to be the greatest in seeds followed by root, leaves, and stem. Methanol was identified to be the best solvent for the extraction of phenolics, flavonoids, and antioxidants. Antioxidant action was evaluated for the aqueous extract of seed coat by utilizing 1, 1-diphenyl-2-picrylhdyrazyl (DPPH) radical scavenging assays and the maximum % of scavenging activity was noticed at 100ug/ml. The plant Abrus precatorius was examined for its antioxidant activity. The extractions were utilized to assay by Reducing power, Nitric oxide scavenging activity, and DPPH techniques for investigation of antioxidant action. The results primarily recommend the existence of potent oxidant inhibitory principles in the roots of Abrus precatorius.

Keywords: Abrus precatorius, antioxidant, DPPH, scavenging activity.

INTRODUCTION

Abrus precatorius seeds are slightly smaller than ordinary peas; ovoid and scarlet with a black spot around the hilum. The root is woody, tortuous, and much branched, with a sweet taste 1. Abrus precatorius leaf is effective in wound healing, infections with acne sores or boils, and wounds. It also supports in capturing rid of itching and other skin-associated complications. The plant kingdom synthesizes diverse active compounds which are valuable in the treatment and control of many diseases. These compounds are principally secondary metabolites 2. It is endemic in the tropics and generally known as the Rosary bean. The roots of Abrus precatorius possess proteins, glycosides, phenolic compounds, fatty acids, fatty acid esters, anthocyanins, and minerals 3. The leaf of Abrus precatorius has long internodes. It has lean branches with round folded stem with polished coarse brown bark. Its roots are extremely and forcefully hard to be eliminated. It increases in population size following a fire 4. Plants have been extensively used to treat various diseases. The practice of using plants as a source of medicine could be traced back as far back as the beginning of human civilization. The earliest mention of the use of plants to treat diseases in Hindu culture is found in Rig-Veda which was written between 4500 -1600 BC 5. Numerous types of research have been described in different parts of Abrus precatorius L. utilizing various extracts for its pharmacological and therapeutic claim. Furthermore, the plant is been a valuable source of natural products for the development of medicine against various diseases and industrial products. Different active components of plant tissue can be separated using appropriate solvents. Despite many studies having been...
reported on this plant species, the climatic and geographic conditions also impose the distribution of highly active components. The origin place of renewed systems of indigenous medicine like Siddha, Ayurvedha, & Unani is India. The activity of medicine depends on the proper parts of plant use and its biological effect which in turn depends on the presence of required quantity dose and nature of secondary metabolite in a raw drug material. The different chemical constituents are detected in crude dry powder of various medicinal plants, while different parts extracts like leaf, stem, fruits, and root were screened for phytochemical constituents by FT-IR spectroscopic analysis technique. The different active functional groups of chemical components in various extracts of medicinal plants were detected using the spectroscopic method.

Medicinal plants are vital biological sources whose parts (leaves, seeds, stems, roots, fruits, foliage, etc.) extracts, decoctions, infusions, and powders are used in the treatment of various diseases of humans, plants, and animals. Plant extracts are highly efficient against microbial infections. It is estimated that over 70,000 plant species, from lichens to tall trees, have been utilized at one time or another for medicinal purposes. Secondary metabolites such as alkaloids, tannins, flavonoids, and phenolic compounds are rich in plants which have been found in-vitro to have antimicrobial properties. One of the endangered plant species of the flora of Saudi Arabia is Abrus precatorius. The percentage frequency and the density per hectare of Abrus precatorius were 0.31 and 0.05, correspondingly. Furthermore, Abrus precatorius is classified as a non-endemic-endangered species in Jabal Fayfa, southwest of Saudi Arabia. Paste of Abrus precatorius seeds was enforced locally in sciatica, stiffness or shoulder joint, and paralysis said to be effective in dysentery, and the paste was utilized against skin ailments. Half-boiled seeds are given as tonic. It also manifests anti-cancerous activity. Trypanosomiasis is one of the main public health issues in sub-Saharan Africa. It is a serious handicap to populations and countries’ struggle for social and economic evolution. Hence, it is an essential priority for biomedical, public agencies, agricultural regions, and the scientific community. The ailment produced by the Trypanosome brucei sub-group is related to anemia, hepatocellular degeneration, and glomerulonephritis. Natural products chiefly from plant kingdoms provide a wide range of biologically active constituents that play as natural antioxidants with recognized potential in drug discovery and development. Seeds are utilized for the treatment of diabetes and chronic nephritis. In Siddha medicine, the white variety is utilized to synthesize oil that is utilized to be an aphrodisiac. Biologically active constituents from herbal medicines are utilized necessarily for curing mild or chronic illnesses. Hot water extract from seeds is consumed orally as an antifertility agent, as an abortifacient, and to stop conception. Decoctions of dried seeds are consumed orally to stimulate abortion. Plant extracts and also their primary and secondary metabolites have an important role in the treatment of numerous human illnesses. There is growing evidence that free radicals generated molecular alterations that are associated with numerous degenerative human illnesses such as arteriosclerosis, cancers, Alzheimer's disease, Parkinson's disease, diabetes, asthma, arthritis, immune deficiency ailments, and aging. Antioxidants are compounds that scavenge free radicals and prevent them from producing cell damage. Plants consist of antioxidant constituents that work as free radical scavengers, reducing agents, and quenchers of singlet oxygen production. Abrus precatorius Linn. is a woody twinning plant of the Fabaceae family, with specific red and black seeds. The leaves are pinnate and glabrous, with many leaflets (>12) ordered in pairs. The leaflets are oblong, calculating 2.5- cm long and 1.5 cm wide. The plant bears orange-pink flowers, which arise as clusters in short racemes that are frequently yellowish or reddish-purple in color, small, and typically pea-like. The plant generates short and stout brownish pods, which curl back on opening to show pendulous red and black seeds, 4 to 6 peas in a pod. This plant breeds wild in thickets, farms, and secondary clearings, and sometimes in hedges. It is most general in rather dry areas at small elevations throughout the tropics and subtropics. For a long time, several experimental and epidemiological findings have revealed that a broad diversity of phytochemicals such as phenolics, flavonoids, isoflavone, flavones, anthocyanins, catechin, iso-catechin, and carotenoids have significant antioxidant actions. These phytochemicals and others are determined in the leaves and seeds of Abrus precatorius which make this plant very important and versatile for its numerous numbers of medicinal features. In the Indian System of Medicine, the seeds are utilized for sciatica, paralysis, headache, dysentery, diarhrea, leprosy, ulcer, nervous disorders, alopecia, and also anti-inflammatory, anti-diabetic, antibacterial, anthelmintic, abortive, antidiarrheal, antimicrobial, inulin, laxative, antipyretic and antiphlogistic. In India, hot water extract from dried leaves and roots is introduced to the eye to treat eye illness. In Brazil, a water extract from dried leaves and roots is consumed orally as a nerve tonic. Seeds are remarked to be emetic, tonic, purgative, aphrodisiac, anti-ophthalmic, and antiphlogistic. In Siddha medicine, the white variety is utilized to produce oil that is utilized to be an aphrodisiac. In Nigeria, Abrus precatorius leaves have been utilized for the therapy of numerous illnesses, including malaria, typhoid, hepatitis, and respiratory tract infections. The leaves have also been documented for their cytotoxicity, anti-diabetic and antimicrobial actions. The root and leaves are utilized in conventional medicine to cure cancer, chronic nephritis, bronchitis, fever, asthma, stomatitis, and diabetes. The stem, root, and leaves of this plant are utilized to cure snake bites, tuberculosis, protozoal infections, and insecticide toxic. The seeds have been documented for their toxic effect on the kidneys, liver, heart, spleen, intestine, and lungs. However, the leaves have been
known to possess fewer concentrations of a deadly poison termed “abrin” 17. Abrus precatorius is a vine originally native to India. The leaves of Abrus precatorius contain a sweetness potency as that of sucrose. The plant is traditionally utilized for the therapy of tetanus and rabies 18. Antioxidants have gained numerous importance because of their potential therapeutic benefit in illnesses produced by free radicals. Plants with medicinal features are being examined for this purpose, because of their significant antioxidant actions, fewer side effects, and economic feasibility 19. Herbal medicines are in more demand in developed as well as developing countries for primary healthcare due to their broad biological and medicinal actions, larger safety margins, and low costs 20. The plant has been utilized in Hindu medicines from very early times, and also in China and other ancient cultures. The seeds have been utilized to treat fever, malaria, headache, and dropsy and to remove worms. A decoction of the seeds is utilized for abdominal complaints, conjunctivitis, trachoma, and malarial fever. Central Africans utilize powdered seed as an oral contraceptive. It is also utilized to reduce high blood pressure and alleviate severe headaches. The seed has purgative features and is utilized as an emetic, tonic, aphrodisiac, and for nervous disorder 21. This review work focuses on the Antioxidant power of the various parts of the Abrus precatorius plant by using various solvents.

MATERIALS AND METHODS

Krishneveni Marimuthu et al, (2014)12

Aqueous extract preparation: Aqueous extract was produced by taking 75 and 100mg of Abrus precatorius seed powder. Each concentration was diffused in 10ml water mixing with a magnetic stirrer at 4°C for 4 hours. The mixture was strained through nylon cloth and centrifuged at 20,000g for 30 minutes. 0.1 ml of supernatant was utilized for the analysis.

Reducing power assay: Aqueous extract was added with phosphate buffer (2.5ml, 0.2M, PH 6.6) & 1%w/v potassium ferricyanide (2.5ml). The mixture was incubated at 50°C for 20 minutes. 1.0 ml of Trichloro acetic acid (10%) was mixed to prevent the reaction, which was then centrifuged at 3000rpm for 10 minutes. The upper layer of solution (1.5ml) was added with distilled water (1.5ml) and FeCl₃ (0.1ml, 0.1%) after adding, the mixtures were incubated for 10 minutes and the optical density was calculated at 700nm. The enlarged optical density of the reaction mixture revealed enlarged reducing power.

Ascorbic acid was utilized as a positive control.

Total antioxidant capacity: Total antioxidant capacity by phosphomolybdenum technique assay is established on the reduction of Mo (V1) to Mo (V) by the sample anlyte and the successive generation of a green phosphate/Mo (V) complex at acidic pH by mixing 4ml reagent solution consisting 0.6M Sulphuric acid, 28mM Sodium phosphate, 4mM Ammonium molybdate. The tubes were incubated in a water bath at 95°C for 90 min. After the samples had been cooled to Room temperature, the optical density of the mixture was calculated at 695nm against blank. The phosphomolybdenum method is quantitative, since, the total antioxidant activity is indicated as the number of equivalents of ascorbic acid.

Nitric oxide scavenging activity: This procedure is established on the principle that sodium nitroprusside in an aqueous solution, at physiological pH spontaneously forms nitric oxide which interacts with oxygen to synthesize nitrite ions that can be determined utilizing Griess reagent. Scavengers of nitric oxide engaged with oxygen, leading to reduced formation of nitrite ions. For the experiment, sodium nitroprusside (10mM), in phosphate-buffered saline, was added with extract and incubated at room temperature for 150 minutes. 0.5ml of Griess reagent was mixed after the incubation period. The optical density of the chromophore produced was read at 546nm. Ascorbic acid was utilized as a positive control.

Metal chelating activity: Mix extract (0.1ml) to a solution of 2mM FeCl₃ (0.05ml). The reaction was initiated by the mixing of 5mM Ferrozine (160µl), the mixture was shaken vigorously and kept standing at room temperature for 10 minutes. The optical density of the solution was then calculated spectrophotometrically at 562 nm. A standard curve was drawn utilizing ascorbic acid. Distilled water (1.6ml) instead of sample solution was utilized as a control. Distilled water (160µl) instead of ferrozine was utilized as a blank, which is utilized for error correction because of the unequal color of the sample solution.

Rashmi Arora et al, (2011)13

Extract preparation: Nearly 300 grams of powdered seeds of Abrus precatorius were extracted with ethanol in the Soxhlet apparatus for seven days. The extract was evaporated by a rotary evaporator at 40 to 50°C under reduced pressure. The concentrated filtrate was added to distilled water and partitioned sequentially with hexane. The aqueous layer was separated and concentrated by a water bath. The yield of the extract was examined. The ethanol extract produces a brown sticky mass. The crude extract was utilized for further analysis.

Antioxidant activity: The antioxidant activity was done by the DPPH assay technique. In this technique the % inhibition of 1,1 diphenyl 2-picryl hydrazyl radical was estimated three times by utilizing the below formula, % scavenging Activity = 100 \( \times (A0 - As) \) Where, AO is the optical density of control, As is the optical density of the sample.

Zahraa Suhall Nassir (2017)14

Preparation of extract: Shade-dried coarsely powdered seeds, and aerial parts (200 grams) were defatted with hexane for 24 hours and then kept to evaporate at room temperature. The defatted plant material was extracted with 80%v/v ethanol (1 Litre) in the soxhlet apparatus until complete exhaustion.
DPPH Radical Scavenging Activity: The antioxidant activity of the extracts was calculated based on the scavenging activity of the stable 1, 1- diphenyl 2-picrylhydrazyl (DPPH) free radical. 2 ml of 0.1 mM DPPH solution in ethanol was added with 1 ml of plant extract solution of Abrus precatorius with different 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 reduction in optical density was calculated at 517 nm after 30 min in the dark. The % inhibition was determined utilizing the following formula:

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\%\text{ inhibition} = \frac{(\text{Abs control} - \text{Abs sample})}{\text{Abs control}} \times 100
\]

Where Ac is the optical density of the control. As is the optical density of the sample.

Durgesh Verma et al, (2011)

Sample preparation: Air-dried (35 – 40 °C) powdered seeds (100 mesh) of Abrus precatorius (5.0 g) were dissolved in methanol (3 × 20 ml, 3 times, 3 days). Extracts were mixed, filtered, and evaporated to dryness by a rotary evaporator and then lyophilized. 10 mg of accurately weighed methanol extract was dissolved in 1 ml of methanol to produce 10 mg ml\(^{-1}\) solution.

Determination of In-vitro anti-oxidant activity (DPPH radical scavenging activity): A solution of 0.135 mM DPPH (2, 2- Diphenyl-2-picrylhydrazyl) in methanol was produced and 1.0 ml of this solution was added with 1.0 ml of extract in methanol consisting of 0.02 - 0.1 mg of extract. The reaction mixture was vortexed thoroughly and kept in the dark at room temperature for 30 minutes. Optical densities of the mixture were estimated at 517 nm utilizing a Double beam UV-Vis Spectrophotometer. Ascorbic acid and Gallic acid were utilized as references. The capacity to scavenge DPPH radicals was determined by the following equation. DPPH radical scavenging activity (% = \{[\text{Abs control} - \text{Abs sample}) / \text{Abs control}]\} \times 100

Tania Tabassum et al, (2017)

Extract preparation: A glass-made jar containing a plastic cover was taken and the jar was washed with methanol and evaporated. Then 370 grams of the dried leaves were placed in the jar and methanol (1500ml) was added into the jar up to 1-inch height above the sample surface. The plastic cover with aluminum foil was closed correctly to prevent the entrance of air into the jar. This process was kept cool, dry, and dark place.

DPPH Free Radical Scavenging Activity: The 1, 1-diphenyl-2-picrylhydrazyl radical (DPPH) has been broadly utilized to examine the free radical scavenging capability of antioxidants. DPPH free radical is reduced to the respective hydrazine when it reacts with hydrogen donors. DPPH can produce stable free radicals in aqueous or methanol solution. With this technique, it was possible to determine the antiradical capacity of an antioxidant action by estimating the decrease in the optical density of DPPH at 517 nm. Resulting of a color change from purple to yellow the optical density was reduced when the DPPH was scavenged by an antioxidant, through the donation of hydrogen to produce a stable DPPH molecule. In the radical form, this molecule had an optical density of 517 nm which disappeared after acceptance of an electron or hydrogen radical from an antioxidant constituent to become a stable diamagnetic molecule. Preparation of Solution: 10 mg extract of Abrus precatorius was taken by electronic balance and added with 10 ml of methanol (99-100%) to produce 1000 mcg/ml solution of extract as stock solution. Another 10 different concentrations of solutions were produced by the 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 method was followed to produce various concentrations of solutions from stock solution: Volume which we have to take from stock solution = Desired concentration X Desired volume / Supplied concentration. Similarly, different concentrations (500 μg/ml – 0.97 μg/ml) of ascorbic acid solutions were produced. 2 mg DPPH powder was taken by electronic balance and mixed with 100 ml of methanol (99-100%) to produce 20 μg/ml DPPH solutions. It should be placed in a cool, dry, and dark place.

Assay of Free Radical Scavenging Activity: 2.0 ml of a methanol solution of the sample (Control/extractives) at various concentrations from 500.0 to 0.977mcg/ml was added with 3.0 ml of a DPPH methanol solution (20 mcg/ml). After 30 min reaction period at room temperature in a dark place the optical density was estimated at 517 nm against methanol as blank by UV spectrophotometer. Inhibition of free radical DPPH in percent (\%) was determined as follows: {((Ao – As)/Ao) X 100} Where Ao is the absorbance of the control reaction (containing all reagents except the test material), and As is the absorbance of the extract/standard. Extract/standard concentration providing 50% inhibition (IC50) was determined from the graph plotted Inhibition % against extract concentration.

Fatima Mohammad madaki et al, (2019)

Sample Preparation and Extraction: The gathered leaves were rinsed and dried out for two weeks at 37 °C and powdered, utilizing a grinding mill. A 50 grams sample of the material was extracted with 200 ml of methanol, utilizing soxhlet apparatus, and the resulting extract was concentrated, utilizing a water bath at low temperature.

Antioxidant Study: The antioxidant activity of the extracts was determined at different concentrations (20-100 mcg/ml), utilizing ascorbic acid as the reference by DPPH assay.


Preparation of extracts: The air-dried leaves of the plant were powdered with a mechanical grinder to get a coarse powder, which was then taken to sequence extraction in a
soxhlet apparatus using hexane, ethyl acetate, ethanol, and water. Each time before extracting with the next solvent, the material was evaporated in a hot air oven at 40°C. Extracts were then strained through a Whatman No.1 filter paper and concentrated to the dry mass with the help of a rotary evaporator. The extraction process was repeated 3 times at various periods. It was examined that there was no larger difference in the % yield and content of phytoconstituents that are believed to act an important role in biological activities. The yield of each extract was calculated and residues were kept in dark glass tubes for further investigation. The various extracts were named APH (for hexane extract), APE (for ethyl acetate extract), APA (for ethanol extract), and APW (for water extract). The evaporated extracts were diffused in dimethyl sulfoxide (DMSO) at 20 μg/ml and diluted with phosphate-buffered saline (PBS, pH 7.4) to produce final concentrations.

Phosphomolybdenum assay: The total antioxidant activity of extracts was examined by green phosphomolybdenum complex. An aliquot of 10 μl of sample solution was mixed with 1 μl of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in a microcentrifuge tube. Tubes were incubated in a dry thermal bath at 95°C for 90 minutes. After cooling, the optical density of the mixture was measured at 695 nm against a blank. Ascorbic acid has been utilized (r2 = 0.964) for reference and the reducing capability of the analyzed extracts was expressed as mg of ascorbic acid equivalents (mg AAE/g of dw).

Ferric-reducing/antioxidant power (FRAP) assay: Extracts and standard (Vitamin C) in 1 ml of appropriate solvents were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide (1%), and then the mixture was incubated at 50°C for 30 minutes. Afterward, 2.5 ml of trichloroacetic acid (10%) was mixed into the mixture, which was then centrifuged at 5000 rpm for 10 minutes. Finally, 2.5 ml of the upper layer solution was mixed with 2.5 ml of distilled water and 0.1 ml of FeCl3 (0.1%). The optical density was calculated at 700 nm and the reducing power of the extracts was presented as mg AAE/g of dw.

DPPH free radical scavenging activity: The DPPH free radical scavenging action of leaf extracts of Abrus precatorius was calculated in terms of hydrogen donating or radical scavenging ability utilizing the stable radical DPPH. 0.004% w/v of DPPH radical solution was produced in methanol and then 900 μl of this solution was added with 100 μl of extract solution consisting of 40–400 μg/ml of dried extract. The optical density was calculated at 517 nm after 30 minutes of incubation. Methanol (95%), DPPH solution, and ascorbic acid were utilized as blank, control, and reference correspondingly.

Hydroxyl radical scavenging activity: The mixture consisting of FeCl3 (10 mM), ascorbic acid (1 mM), H2O2 (10 mM), deoxyribose (28 mM), and various concentrations of test samples (40–400 μg/ml) in 500 μl phosphate-buffered saline (PBS, 20 mM, pH 7.4) was incubated for 30 minutes at 37°C. After mixing 1 ml of trichloroacetic acid (10%, w/v) and 1 ml thiobarbituric acid (2.8% w/v; in 25 mM NaOH), the reaction mixture was boiled for 15 minutes. The extent of oxidation was calculated at 532 nm and the scavenging activity of the test sample was expressed as the % inhibition of the deoxyribose degradation to malondialdehyde. Ascorbic acid was utilized as the positive control.

Hydrogen peroxide scavenging assay: A 40 mM of H2O2 solution was added with various concentrations of plant extracts (20–200 μg/ml) and incubated for 3.5 hours at room temperature. After incubation, 90 μl of the H2O2 sample solution was added with 10 μl of HPLC-grade methanol, and 0.9 ml of FOX reagent was added (prepared by mixing 9 volumes of 4.4 mM BHT in HPLC-grade methanol with 1 volume of 1 mM xylene orange and 2.56 mM ammonium ferrous sulfate in 0.25 M H2SO4). The reaction mixture was vortexed and then incubated at room temperature for 30 minutes. The optical density of the ferric-xylene orange complex was calculated at 560 nm. Ascorbic acid was utilized as the reference compound.

Nitric oxide scavenging activity: The reaction solution (50 μl) consisting of 10 mM sodium nitroprusside in PBS (pH 7.0) was added with various concentrations (40–400 μg/ml) of sample extracts, followed by incubation at 37°C for 20 minutes under the light. After incubation, the samples were added with 300 μl of Griess reagent (1% sulfanilamide, 2% H3PO4). The samples were again incubated for 30 minutes at room temperature under light conditions followed by the mixing of 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride. The optical density was documented at 546 nm and the results were expressed as percent of scavenged nitric oxide concerning the negative control without mixing of any antioxidant. Ascorbic acid was utilized as a positive control.

Superoxide radicals scavenging activity: The scavenging activity of the plant extracts towards superoxide anion radicals was calculated by the nitro-blue tetrazolium (NBT) reduction technique with minor modifications. Superoxide anions were produced in a non-enzymatic phenazine methosulfate–nicotinamide adenine dinucleotide (PMS–NADH) system through the reaction of PMS, NADH & oxygen. It was assayed by the reduction of nitroblue tetrazolium. In the experiment, the superoxide anion was produced in 2 ml of phosphate buffer (100 mM, pH 7.4) consisting of 500 μl of 156 μM nitroblue tetrazolium (NBT solution), 500 μl of 468 μM nicotinamide adenine dinucleotide (NADH) solution and 300 μl of various concentrations (40–400 μg/ml) of extracts. DMSO and L-ascorbic acid were utilized as a solvent and positive control respectively. The reaction was initiated by mixing 100 μl of 60 μM phenazine methosulfate (PMS) into the mixture. After 5 minutes of incubation at room temperature, the absorbance was calculated at 560 nm against blank. The reduced optical density of the reaction mixture revealed raised superoxide anion scavenging activity.
Inhibition of lipid peroxidation assay: Fe³⁺-induced lipid peroxidation is one of the established systems for evaluating the antioxidant activity of different plant extracts. A modified thiobarbituric acid-reactive species (TBARS) assay was utilized to calculate the lipid peroxide produced utilizing rat liver homogenate as lipid-rich media. Malondialdehyde (MDA), a secondary final product of the oxidation of polyunsaturated fatty acids, combined with 2 molecules of TBA yielding a pinkish-red chromogen. Healthy albino rats of the wister strain (250 grams) were sacrificed and the liver was perfused with 0.15 M potassium chloride, the homogenate was centrifuged at 800 g for 15 min at 4 °C and the supernatant was utilized for thiobarbituric acid assay. The extracts of Abruṣ precatorius at various concentrations (40–400 μg/ml) were added with the liver microscope preparation and incubated at room temperature for 10 minutes. Then, 50 μl Fenton’s reagent (10 mM FeCl₃; 10 μl of 2.5 mM H₂O₂; 0.1 M L-ascorbic acid) in phosphate buffer (0.2 M, pH 7.4) was mixed, and the volume was made to 1 ml. The tubes were then incubated for 30–45 minutes at 37 °C to stimulate lipid peroxidation. Thereafter, 2 ml of ice-cold HCl (0.25 N) containing 15% trichloroacetic acid, 0.5% thiobarbituric acid, and 0.5% butylated hydroxytoluene (BHT) were added to each sample accompanied by heating at 100 °C for 15 minutes. The reaction mixture was placed in an ice bath for 10 minutes. The mixture was centrifuged at 1000 rpm for 10 minutes and the extent of lipid peroxidation was subsequently monitored by the production of thiobarbituric acid reactive substances (TBARS) as pink chromogen in the presence or absence of extracts and standard (L-ascorbic acid). The optical density of the supernatant was calculated spectrophotometrically at 532 nm and the decline in the formation of pink chromogen in pre-treated reactions was declared as inhibition of lipid peroxidation.

Amita Jain et al, (2015)¹⁹

Extract preparation: The fresh and dried plant parts were compressed into fine powder utilizing liquid nitrogen. 10 grams of the sample were dissolved in 100 ml of 4 different extracting solvent systems like Distilled water, Ethanol, Methanol & Acetone correspondingly for overnight extraction. Extracts were strained utilizing Whatman No.1 paper and the filtrates were concentrated to 10 ml by utilizing the rotary evaporator at 40 °C. Extracts were re-diffused in each respective solvent to make the stock solution of 100 mg/ml for investigation and stored at 20°C refrigerator as far as further use.

DPPH radical scavenging Assay: The radical scavenging potential of various extracts was examined by DPPH assay. The radical scavenging capacity of antioxidants constituents presents in the extract, reduces the optical density and produces a stable DPPH (2,2-diphenyl-1-picrylhydrazyl) radical providing it colorless. The optical density of the DPPH reagent was documented as the control and the radical scavenging activity of the samples was determined utilizing the following formula: % Inhibition = [(Absorbance of control – Absorbance of the sample) / Absorbance of control] X 100.

ABTS⁺ radical scavenging Assay: Optical density of the stable ABTS radical scavenging assay reagent was documented as the control and the ABTS⁺ radical scavenging activity of the sample was examined utilizing the following formula: ABTS⁺ radical scavenging activity = [(Absorbance of control – Absorbance of the sample) / Absorbance of control] X 100.

FRAP Assay: It quantitates the number of antioxidants in the sample, dependent on its capacity to reduce Fe³⁺ to Fe²⁺. The antioxidant content dependent on ferric ion reducing capacity was determined utilizing a standard curve of Trolox and the calibration equation y = 1.32x−0.0044 (R² = 0.9717) where ‘x’ is the mg Trolox Equivalent and ‘y’ is the optical density at 595 nm and the content were expressed as mg Trolox Equivalent Antioxidant Capacity per Gram of sample (mg TEAC/g).

B.Y.Sathish Kumar et al, (2017)²⁰

Preparation of the extract: 1g of the seed coat was boiled in 20 ml of water and the extract was investigated for various biological actions.

Determination of antioxidant activity using DPPH radical scavenging activity: The antioxidant action of the extracts was examined by estimating the hydrogen donating or radical scavenging capacity, utilizing the stable DPPH radical recorded prepared followed by preparation of working standard solutions of needed concentration.

Narendra Boggula et al, (2017)²¹

Extraction: 10 grams of each plant fine powder of indigenous plants weighed into a 250 ml conical flask and 100 ml of solvents were mixed separately for each plant powder and then on a rotary shaker at 190 – 220 rotation per minute for 24 hours. This was strained with Whatman no. 1 filter paper, the residue was discarded and the filters were dried to dryness in a water bath temperature of 80°C.

Preparation of stock solution: The stock solution was formed by weighing 10 mg of each dried solvent extract diffused in 1 ml of dimethyl sulphoxide (DMSO) giving a final concentration of 10,000 μg/ml. The stock solution was placed in screw-capped bottles for further investigation.

Anti-oxidant activity: The total antioxidant activity of the extract was investigated by the DPPH radical scavenging assay, Nitric oxide radical scavenging assay & Reducing power assay.

DPPH Radical Scavenging Assay: DPPH radical scavenging activity was estimated by the spectrophotometric technique. A stock solution of 25mg of DPPH (150μM) was formed in 100ml of ethanol, 0.1ml of extract of various concentrations was diffused in DMSO, and 1.9ml of DPPH was mixed. 0.1ml of DMSO was mixed with 1.9ml of DPPH in the case of control and 0.1ml of DMSO was mixed with 1.9ml of ethanol in the case of blank. The reaction proceeded to be finished in the dark for about 20 minutes.
Then the optical density of test mixtures was measured at 517nm. The % inhibition was determined and expressed as the percent scavenging of DPPH radical. Curcumin (50, 100, 200μg) was utilized as standard.

**Nitric Oxide Radical Scavenging Assay:** The nitric oxide radical scavenging activity of *Abrus precatorius* was examined as per this method. An aqueous solution of sodium nitroprusside spontaneously produces nitric oxide (NO) at physiological pH, which interacts with oxygen to form nitrate ions and was calculated colorimetrically. 3ml of reaction mixture consisting of 2ml sodium nitroprusside, 10mM in phosphate-buffered saline (PBS), and 1ml of different concentrations of the extracts were incubated at 37°C for 4 hours. Control without test constituents was maintained identically. After incubation 0.5ml of Griess reagent was treated. The optical density of the chromophore produced was read at 546nm. The % inhibition of nitric oxide formation was calculated by comparing the optical density values of the control and those of test constituents. Curcumin (50, 100, 200μg) was utilized as standard.

**Reducing Power Assay:** The reducing power assay of *Abrus precatorius* was examined according to this method. Extracts of various concentrations were formed in 1ml of DMSO and added with 2.5ml of phosphate buffer (pH 6.6, 0.2M) and potassium ferricyanide (2.5ml, 1%). The mixture was incubated at 50°C for 20 minutes. Aliquots of trichloroacetic acid (TCA) (2.5ml, 10%) were treated to the mixture, which was then centrifuged at 1,500 rotation per minute for 10 minutes. The upper layer of the reaction mixture was added with distilled water (2.5ml) and freshly produced FeCl₃ solution (0.5ml, 0.1%). The optical density was calculated at 640nm. Elevation in the optical density of the reaction mixture indicates the elevation in reducing power. Reducing power is given in terms of ascorbic acid equivalent (as Emg⁻¹).

**RESULTS AND DISCUSSION**

Among the different antioxidant activities evaluated, the reducing power, as well as total antioxidant activity, was greater when compared to nitric oxide scavenging and metal chelating activity. All antioxidant activities were higher at 100mg concentration. Aside from their role as health benefactors, antioxidants are added to foods to stop or delay the oxidation of food, produced by free radicals generated during their exposure to environmental factors such as air, light, and temperature. The quantitative antioxidant capacity of the ethanolic extract of the *Abrus precatorius* seeds was larger than the standard vitamin C. The % inhibition of the DPPH free radical was examined to be 80.1 + 0.34 at 300 μg/ml. DPPH assay gives fundamental information on the antiradical action of extracts and its results can reveal the presence of phenolic and flavonoid constituents in plant extracts. Very significant antioxidant actions existed in ethanolic extract and positive control (Vitamin C), which raised with increasing concentration. IC₅₀ of ethanolic extract of *Abrus precatorius* plant, (which is the concentration of the sample required to scavenge 50% of the free radicals present in the system), was determined by the linear regression equation as the following: $y = 5.8991ln (x) + 66.89$. $y = \text{percentage of DPPH scavening activity}$ and represented by 50%. X= concentration. The IC₅₀ DPPH scavenging activity for ethanolic extract 57.08 mg/ml. It was noticed that at a concentration of 0.1 mg/ml, the DPPH free radical scavenging activity of seeds was observed 63.70%. The DPPH test is established on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. The % scavenging of DPPH radical was determined to increase with increasing concentration of the samples. The IC₅₀ value of positive control ascorbic acid is 7.61μg/ml. On the other hand, the methanol extract revealed promising DPPH free radical scavenging activity with an IC₅₀ value of 14.87μg/ml. The extract of *Abrus precatorius* inhibited DPPH radicals with an IC₅₀ value of 106.22μg/ml while ascorbic acid had an IC₅₀ value of 33.97μg/ml. The reducing power analysis of the extract also revealed some antioxidant activity, resulting in a rise in absorbance with the increasing concentrations of the extract. The IC₅₀ values of Abrus precarious was also far better than the scavenging activities documented for some other medicinal plants, such as Padina pavonica (IC₅₀ = 5.59 mg/ml), Laurenica majusculare (IC₅₀ =14.3 mg/ml), and Laurencia catarinensis (IC₅₀ = 53.8 mg/ml). The more antioxidant activities could be translated to a larger therapeutic value of the plant extract. In phosho, molybdenum Assay APA has a larger antioxidant capacity (24.73 ± 0.72 mg ascorbic acid equivalent/g dw) than the other three extracts which revealed antioxidant capacity in the order: APE (17.92 ± 0.38 mg AAE/g dw) > APW (16.66 ± 0.68 mg AAE/g dw) > APA (7.16 ± 0.43 mg AAE/g dw). In FRAP Assay the reducing ability of the extracts was in the range of 13.34 ± 0.35 to 2.67 ± 0.40 AAE mg/g dw. The FRAP values for APW were observed to be higher than the other three extracts. This suggests that APW has a significant ability to react with free radicals to alter them into more stable non-reactive species and to terminate radical chain reactions. DPPH assay provides basic information on antiradical activity of extracts and its results can reveal the existence of phenolic and flavonoids compounds in plant extracts. Very potent antioxidant activities were observed in all four extracts and positive control, which increased with increasing concentration. DPPH activity values for APE, APA, and APW were examined to be nearer to each other. The APE and APW were able to reduce the formation of DPPH radicals with a percentage inhibition of 96.35 ± 2.37 and 92.63 ± 4.63% correspondingly at the largest concentration of 400 μg/ml with the IC₅₀ values of 57.66 ± 1.32 and 79.97 ± 1.84 μg/ml correspondingly. Previous reports have described that ethyl acetate fractions are good origins of antioxidant compounds. APA also produced potent DPPH scavenging activity (95.14 ± 3.44%) in this study and its IC₅₀ (60.67 ± 1.03 mcg/ml) was close to that of APE, in which phenolic levels were nearly 3.5 times larger. Many groups have pronounced a positive correlation between phenolic content and antioxidant action utilizing the same assay.
systems, but our research work could not establish a correlation in the same manner. It could be because of the existence of other reducing constituents that probably interfere with the Folin-Ciocalteau assay and/or the existence of other non-phenolic constituents with antioxidant effects. APH revealed less inhibitory action as compared to other extracts with the % inhibition of 50.84 ± 5.68 at a similar concentration with the IC50 value of 196.70 ± 1.84 μg/ml. The IC50 values of Vitamin C and quercetin (positive controls) were examined to be 3.80 ± 0.43 and 9.84 ± 0.6 μg/ml correspondingly. The hydroxyl radical is understood to be the most reactive oxygen radical and it seriously spoils neighboring bio-molecules in the body, such as protein and DNA, developing into mutagenesis, carcinogenesis, and cytotoxicity. Therefore, the elimination of hydroxyl radicals is possibly one of the most effective defenses of a living body against numerous illnesses. A significant reduction in the concentration of hydroxyl radical was noticed due to Abrus precatorius extracts. All the extracts produced significant activity, above 40% in a concentration-dependent manner with greater inhibition of 79.52 ± 2.57% at 400 mcg/ml by APE with IC50 value of 205.51 ± 3.08 mcg/ml. APA (78.97 ± 1.60%; IC50 = 209.33 ± 4.13 mcg/ml) and APW (68.18 ± 3.14%; IC50 = 309.90 ± 5.21 mcg/ml) extracts were also observed to be significant powerful quenchers of •OH radical as compared to Vitamin C (IC50 = 62.40 ± 3.72). APH was observed to be a weak scavenger of •OH with the IC50 value of 464.25 ± 4.43 μg/ml. Our results recommended that the hydroxyl radical scavenging ability of extracts of Abrus precatorius is comparable to or even larger than earlier published reports and could help in stopping oxidative damage in the human body. Hydrogen peroxide (H2O2) is a biologically relevant, non-radical reactive oxygen species and is inevitably produced as a by-product of normal aerobic metabolism. However, when concentration rises under stress conditions, H2O2 could be detrimental for cells and could be changed into other ROS such as hydroxyl radicals. Thus, H2O2 scavenging activity becomes a crucial property of total antioxidant activity. In this research work, APA extract (IC50 = 92.83 ± 3.23 μg/ml) was observed to be an efficient scavenger of hydrogen peroxide radical, while APW extract (IC50 = 152.35 ± 1.06 μg/ml) was the least efficient. The APH and APE extracts also produced comparable efficiency with IC50 = 112.59 ± 3.24 and 121.02 ± 3.14 μg/ml correspondingly. The H2O2 scavenging power of all extracts was also better than that of quercetin examined in similar conditions. The results strongly suggest that these extracts consist of essential constituents for radical elimination. Several reports have already manifested that nutritive phenols act a significant role in preserving mammalian and bacterial cells from cytotoxicity stimulated by H2O2 revealing that the noticed activity of plant extracts could be because of the existence of phenols. Initially, NO was regarded to have only useful activities. A significant scavenging action on superoxide radicals was observed in the inhibitory effect of Abrus precatorius extracts on superoxide radicals in a concentration-dependent manner. Large inhibitions were found at very few extract concentrations. At 400 μg/mm of tested extract, the scavenging effects on superoxide radicals were observed to be 95.01 ± 4.29%; IC50 value = 143.44 ± 3.28 μg/ml for APE and 93.15 ± 4.36%; IC50 value = 157.07 ± 2.56 μg/ml for APA. Moreover, APW and APH also possess the significant scavenging effect 73.25 ± 4.50%; IC50 value = 201.45 ± 6.23 μg/ml and 46.81 ± 2.87%; IC50 value = 427.26 ± 5.72 μg/ml correspondingly, however with larger IC50 values.

Lipid peroxidation involves the production and propagation of lipid radicals with various deleterious effects, besides the demolition of membrane lipids, metabolic disorders, and inflammation. The formation of malondialdehyde (MDA) is a hallmark of this procedure. This process is started by hydroxyl and superoxide radicals leading to the production of peroxy radicals (LOO·) that ultimately propagates chain reaction in lipids. Thus, antioxidants that are capable of scavenging peroxy radicals could stop lipid peroxidation. In this research work, we calculated the potential of Abrus precatorius extracts to reduce lipid peroxidation in rat liver microsomes, stimulated by the Fe2+/ascorbate system. Different
extracts preserved against lipid peroxidation stimulated by Fe$^{2+}$, considerably decreased MDA content in a concentration-dependent manner. APE had the largest inhibiting activity (98.70 ± 2.56%; with the lowest IC$_{50}$ value at 45.46 ± 3.71 µg/ml. When compared to the activity of standard, (Vitamin C, IC$_{50}$ = 48.72 ± 4.23 µg/ml), inhibiting activity against lipid peroxidation of APE was very large considering that the extract was a mixture of a large number of constituents against pure constituents utilized as standards. The other extracts (APA, APW, and APH) manifested to be many poor inhibitors of lipid peroxidation than APE with the % inhibition of 73.75 ± 1.60%, 68.09 ± 3.26%, and 56.73 ± 3.81% correspondingly with larger IC$_{50}$ values than that of APE. DPPH assay gives fundamental information on the antiradical activity of extracts and its reports can reveal the existence of phenolic and flavonoid constituents in plant extracts. The methanolic extract of fresh seed revealed large scavenging activity (88.34±0.08 %) compared to dry seed (56.92±0.04 %) Fresh and dried condition of plant parts did not indicate significant variance in antioxidant action. However, amongst solvents methanol was found best in terms of extracting large amounts of antioxidative constituents. FRAP assay revealed a broad range of variation among the plant parts and solvents utilized Fresh seeds (110.10±5.70) and dry roots (59.39±0.43) mg TEAC/g revealed the largest FRAP activity when dry stem (1.77 ± 0.27 mg TEAC/g) and dry seeds (1.84 ± 0.02 milligram TEAC/g) produced the minimum FRAP value. Fresh seeds revealed 110.10 ± 5.70 mg TEAC/g while extracts were produced in methanol. The largest FRAP activity was observed in seeds followed by root > leaf > stem. The FRAP values for methanolic extracts were observed to be higher than the other 3 extracts. Extracts produced in acetone and methanol were fast and effective scavengers of the ABTS$^+$ radicals. Fresh seed revealed the greatest % inhibition of the radical. There was no significant variance in % inhibition of ABTS$^+$ radical in fresh and dry tissue extracts, but there was variance amongst different plant parts. The largest activity was found in acetone extract in fresh and also dry tissue followed by methanol. The DPPH test is established on the exchange of hydrogen atoms between the antioxidant and the stable DPPH free radical. It is apparent from the graph that the % scavenging of DPPH radical was observed to increase with increasing concentration of the samples. The results of the DPPH radical scavenging activity of seed coat extract of A. precatorius reveal that it contains very high % antioxidant activity when compared to Vitamin C (standard). The maximum % of scavenging activity of the aqueous extract of the seed coat was found at 100µg/ml. The extracts of Abru$\mathrm{s}$ precatorius were assayed for antioxidant action for petroleum ether extract and ethanolic extracts equivalent to Vitamin C. The anti-oxidants act either by scavenging different types of free radicals obtained from oxidative processes, by stopping free radical formation through reduction precursors, or by chelating agents. In this work, the extracts significantly contain anti-oxidant action. Petroleum ether extract of Abru$\mathrm{s}$ precatorius revealed large anti-oxidant action. These revelations are significantly observable as various metabolic disorders and functional defects might be attributed to its antioxidant potential. However, scientific confirmation of traditional claims is essential for exploiting the medicinal uses of this wonder herb. In light of the reports of current evaluation, it can be concluded that the antioxidant potentials of petroleum ether extract of Abru$\mathrm{s}$ precatorius in vitro are promising, however, the plant extracts should be examined to find out the numerous phytochemicals characteristic for antioxidant and pharmacological action. However, further studies are needed to evaluate the molecular mechanism of the antioxidant action of the Abru$\mathrm{s}$ precatorius L. roots.

**CONCLUSION**

In conclusion, this work reveals that Abru$\mathrm{s}$ precatorius seeds can be utilized as an antioxidant as it possesses flavonoids and a moderate quantity of phenolics-induced antioxidant actions. Even though the seeds are not utilized as edible seeds, according to reports, Abru$\mathrm{s}$ precatorius seeds find their use in therapeutic largely. The ethanolic extract of Abru$\mathrm{s}$ precatorius seeds was observed to have significant Antioxidant action. This work has collected experimental proof that the ethanolic extract of the Iraqi plant Abru$\mathrm{s}$ precatorius possessed diverse phytochemicals particularly the alkaloids, flavonoids, terpenoids, and others that produced significant antioxidant action by scavenging DPPH free radicals. Therefore, it can be utilized as an origin of natural antioxidants and utilized in drug formulations for the therapy of ailments resulting from oxidative stress. A consequence of antioxidants on DPPH is thought to be because of their hydrogen-donating capacity. Though the DPPH radical scavenging capacities of the extracts were significantly small than those of ascorbic acid (86.8%) and gallic acid (78.8%), the study revealed that extracts have proton donating ability and could act as free radical inhibitors or scavengers, playing possibly as primary antioxidants. Plant extract revealed 63.70% radical scavenging capacity at 0.1 mg/ml concentration. Abru$\mathrm{s}$ precatorius leaves have significant antioxidant action. The methanol extract of Abru$\mathrm{s}$ precatorious leaves has significant antioxidant action. This work has gathered experimental proof that Abru$\mathrm{s}$ precatorius leaf extracts contained significant antioxidant activity by efficiently scavenging numerous free radicals. In the current investigation, it has been shown that the methanol extracts of seed, root, leaves, and stem are observed to be good origins of antioxidant action. Total antioxidant power was observed to be greater in methanolic seed extracts followed by root > leaves > stem. The aqueous extract of the seeds coat consists of significant antioxidant action.

**Declaration of Interest**

The author describes no conflicts of interest. The authors solely are liable for the content and writing of this article.
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REFERENCES

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