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Antioxidant Potential and GC-MS Analysis of *Abrus precatorius* Linn Leaves Ethanol Extract

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of present study to evaluate the antioxidant potential of *Abrus precatorius* Linn leaves ethanol extract containing bioactive compounds on free radical scavenger using DPPH. Ethanol extract of *Abrus precatorius* Linn leaves was subjected to preliminary phytochemical screening and fraction of extract was detected by Gas Chromatography-Mass Spectrometry analysis.

Methodology and Results: GC-MS analysis of ethanol leaves extract was carried out on Shimadzu GC-MS model number QP 2010S and revealed the presence of 18 phytochemical compounds, with mome inositol as a dominant component. Antioxidant potential of leaves ethanol extract was evaluated using DPPH free radical scavenging assay at five different doses as 5, 10, 20, 30, 40 and 50 µg/mL and showed significant DPPH free radical scavenging potential with the IC_{50} value of 33.37 µg/mL. The bioautography of extract showed that fractions with the most prominent antioxidant potential tended to contain secondary metabolites reported in preliminary phytochemical screening such as alkaloids, carbohydrates, protein and amino acids, glycoside, tannins, flavonoids, triterpenoids and phenolic compounds. The results evaluate and justify the traditional relevance of *Abrus precatorius* Linn leaves ethanol extract for free radical scavenging potential as a antioxidant and can be used as a lead for the isolation of the antioxidant bioactive chemical constituents in further study.

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Conclusion: Preliminary phytochemical screening reported various bioactive compounds in the ethanol extract of *Abrus precatorius* Linn leaves and identified by GC-MS. The extract exhibited greater free radical scavenging activity i.e. antioxidant potential. The presence of various bioactive phytocompounds justifies the therapeutic use of the *Abrus precatorius* Linn plant leaves for various ailments by traditional practitioners.

Keywords: Abrus precatorius Linn; phytochemical; DPPH; antioxidant; GC-MS.

1. INTRODUCTION

Medicinal plants are rich sources of natural products. This free gift of the nature has been the backbone of traditional medicinal system of healing throughout the world and has also been an integral part of history and culture bestowed upon humanity with numerous therapeutic properties. The well documented natural products are played critical roles in modern drug development. Most of the natural products derived compounds in various stages of clinical development highlighted the existing significant bioactivity of use as sources of new drug candidates [1].

The phytochemical compounds present in the different parts of medicinal plants are getting attention for their active role in the prevention of several human illnesses [2]. *Abrus precatorius* Linn is a woody twinning plant with characteristic red seeds with black mark at the base commonly found in grassland and widely distributed tropical medicinal plant with several therapeutic properties. The seeds, leaves and roots of *Abrus precatorius* Linn are rich in phytoconstituents and several identified compounds having significant pharmacological activity [3].

Abrus precatorius Linn leaves have been used to treat numerous illnesses, including to cure fever, cough and cold, graying of hair, laxative, expectorant and aphrodisiac, urticaria, eczema, stomatitis, conjunctivitis, alopecia areata, migraine, lymphomas, leukemia, dysmenorrhoea, malaria, typhoid, hepatitis, cytotoxicity, anti-diabetic and antimicrobial activities [4].

The stem, root and leaves are used in traditional medicine to treat cancer [5], bronchitis, fever, asthma, chronic nephritis [6], stomatitis, diabetes [7]; also used to treat snake bites, to treat jaundice, haemoglobinuric bile, abdominal pains and tumors, abortion, hot water extract antimalarial and anticonvulsant, decoction used to treat bronchitis, hepatitis, tuberculosis, protozoal infections and insecticide poisoning [8,9]. The seeds of this plant are used for graying of hair, worm infection and treatment of fractures, insecticide, antimicrobial, contraceptives, tuberculosis and painful swellings, purgative, emetic, tonic, antiphlogistic, aphrodisiac, antiophthalmic and reported for the toxic effects on kidneys, liver, spleen, intestine, heart and lungs [10].

Due to the wide therapeutic properties of the leaves in various illnesses, the present study of ethanol leaves extracts of *Abrus precatorius* Linn were selected to study antioxidant potential by DPPH free radical scavenging in vitro assay in order to identify the phytochemical constituents for discover resources of new lead structures, which improve the traditional medicine.

2. MATERIALS AND METHODS

2.1 Standards and Reagents

All chemicals, reagents, reference standards and solvents used were extra pure and A.R. grade. 2, 2-diphenyl picrylhydrazyl (DPPH) free radical was purchased from Sigma-Aldrich, USA, Ascorbic acid was purchased from SDF, Mumbai and ethanol 99% absolute.

2.2 Collection of Plants Leaves

The leaves of *Abrus precatorius* Linn medicinal plant were collected from Limbodi dam area and authenticate with the help of our institute botanists.

2.3 Extraction of Plants Leaves

The leaves of plant were cleaned and air dried under shade, then powdered with the help of grinder. 5 g of powdered material was added in 50 mL of ethanol solvents and then kept on a magnetic stirrer for 1 hrs. Thereafter, it was extracted by using a soxhlet apparatus sequentially at 80ºC. The extracts were collected and the solvents were evaporated out to dryness. The obtained materials were stored in airtight bottles at 5ºC for further studies.

2.4 Phytochemical Analysis

The extract was preliminary qualitatively analysed for the bioactive phyto-constituents according to the standard protocols [11, 12] as

2.4.1 Alkaloids

Extracts were dissolved in 5 mL 1N HCl, filtered and treated with Mayer's reagent. Yellow coloured precipitate formation indicates the presence of alkaloids.

2.4.2 Carbohydrates

In 5 mL distilled water extracts were dissolved, filtered and treated with Molisch's reagent. The violet ring formation at the junction indicates the presence of carbohydrates.

2.4.3 Proteins and amino acids

To test for amino acids and proteins, extracts were dissolved in 5 mL of distilled water. The extracts solution was treated with Biuret reagent. Purple colour formation indicates the presence of protein; where as in 1 mL of extract solution, 1 mL of 0.25 % w/v Ninhydrin reagent was added and boiled for 5 minutes. Blue colour formation indicates the presence of amino acid.

2.4.4 Glycosides

Extracts were hydrolysed with 2 mL 1N HCl and tested with sodium nitropruside in pyridine and NaOH solution. Pink to blood red colour formation indicates the presence of cardiac glycosides.

2.4.5 Tannins

In 2 mL of extract solution 1 mL of 5% w/v $FeCl_3$ solution was added. Formation of blue-black colour indicates the presence of tannins.

2.4.6 Saponins

In 10 mL distilled water extracts were dissolved and shaken in a graduated cylinder for 10 minutes. Development of 1cm layer of foam in cylinder indicates the presence of saponins.

2.4.7 Flavonoids

Extracts were treated with 2% NaOH solution. Formation of yellow colour, which became colourless when 2 drops of 1N HCl added, indicates the presence of flavonoids.

2.4.8 Steroids

Extracts were treated with chloroform and filtered. The filtrates were tested with 2-3 drops of Conc. H_2SO_4, shaken and allowed to stand. Appearance of red colour at lower layer indicates the presence of steroids.

2.4.9 Triterpenoids

Extracts were dissolved with chloroform and filtered. The filtrates solution was tested with 2-3 drops of Conc. H_2SO_4, shaken and allowed to stand. Yellow colour appearance at lower layer indicates the presence of triterpenes.

2.4.10 Phenolic compounds

Extracts solution was tested with 2-3 drops of 10% w/v $FeCl_3$ solution. Formation of bluish colour indicates the presence of phenolic compounds.

2.5 Antioxidant Activity

The antioxidant activity was studied by using stable free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) with the help of UV-spectrophotometer in vitro assay [13-16]. 0.1 mM DPPH solution was prepared in ethanol solvent. 1.0 mL of DPPH solution was added to 1.0 mL of extract solution in water at different concentrations (5-50 μg/mL) and final volume 3 mL was made by adding distilled water. After 10 minutes, the absorbance of each concentrations of solution was measured at 517 nm. Ascorbic acid was used as standard. Percentage of scavenges DPPH free radical by test compounds were determined as

$$\% \text{ Free Radical Scavenged Activity} = (A_{Control} - A_{Test} / A_{Control}) \times 100$$

IC_{50} values were calculated by using graphical method.

2.6 GC-MS Analysis

GC-MS analysis of ethanol extracted sample were carried out on Shimadzu GC-MS model number QP 2010S.The column Rxi-5Sil MS, 30 meter length, 0.25 mm ID, 0.25 μm thickness was used. The reported compounds were identified by inbuilt libraries NIST-11 and WILEY-8.

2.7 Statistical Analysis

The experimental tests were performed triplicate in three sets and the results expressed in mean ± SD. Values of $p<0.05$ were considered as statistically significant.

3. RESULTS AND DISCUSSION

The results showed that the *Abrus precatorius* Linn leaves ethanol extract is a rich source of natural phytochemical constituents and exhibited significant dose dependent antioxidant potential.

3.1 Phytochemical Analysis

The ethanol extract of *Abrus precatorius* Linn leaves was investigated qualitatively for the active phytochemical analysis by using standard protocols. The qualitative phytochemical analysis of the ethanol extract were showed the presence of alkaloids, carbohydrates, protein and amino acids, glycoside, tannins, flavonoids, triterpenoids and phenolic compounds [17] etc.(Table 1).

3.2 Antioxidant Potential

Table 2 represents experimental data of ethanol extract of leaves and ascorbic acid as a standard. This extract showed DPPH free radical scavenging activity at higher concentration tested.

Table 1. Phytochemical Tests Performed for *Abrus precatorius* Linn leaves extract

Phytochemicals	Result
1. Alkaloid	+
2. Carbohydrate	+
3. Protein and amino acids	+
4. Glycoside	+
5. Tannin	+
6. Saponin	-
7. Flavonoids	+
8. Steroids	-
9. Triterpenoids	+
10. Phenolic compounds	+

(+) for present and (-) for absent

The leaves of *Abrus precatorius* Linn exhibited greater free radical scavenging activity i.e. antioxidant potential [18] by about IC_{50} values of ethanol extract 33.37 μg/mL and ascorbic acid as a standard 15.90 μg/mL at concentrations ranging from 5 μg/mL to 50 μg/mL as shown in Fig. 1.

3.3 GC-MS Analysis

GC-MS analysis of *Abrus precatorius* Linn leaves ethanol extract were carried out and detected presence of 18 bioactive phytocompounds which contribute to antioxidant potential as shown in chromatogram (TIC) Fig. 2) and tabulated in Table 3.

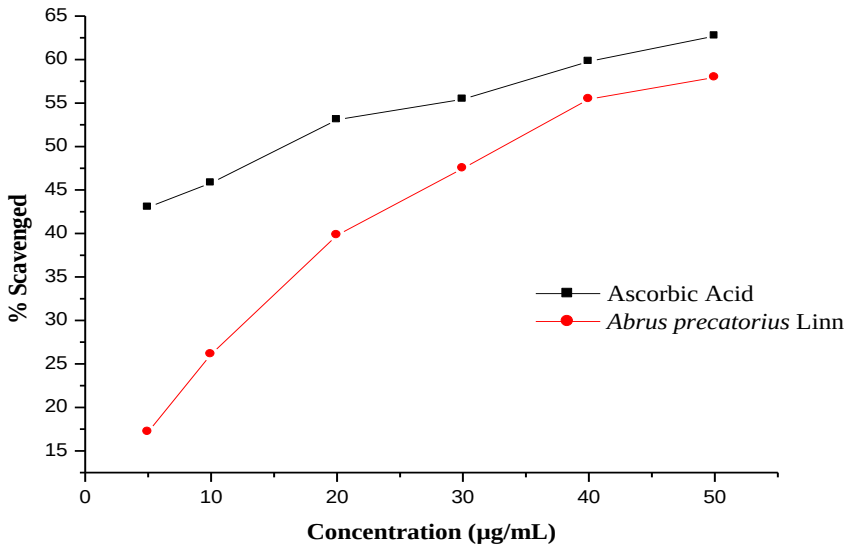

Fig.1 Antioxidant activity of *Abrus precatorius* Linn extract

Table 2. Antioxidant potentials of standard and *Abrus precatorius* Linn leaves extract

Concentration (µg/mL)	% Scavenged standard	% Scavenged of extract
5	43.02±0.04	17.17±0.03
10	45.81±0.02	26.11±0.04
20	53.07±0.03	39.80±0.00
30	55.45±0.00	47.48±0.02
40	59.78±0.02	55.44±0.01
50	62.71±0.01	57.96±0.04
IC_{50} Value (µg/mL)	15.90±0.02	33.37±0.03

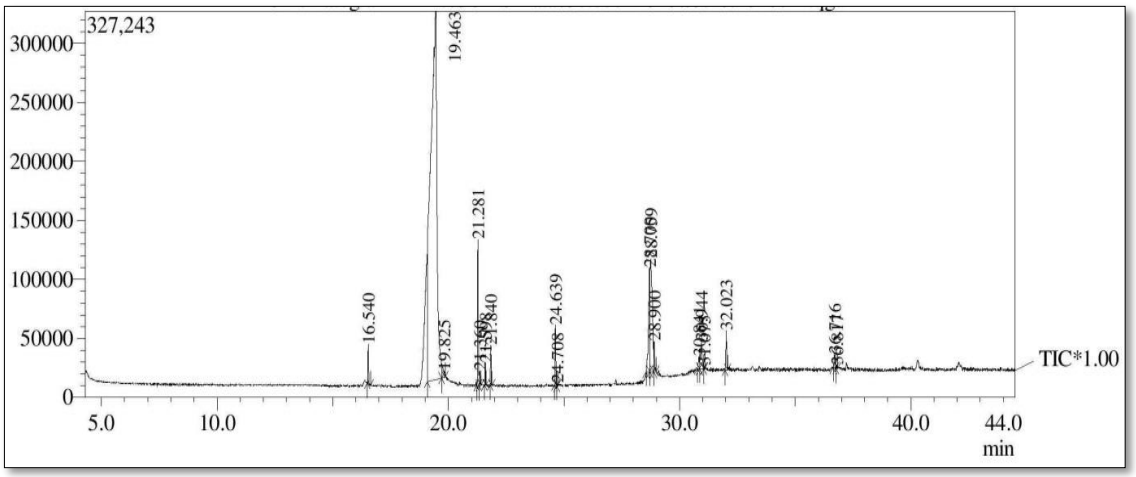

Fig. 2. GC-MS chromatogram (TIC) of *Abrus precatorius* Linn leaves

Table 3. Chemical constituents detected in *Abrus precatorius* Linn leaves

Peak#	R. Time	Detected Compounds	Base m/z	Molecular formula
1	16.540	Phenol, 3,5-bis(1,1-dimethylethyl)-	191.05	$C_{14}H_{22}O$
2	19.463	Mome Inositol	87.00	$C_6H_{12}O_6$
3	19.825	Pentanethioic acid, S-propyl ester	57.00	C_8H_{16}
4	21.281	Neophytadiene	68.05	$C_{20}H_{38}$
5	21.360	2-Methylbutyl Methacrylate	70.00	$C_9H_{16}O_2$
6	21.598	6-Octen-1-ol, 3,7-Dimethyl-, Propanoate	82.05	$C_{13}H_{24}O$
7	21.840	16-Heptadecenal	81.05	$C_{17}H_{32}O$
8	24.639	Tetradecanal	71.00	$C_{14}H_{28}O$
9	24.708	Propane, 2-nitro-	42.95	C_3H_7NO
10	28.700	Bicyclo[4.1.0]Heptane, 4,4-Dimethyl-3-(3-Methyl-3-Butenylidene)-2-Methylene-	77.00	$C_{15}H_{22}$
11	28.759	3-Oxatricyclo [20.8.0.0(7,16)] triaconta-1(22), 7(16), 9,13,23,29-hexaene	189.05	$C_{29}H_{42}O$
12	28.900	2-Tetradecynal, 4-Hydroxy-	71.00	$C_{14}H_{24}O_2$
13	30.841	2-Cyclohexyl-1,3-dioxolane-4,5-dicarboxylic acid, dimethyl ester	145.05	$C_{13}H_{20}O$
14	30.944	Tricyclo[4.3.0.0(7,9)]non-3-ene, 2,2,5,5,8,8-hexamethyl-, (1.alpha.,6.beta.,7.alpha.,9.alpha.)-	189.10	$C_{15}H_{24}$
15	31.075	1-Hexen-3-ol	57.00	$C_6H_{12}O$
16	32.023	Bicyclo(10.3.0)Pentadec-1(12)-en-13-one Tosylhydrazone	69.05	$C_{22}H_{32}O_2N_2S$
17	36.716	Carbonic acid, neopentyl cyclohexylmethyl ester	83.10	$C_{13}H_{24}O_3$
18	36.817	3,4-Pentadien-2-one, 1,1,1-Trifluoro-	69.00	$C_5H_3F_3$

The free radical scavenging activity of *Abrus precatorius* Linn leaves ethanol extract was tested for antioxidant potential by DPPH assay method. The extract exhibited dose dependent free radical scavenging activity. This indicates that the leaves extract has potent antioxidant activity [19]. The specific bioactive compounds which are responsible for this activity were studied through GC-MS and preliminary qualitative phytochemical analysis [20].

The GC-MS analysis were detected presence of 18 bioactive phytocompounds [21]; while preliminary qualitative phytochemical analysis of *Abrus precatorius* Linn leaves ethanol extract were confirmed the presence secondary metabolites like alkaloids, carbohydrates, protein, tannins, flavonoids, steroids, triterpenes and phenolic compounds [22-23]. Seeds and root of *Abrus precatorius* Linn also predominantly contain flavanoid, phenolics, antioxidative isoenzymes and other active biological active compounds were detected in GC-MS analysis [24]. The review of literature shows that there is an ability to scavenge free radical in these active phytochemicals [25].

The largest proximate content of the leaves is carbohydrate and minerals. The leaves are rich in potassium and calcium than other minerals [26]. Flavonoid glycoside was isolated and characterized by using thin layer chromatography in the leaves [27].

Several bioactive constituents like abrine, trigonelline, abruslactone A, hemiphloin, abrusoside A, abrusoside B, abrusoside C, abrusoside D, arabinose, galactose, xylose, choline, hypaphorine, precatorine , glycyrrhizin, montanyl alcohol, inositol, D-monomethyl ether, pinitol, 3,4-Dihydroxy Benzoic Acid [28] are identified in the leaves of *Abrus precatorius* Linn [29]. The compounds identified in the leaves of *Abrus precatorius* Linn find themselves in the traditional preparation.

Leaves were reported to have a broad range of therapeutic effects like antibacterial, antifungal [30], antitumor, analgesic, anti-inflammatory, antispasmodic, anti-diabetic, antiserotonergic, anti-migraine, including treatment of inflammation, ulcers, wounds, throat scratches and sores [31-32].

The naturally occurring plant derived antioxidants especially polyphenols and flavonoids in health products were clearly indicate as a promising avenue for the prevention of oxidative stress related disorders [33-35]. Similarly in ethanol extract of leaves showed activity at higher concentration tested. The dose dependent maximum scavenging of free radicals reported by ethanol extract in vitro study. Therefore, *Abrus precatorius* Linn herbal local medicinal plant leaves have been used as potent antioxidant to treat and prevent pathological conditions [36].

4. CONCLUSION

In the present study Preliminary phytochemical screening reported various bioactive compounds. 18 chemical compounds identified in the ethanol extract of *Abrus precatorius* Linn leave by GC-MS analysis. The leaves of *Abrus precatorius* Linn exhibited greater free radical scavenging activity i.e. antioxidant potential. The presence of various bioactive phytocompounds justifies the therapeutic use of the *Abrus precatorius* Linn plant leaves for various ailments by traditional practitioners. By isolating and identifying these phytochemical compounds, new drugs can be formulated to treat different illnesses. It could be concluded that leaves of *Abrus precatorius* Linn plant is of phytopharmaceutical significance and recommended to undertake further studies to find out bioactivity of components with potential therapeutic benefits.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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