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Original Article

ESTIMATION OF FLAVONOID CONTENT, POLYPHENOLIC CONTENT AND ANTIOXIDANT POTENTIAL OF DIFFERENT PARTS OF *ABRUS PRECATORIUS* (L.)

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ABSTRACT

Objective: *Abrus precatorius* (L.) is a tropical plant and is used in traditional medicine for treatment of a wide range of ailments. Lately, plants with medicinal properties have gained importance for their potential therapeutic use in diseases caused due free radicals. Hence, the present investigation was carried out to estimate the total phenolic and flavonoid content and free radical scavenging activity of fresh and dry parts of *Abrus precatorius*.

Methods: Plant material was collected from Karnala forest of Maharashtra. Extracts of leaves, stem, root and seed (fresh and dry) were prepared using four different solvents i.e. Distilled water, Ethanol, Methanol and Acetone. Each extract was tested for total phenolic content, flavonoid content, and antioxidant activity (by FRAP, $DPPH^{\cdot}$ and $ABTS^{\cdot+}$assays), while phenolic compounds like Gallic acid, Catechol, Vanillin, Caffeic acid, p-Coumaric acid and Ferulic acid were detected and quantified using RP-HPLC.

Results: Seeds showed highest phenolic content (8.99±0.27 mg GAE/g) and $DPPH^{\cdot}$ radical scavenging activity (88.34±0.08 %) in methanolic extracts. The leaves had the highest flavonoid content (145.68±0.99 mg RE/g). 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.

Conclusion: This study substantiates the high antioxidant activity of different plant parts of *A. precatorius*. Therefore, it can be used as a source of natural antioxidants and used in drug formulations for treatment of diseases resulting from oxidative stress.

Keywords: *Abrusprecatorius*, TPC, TFC, Antioxidant activity, RP-HPLC.

INTRODUCTION

Abrus precatorius (L.) (Family Fabaceae) is a perennial climber, native to India and found throughout the tropical regions of the world. It is used in traditional medicine for the treatment of the wide range of ailments. It is used as anti-helmentic, anti-diarrhoel, anti-emetic, prevent rabies and to treat tetanus [1]. More recently, *A. precatorius* is found to have anti-protozoan activity [2], anti-trypanosomal activity [3] and has potential in treatment of tuberculosis [4].

The leaves are used as sweeteners and also have therapeutic effects in treating fever, cough and cold while the seeds have been used in treatment of worm infection, vitiligo patches, baldness [5], aphrodisiac, anti-diabetic, anti-cancer, anti-oxidative, anti-inflammatory, anti-microbial, anti-fertility in males, abortifacient in females [1,6,7]. The seed kernels are found to contain flavonoids, abrectorin and glycoside semethoxycentaureidin 7-O-rutinoside [8], 8-C glucosylscutellarine 6, 7-dimethyl ether, 2-O-apioside flavones C-glycoside [9], alkaloids, methyl ester of N-N dimethyltryptophan methocation and precatorine [10], indole derivatives, anthocyanins, sterols, terpenes. The roots contain precol, abrol, glycyrrhizin triterpenoids and alkaloids, which are also found in the leaves and stem [5]. Leaves and ethanolic extracts of seeds of *A. precatorius* are also reported having potent anti-oxidative activity [10-12].

Antioxidants have the capacity to slow down or prevent the harmful effects of oxidative stress imposed by free radicals and other ROS in the body. Reactive Oxygen Species (ROS) including free radicals are produced during the normal metabolism of the body and when not sufficiently scavenged by natural defense mechanisms of the body, they cause various diseases [13]. Thus, antioxidants have gained a lot of importance due to their potential therapeutic use in diseases caused by free radicals. Plants with medicinal properties are being investigated for this purpose, due to their potent antioxidant activities, low side effects and economic feasibility [14]. Compounds like flavonoids and phenolic compounds found in plants, exert multiple biological effects including antioxidant and free radical scavenging abilities [15]. Hence this study aims to carry out a thorough investigation of the phenolic and flavonoid content and antioxidant capacity of *Abrus precatorius* and determines the best plant part (in fresh or dry state) and solvent for its extraction.

MATERIALS AND METHODS

Plant material

Leaves, stem, root and seeds of *A. precatorius* (L.) were collected from Panvel region of Raigad District, Maharashtra, India. The plant was authenticated by Dr. Milind Sardesai, Botany Department, (Dr. B. R. Ambedkar Marathwara University), Maharashtra, India. The plant parts were separated and half of each explants were air dried for around two weeks at room temperature (for dry plant extracts), while the rest was used for fresh extracts.

Chemicals

Methanol, Acetone, Folin-Ciocalteu reagent, Sodium Acetate, Sodium Carbonate were purchased from SRL Pvt. Ltd.(Mumbai, India); Trolox, Rutin, TPTZ, DPPH, ABTS and Potassium Persulfate were obtained from Sigma-Aldrich Chemical Co. (St. Lois, MO, USA); Aluminum chloride, Gallic Acid, Acetic Acid were acquired from Molychem (Mumbai, India); Ferric Chloride was from Hi Media Laboratories (Mumbai, India);Ethanol, HPLC grade water, Methanol and Acetic acid were procured from Merck (Darmstadt, Germany);

Extract preparation

The fresh and dried plant parts were crushed into fine powder using liquid nitrogen. Ten grams of the sample were suspended in 100 ml of four different extracting solvent systems like Distilled water, Ethanol, Methanol and Acetone respectively for overnight extraction. Extracts were filtered using Whatman No.1 paper and the filtrates were concentrated to 10 ml by using the rotary evaporator at 40 ^{o}C. Extracts were re suspended in each respective solvent to make the stock solution of 100 mg/ml for analysis and stored at-20 ^{o}C refrigerator until further use [16].

Phytochemical assays

All methods described below are spectrophotometric methods and absorbance at specific wavelengths was taken using a UV spectrophotometer (Shimadzu–Model UV-1700 Pharma spec, Kyoto, Japan). All readings were taken in triplicates and the observations were recorded.

Total phenolic content (TPC)

The TPC was determined by a spectrophotometric method using Folin-Ciocalteu (FC) reagent [17]. The Phenolic content of each sample were determined from the standard curve of Gallic acid using the calibration equation y = 3.1368x–0.0023 (R^2 = 0.9903) where 'x' is the GAE in mg and 'y' is the absorbance at 760 nm and the content were expressed as milli gram Gallic acid Equivalent per Gram of Sample (mg GAE/g).

Total flavonoid content (TFC)

The total flavonoid content was estimated by a spectrophotometric method described by Jain *et al.* [17], which detects the amount of colored complex formed between the flavonoids and aluminum ions. The flavonoid content was estimated from standard curve of Rutin using the calibration equation of y = 6.3771x–0.0084 (R^2= 0.9964) where 'x' is the mg Rutin Equivalent and 'y' is the absorbance at 368 nm, and the content were expressed as milli gram Rutin Equivalent per Gram of sample (mg RE/g).

DPPH Radical scavenging activity assay

The radical scavenging potential of various extracts was determined by $DPPH^{\cdot}$ assay as described by Jain *et al.* [17]. The radical scavenging ability of antioxidants compounds presents in the extract, decreases the absorbance and forms stable DPPH (2, 2-diphenyl-1-picrylhidrazyl) radical rendering it colorless. The absorbance of the $DPPH^{\cdot}$ reagent was recorded as the control and the radical scavenging activity of the samples were calculated using following formula:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

$ABTS^{+}$ radical scavenging assay

The free radical scavenging activity was determined by ABTS radical cation de-colorization assay described by Jain *et al.* [17]. Absorbance of the stable $ABTS^{\cdot+}$reagent was recorded as the control and the $ABTS^{\cdot+}$radical scavenging activity of the sample was calculated using following formula:

$$ABTS^{\cdot+}\text{radical Scavenging activity (\%)} = \frac{(\text{Absorbance of control} - \text{Absorbance of sample}) \times 100}{\text{Absorbance of control}}$$

Ferric reducing antioxidant power (FRAP) assay

It quantitates the amount of antioxidants in the sample, based on its ability to reduce $Fe^{3+}$to $Fe^{2+}$and the assay was carried out as described by Chanda and Dave [13]. The antioxidant content based on ferric ion reducing ability, was calculated using a standard curve of Trolox and the calibration equation y = 1.32x–0.0044 (R^2 = 0.9717) where 'x' is the mg Trolox Equivalent and 'y' is the absorbance at 595 nm and the content were expressed as milli gram Trolox Equivalent Antioxidant Capacity per Gram of sample (mg TEAC/g).

Detection and quantification of phenolic compounds using RP-HPLC

Analysis of individual phenolic compounds present in the different parts of plant extracts was performed on a Waters HPLC (Model 2487) using a hypersil C18 reversed phase column 15 cm with 5µ particle size. A constant rate of 1 ml/min of mobile phase (20% Methanol, 1% Acetic acid and 79% water) was maintained through the column. The elution gradient was linear over 60 min, using an UV detector set at wavelength 280 nm. The phenolic compounds from each sample were identified by comparing their relative retention time with the standard chromatogram of a mixture of standard phenolic compounds, obtained from Sigma (USA). The concentration of an individual compound was calculated on the basis of peak area measurements and then converted to ppm or µg/g. All the solvents and chemicals used were HPLC spectral grade.

Data analysis

The mean±standard deviation of triplicate readings of each sample for each assay was determined using Microsoft excel. Significant differences between samples were analyzed by Duncan's Multiple Range Test (DMRT) at P<0.05 and graphs were generated with Bonferroni Post-Hoc test. Correlation was determined using Pearson correlation coefficient (R) and Coefficient of Determination (R^2) between different assays i.e. total phenolic, flavonoid content and the antioxidant assays were carried out using SPSS software version 19.0.

RESULTS

In the present investigation, the effect of various solvents on the extraction efficiency of phenolics, flavonoids and antioxidants was studied, as the yield of antioxidant compounds is depends on the solvent used to separate the soluble fraction from the permeable solid [18].

Total phenolic content

Phenolics or polyphenols are secondary plant metabolites that are ubiquitously present in plants and their products. TPC of different extract in various solvents was determined by FC assay using Gallic acid as a standard phenolic compound. The TP content varied in the different extracts and ranged from (0.81±0.04) to (8.99±0.27) mg GAE/g among fresh and dried plant parts (table 1 and fig. 1). Fresh and dried states of plant parts did not show significant difference except in the case of seeds. Fresh seeds showed the highest (8.99±0.27 mg GAE/g) phenolic content when prepared in methanol whereas dried seeds showed (2.80±0.04 mg GAE/g). Stem explants showed least TP content. The TP content was found in following order Seed>Leaf>Root>Stem and was confirmed using statistical methods.

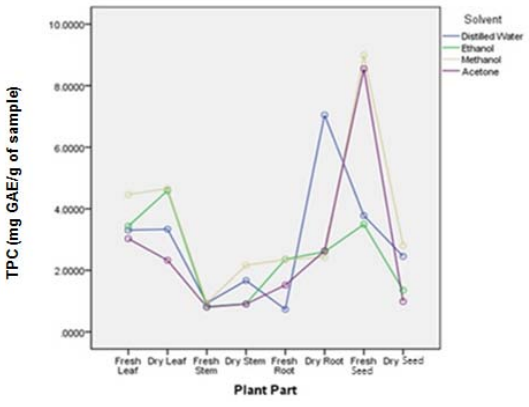

Fig. 1: Total Phenolic content in fresh and dry parts of *Abrus precatorius* in different solvents

Total flavonoid content

It has been recognized that flavonoids show antioxidant activity and has considerable effects on human health. Flavonoids act as ROS either by scavenging or by chelating process [19]. The flavonoid contents of the extracts were between 2.14±0.05 and 145.68±0.99 mg RE/g (table 1 and fig. 2). Among different plant parts, fresh and

dried leaves showed the highest content (145.68±0.99 and 71.15±0.09 mg RE/g) in acetone and ethanol respectively. Maximum flavonoid content was observed in leaves as these are involved in pigment formation whereas other plant part, flavonoid content ranged from 2.14±0.05 to 22.73±0.22 mg RE/g. It was observed that fresh and dried seeds, root and stem did not show significant variation in total flavonoid content whereas fresh leaves have the significantly high amount of flavonoids than dried leaves. Acetone and methanol solvents are proved to be best in extracting maximum amount of flavonoid (table 1).

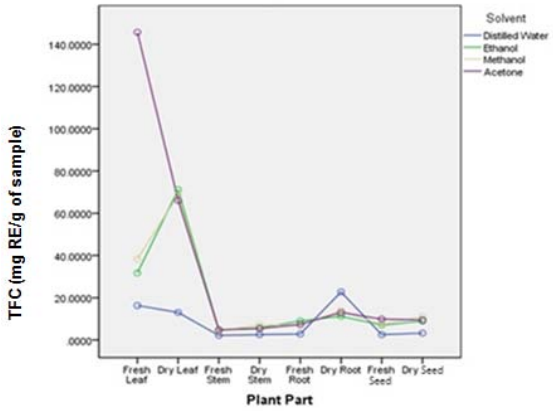

Fig. 2: Total flavonoid content in fresh and dry parts of *Abrusprecatorius* in different solvents

DPPH˙ (2, 2-diphenyl-1-picrylhidrazyl) assay

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 [20]. The methanolic extract of fresh seed showed high scavenging activity (88.34±0.08 %) compared to dry seed (56.92±0.04 %) (table 1 and fig. 3). Fresh and dried condition of plant parts did not show significant difference in antioxidant activity. However, amongst solvents methanol observed best in terms of extracting maximum amount of antioxidative compounds (table 1).

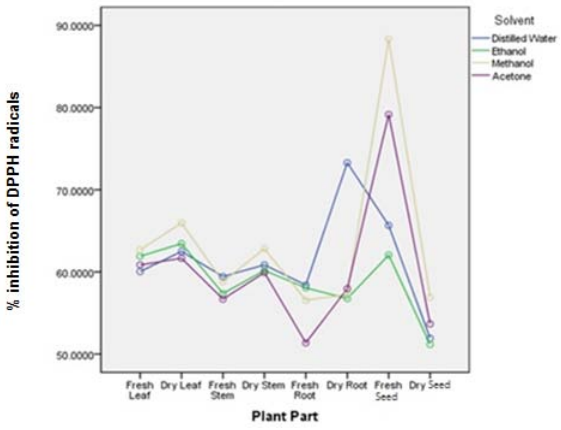

Fig. 3: DPPH˙ radical scavenging activity in fresh and dry parts of *Abrus precatorius* in different solvents

Ferric reducing antioxidant power (FRAP) assay

FRAP assay showed a wide range of variation among the plant parts and solvents used (table 1 and fig. 4). Fresh seeds (110.10±5.70) and dry roots (59.39±0.43) mg TEAC/g showed the highest FRAP activity, while dry stem (1.77±0.27 mg TEAC/g) and dry seeds (1.84±0.02 mg TEAC/g) recorded the lowest FRAP value.

Fresh seeds showed 110.10±5.70 mg TEAC/g when extracts prepared in methanol. Highest FRAP activity was found in seeds followed by root>leaf>stem. The FRAP values for methanolic extracts were found to be higher than other three extract (table 1 and fig. 4).

ABTS˙⁺ (2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) radical scavenging assay

Extracts prepared in acetone and methanol was fast and effective scavengers of the ABTS⁺ radicals (table 1 and fig. 5). Fresh seed showed the highest % inhibition of the radical. There was no significant difference in % inhibition of ABTS˙⁺radical in fresh and dry tissue extracts, but there was difference amongst various plant parts. Maximum activity was observed in acetone extract in fresh as well as dry tissue followed by methanol (table 1 and fig. 5).

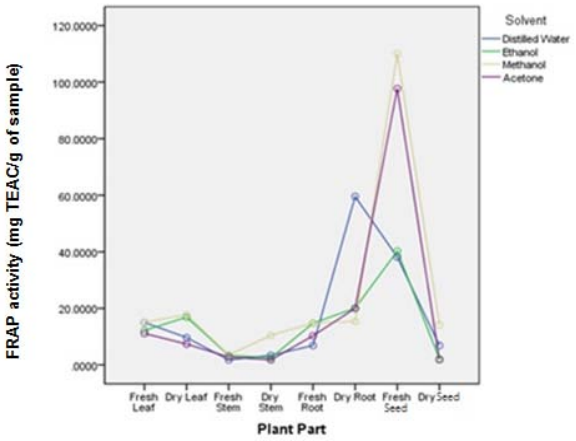

Fig. 4: FRAP activity in fresh and dry parts of *Abrus precatorius* in different solvents

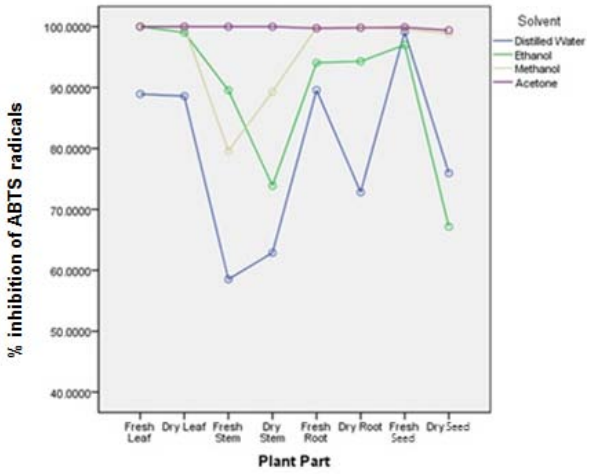

Fig. 5: ABTS˙⁺radical scavenging activity in fresh and dry parts of *Abrus precatorius* in different solvents

Correlation analysis between TPC, TFC and Antioxidant activity (DPPH˙, FRAP and ABTS˙⁺assays)

Studies have reported the strong correlation between phenolic content and antioxidant activity in medicinal plants [21-23] while other reports do not [16, 24]. To establish reproducibility, suitability and relationship amongst TPC, TFC and the total antioxidant potential, linear regression and correlation analysis was performed using the Pearson correlation coefficient (R) and Correlation of

Determination (R^2) for fresh and dry plant parts (table 2). All the R values were found significant at P<0.01.

The antioxidant activity of plant extracts showed the correlation with the total phenolic content. The TPC in fresh plant parts showed high correlation with DPPH$^{\cdot}$ (R = 0.924**), FRAP (0.939**) and moderate with ABTS$^{\cdot+}$(R = 0.434) whereas TFC did not show significant correlation with antioxidant potential measured by different assays. Amongst DPPH$^{\cdot}$, FRAP and ABTS assay DPPH$^{\cdot}$ showed correlation with FRAP (R = 0.943**) but none of them showed affinity to ABTS. Similarly, antioxidant potential of dried plant parts showed significant positive correlation amongst them. TPC showed correlation with TFC (R= 0.506*), FRAP (R= 0.884**) and DPPH$^{\cdot}$(R=0.744**). DPPH and FRAP assay are correlated (R = 0.684**) while they did not show any positive correlation with ABTS assay in dried condition of various plant parts.

Table 1: Phenolic content, Flavonoid Content and Antioxidant potential (DPPH, FRAP and ABTS Assay) of different parts of *Abrus precatorius* (L.) in various solvents

Plant Part	Solvent	TPC Assay (mg GAE/g)		TFC Assay(mg RE/g)		DPPH Assay (% Inhibition)		FRAP Assay (mg TEAC/g)		ABTS Assay (% Inhibition)	
		Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry	Fresh	Dry
Leaves	D/W	3.30±0.04^{ef}	3.33±0.81^{ef}	16.31±0.5^{h}	13.02±0.09^{i}	60.04±3.6^{hij}	62.48±0.97^{efg}	15±0.76^{fgh}	9.67±0.87^{ijk}	88.93±0.49^{c}	88.6±0.82^{c}
	Ethanol	3.43±0.21^{ef}	4.59±0.11^{d}	31.73±0.31^{f}	71.16±0.09^{b}	61.91±0.16^{efg}	63.44±1.17^{e}	12.15±0.15^{hi}	16.87±1.09^{fg}	99.4±0^{a}	99.04±0.58^{a}
	Methanol	4.46±0.13^{d}	4.65±0.16^{d}	38.32±0.54^{e}	68.58±4.24^{c}	62.67±0.65^{ef}	65.96±0.37^{d}	15.1±0.2^{fgh}	17.75±0.39^{ef}	99.89±0^{a}	99.86±0^{a}
	Acetone	3.02±0.3^{f}	2.32±0.05^{gh}	145.68±0.99^{a}	66.08±2.17^{d}	60.85±0.66^{ghi}	61.65±0.36^{fgh}	11.09±0^{i}	7.37±0.59^{jk}	99.72±0^{a}	99.4±0^{a}
Stem	D/W	0.93±0.12^{jk}	1.66±0.03^{i}	2.14±0.05^{q}	2.52±0.03^{q}	59.43±1.08^{ijk}	60.85±0.12^{ghi}	1.74±1.0^{l}	3.38±0.15^{l}	58.52±0.84^{i}	62.88±2.9^{h}
	Ethanol	0.81±0.04^{k}	0.91±0.06^{jk}	4.77±0.28^{no}	5.7±0^{mn}	57.37±1.23^{mno}	60.17±0.81^{hij}	3.38±0.4^{l}	2.47±0.15^{l}	89.56±0.54^{c}	73.89±4.6^{ef}
	Methanol	0.94±0.01^{jk}	2.16±0.16^{h}	4.34±0.15^{nop}	7.08±0.01^{lm}	58.81±1.21^{jklm}	62.82±0.77^{ef}	3.54±0.08^{l}	10.43±0.48^{ij}	79.59±5.97^{d}	89.31±0.73^{c}
	Acetone	0.80±0.04^{k}	0.9±0.01^{jk}	4.76±0.36^{no}	5.39±0^{n}	56.69±0.13^{no}	59.91±1.2^{ijk}	2.78±1.0^{l}	1.77±0.27^{l}	99.23±0^{a}	99.02±0^{a}
Root	D/W	0.73±0^{k}	7.04±0.08^{c}	2.84±0.04^{pq}	22.73±0.22^{g}	58.39±0.12^{klm}	73.29±0.9^{c}	6.86±0.23^{k}	59.55±0.43^{c}	89.57±0.2^{c}	72.81±0.47^{f}
	Ethanol	2.35±0.05^{gh}	2.6±0.03^{gh}	9.02±0.23^{k}	11.24±0.51^{j}	58.06±0.2^{lmno}	56.75±0.99^{no}	14.75±0.41^{fgh}	20.08±1.42^{e}	94.08±4.5^{b}	94.3±1.02^{b}
	Methanol	2.34±0.1^{gh}	2.42±0.03^{gh}	7.1±0.54^{lm}	13.9±0.12^{i}	56.56±0.04^{o}	57.24±0.1^{mno}	14.82±0.74^{fgh}	15.4±0.59^{fg}	99.81±0.07^{a}	99.86±0.13^{a}
	Acetone	1.51±0.06^{i}	2.63±0.12^{g}	7.34±0.06^{l}	12.99±0.23^{i}	51.37±0.08^{q}	57.95±0.45^{lmno}	10.3±1.29^{ij}	20±1.85^{e}	99.72±0.13^{a}	99.81±0.15^{a}
Seeds	D/W	3.77±0.4^{e}	2.45±0^{gh}	2.49±0.01^{q}	3.24±0.05^{opq}	65.66±0.2^{d}	51.92±0.04^{q}	38.13±1.31^{d}	6.87±0.28^{k}	99.13±0.15^{a}	75.95±1.2^{e}
	Ethanol	3.49±0.02^{e}	1.35±0.02^{ij}	6.98±0.06^{lm}	8.91±0.31^{k}	62.05±0.24^{efg}	51.17±0.09^{q}	40.23±3.0^{d}	2.12±0.24^{l}	96.99±1.4^{ab}	67.16±2.0^{g}
	Methanol	8.99±0.27^{a}	2.8±0.04^{gh}	7.37±0.08^{l}	11.14±0.18^{j}	88.34±0.08^{a}	56.92±0.04^{no}	110.1±5.7^{a}	14.22±0.31^{gh}	99.63±0.15^{a}	98.86±1.73^{a}
	Acetone	8.54±0.67^{b}	0.98±0^{jk}	9.86±0.15^{jk}	9.47±0.38^{k}	79.15±0.31^{b}	53.67±0.04^{p}	97.75±6.10^{b}	1.84±0.2^{l}	99.9±0.07^{a}	99.4±0.41^{a}

*Means of homogeneous subsets are marked with same letters for each assay determined by DMRT at P<0.05, Total Phenol Content (TPC), expressed in milligrams of Gallic Acid Equivalent per gram of sample, Total Flavonoid Content (TFC), expressed in milligrams of Rutin Equivalent per gram of sample, DPPH radical scavenging activity, expressed in terms of % inhibition of DPPH$^{\cdot}$ radicals, Ferric Reducing Antioxidant power (FRAP), expressed in milligrams of Trolox Equivalent Antioxidant Capacity per gram of sample, ABTS$^{\cdot+}$radical scavenging activity, expressed in terms of % inhibition of ABTS^{+} radicals.

Table 2: Correlations (R and R^2) between total phenolic content (TPC), Total Flavonoid Content (TFC) and antioxidant capacity (determined by ABTS, DPPH, FRAP assays) of *A. precatorius* fresh and dry plant parts

Fresh plant parts				
R(R^2)	**TPC**	**TFC**	**DPPH**	**FRAP**
TFC	0.069 (0.005)			
DPPH	0.939** (0.882)	-0.122 (0.015)		
FRAP	0.924** (0.854)	-0.28 (0.001)	0.943** (0.889)	
ABTS	0.434 (0.189)	0.242 (0.058)	0.353 (0.124)	0.221 (0.049)
Dry plant parts				
R(R^2)	TPC	TFC	DPPH	FRAP
TFC	0.506* (0.256)			
DPPH	0.884** (0.782)	0.203 (0.041)		
FRAP	0.744** (0.554)	0.449 (0.201)	0.684** (0.467)	
ABTS	0.040 (0.002)	0.426 (0.181)	-0.025 (0.001)	0.006 (0.00)

R = Correlation coefficient; R^2 = Coefficient of Determination. The values in parenthesis represent the R^2 value, ** Significance at P<0.01, * Significance at P<0.05

RP-HPLC analysis of phenolic compounds

Phenolic compounds that possess antioxidant activity are known to be mainly phenolic acids and flavonoids [23]. Individual phenolic acids like Gallic acid, Cathechol, Vanillin, Caffeic acid, p-Coumaric acid and Ferulic acid in *A. precatorius* were analyzed and quantified using RP-HPLC (fig. 6) for all the plant parts (fresh and dried) prepared in different solvents (table 3). It was observed that most of the plant parts showed the presence of Gallic acid and Catechol, while Vanillin, Caffeic acid and p-Coumaric acid were found in leaves and stem in very less quantity. The methanol extract showed high amount of Catechol around 6.3 µg/g to 1309.0 µg/g of dry weight to that of other solvent system.

Thus methanol, found to be the best solvent system for the extraction and quantification of poly phenolic compounds in *A. precatorius*.

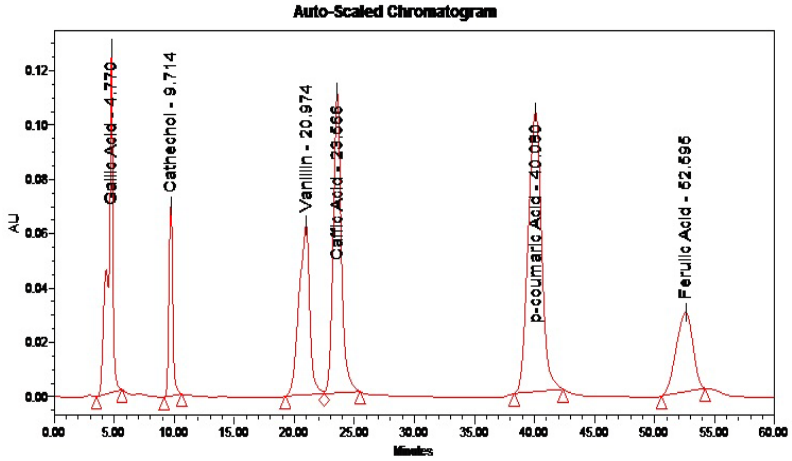

Fig. 6: HPLC Fingerprinting: HPLC chromatogram of standards at 280 nm

Table 3: RP-HPLC quantification of different phenolic compounds from different parts of *A. precatorius* using various solvents

Plant part	Solvent	Gallic acid (µg/g)	Catechol (µg/g)	Vanillin (µg/g)	Caffeic acid (µg/g)	p-Coumaric acid (µg/g)	Ferulic acid (µg/g)
Fresh Leaf	D/W	-	-	-	8.2	-	3.5
	Ethanol	269	-	-	-	-	-
	Methanol	35.7	6.3	1.7	-	1.3	
Dry leaf	D/W	13.7	-	1.3	-	-	-
	Ethanol	-	1.04	-	-	-	-
	Methanol	-	44.3	-	-	-	342.2
Fresh Stem	D/W	-	-	-	-	-	-
	Ethanol	-	-	-	-	-	48.8
	Methanol	-	-	-	-	-	-
Dry Stem	D/W	33.2	957.7	-	-	-	27.5
	Ethanol	-	-	-	-	-	-
	Methanol	-	-	7.8	-	-	-
	D/W	-	133.8	-	1.4	-	-
Fresh Root							
	Ethanol	46.6	296.4	-	-	-	-
	Methanol	-	954.1	-	-	-	-
Dry Root	D/W	-	-	-	-	-	-
	Ethanol	131.2	441.4	1.2	-	6.9	-
	Methanol	85.5	1309	-	-	13.1	-
Fresh Seed	D/W	-	126	-	-	-	-
	Ethanol	248.5	674.5	-	-	-	-
	Methanol	-	-	-	-	-	-
Dry Seed	D/W	-	533.7	-	-	-	-
	Ethanol	11.7	-	-	-	-	-
	Methanol	142.3	972.1	-	-	-	-

Values of phenolic compounds are expressed in micrograms per gram of sample (µg/g)

DISCUSSION

Medicinal plants have long been a vital source of antioxidants. *A. precatorius* is known for its therapeutic properties. The seeds and roots are used in the traditional system of medicine 'Ayurveda' [11]. In this study, we demonstrated the antioxidant potential of various fresh and dried plant parts using different solvents for various assays. As the the antioxidant activity of the phenolic compounds depends on the type of the extracting solvent [25].

Significantly high amount of phenolic compounds was found in methanolic extract of fresh seeds but it was very less in dry seeds (table 1 and fig. 1). The dry seeds of *A. precatorius* are hard coated and become impermeable to different solvents leading to poor extraction of phenolic compounds, while there was not much variation in total phenolic content in other dry and fresh plant parts.

High amount of phenolics detected in leaves when extracts were prepared in methanol (table 1) while Gul, *et al.*, [1] found maximum

amount of phenolic compounds in aqueous leaf extracts. Methanol has the ability to inhibit the action of polyphenols oxidases that causes oxidation of phenolic compounds, therefore maximizes availability of active phenolic compounds [26]. Similar results also reported in *Moringa oleifera* and in *Cassia fistula* [27, 28]. Ethanol extracts of leaves showed high TFC content whereas previous studies by Gul, *et al.*, [1] reported very less. In present study DPPH$^{\cdot}$ radical inhibition was observed with seed ethanolic and methanolic extract as also observed by Arora, *et al.*, [11]. This variation in amount of polyphenolic and flavonoid content is due to the fact that these are affected by various environmental factors in plants [16].

Phenolic compounds are potential antioxidants and free radical scavengers, hence, there is a close correlation between the total phenolic content and antioxidant activity which has been widely studied in different medicinal plants [23, 29, 30] and demonstrated a linear correlation between the content of phenolic compounds and their antioxidant capacity while others [31, 32] show poor linear correlation. The results obtained in our study showed the positive correlation in both the states of tissue (table 2). There was no correlation found between TFC and different antioxidant activity as also reported by previous studies [27, 33, 34].

The antioxidant activity measured by DPPH and FRAPS assays showed similar results and was highly correlated with TPC as also observed by previous studies [35, 36]. It was apparent that extracts displaying good antioxidant behavior in both the DPPH and FRAP assays could be identified by the high phenolic content. Huang, *et al.*, [37] proposed that the DPPH and FRAP assays share a similar mechanism, that is transfer of electrons from the antioxidant to reduce an oxidant.

The results from the present and previous studies, suggest a high degree of redundancy in use of both assays for screening plant extracts. This redundancy has also been proved with the positive correlation between these assay and total phenol content. Therefore, only one assay can be performed to screen for antioxidant activity, so as to reduce the use of potentially valuable plant extracts.

Based on the standardized conditions (fig. 6), six phenolic compounds were identified in varying concentrations, which represent diverse structural types that might contribute to the antioxidant behavior of the extracts. Under our experimental conditions, catechol was the major free phenolic compound detected in roots and seeds (fresh and dry). On the other hand, Gallic acid was also identified. In addition, Vanillin, Caffeic acid, p-Coumaric acid and Ferulic acid were found as minor compounds in most of the plant parts (table 3).

Although whole plant is used as potential source of antioxidants and to cure various ailments, seed extracts being used against antibacterial, anticancer, anti-diabetic, anti-arthritic and antioxidant activity [1, 5, 12], while root, leaf and stem are also used in traditional medicine [38]. Amongst solvent systems, methanol was found to be good solvent system to extract high amount of polyphenolic compounds.

CONCLUSION

Though *Abrus precatorius* is known for its various medicinal activities, but use of different plant parts as the potential source of antioxidant compounds was not very clear. In present investigation, it has been shown that, the methanol extracts of seed, root, leaves and stem are found to be good source of antioxidant activity.

TPC and total antioxidant capacity was found to be maximum in methanolic seed extracts followed by root>leaves>stem. A group of phenolic compounds were detected and quantified by RP-HPLC. Thus, *Abrus precatorius* can be used as an accessible source of natural antioxidant and for treatment of diseases resulting from oxidative stress.

ABBREVIATION

DPPH-2,2-diphenyl-1-picrylhidrazyl, FRAP-Ferric Reducing Antioxidant Power, ABTS-2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), RP-HPLC-Reverse Phase–High Performance Liquid Chromatography, GAE-Gallic Acid Equivalent, RE-Rutin Equivalent, TPC-Total Phenolic Content, TFC-Total Flavonoid Content, TEAC-Trolox Equivalent Antioxidant Capacity

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CONFLICT OF INTERESTS

The authors have no conflict of interest

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