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Acalypha brachystachya: A Valuable Source for Natural Antioxidants

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In present investigation, the crude methanolic extracts of seeds, shoot and leaves of *Acalypha brachystachya* hornem. were fractionated separately with *n*-hexane, chloroform, ethyl acetate and *n*-butanol sequentially to obtain various fractions. These fractions were screened for their phyto-constituents and generally found to contain important bioactive substances such as flavonoids, alkaloids, phenols, terpenoids, tannins, saponins and sugars. Then the antioxidant potential of these fractions was assayed by the four methods, namely, DPPH assay, FRAP assay, total phenolic assay and phosphomolybdenum complex assay. Ethyl acetate soluble fraction of seeds exhibited good antioxidant activity, chloroform soluble fractions of seeds and leaves possessed medium activity while all the remaining fractions showed poor activity.

Key Words: *Acalypha brachystachya* hornem., DPPH, FRAP assay, Total phenolics, Phosphomolybdenum complex, Phytochemical screening.

INTRODUCTION

Antioxidants are the agents that neutralize harmful compounds called free radicals which damage living cells, spoil food and degrade materials such as rubber, gasoline and lubricating oils. Antioxidant can take the form of drugs *e.g.*, enzymes in the body, vitamins supplements or industrial additives[1]. The principle function of antioxidants is in delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and they may reduce oxidative damage to the human body[2]. Free radicals may be defined as any chemical species that are capable of existing with one or more unpaired outer shell electrons. They are extremely reactive and generally highly unstable[3]. Reactive oxygen species, such as superoxide radical, hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$) and singlet oxygen ($^1O_2$), are of the greatest biological significance[3,4]. They are extremely reactive and potentially damaging transient chemical species. In addition to exogenous sources of free radicals, such as ionizing radiation, tobacco smoke, pesticides, pollutants and some medications, they are produced continuously in all cells, as metabolic byproducts by a number of intracellular systems: small cytoplasmic molecules, cytoplasmic proteins, membrane enzymes, peroxisomes, mitochondrial electron transport systems and microsomic electron transport systems[3].

All cellular components, proteins, polyunsaturated fatty acids, nucleic acids and carbohydrates, are prominent biological targets of reactive oxygen species, giving rise to metabolic and cellular disturbances[3]. Fortunately, within biological systems, there are enzymatic systems and chemical scavengers: dietary antioxidants (α-tocopherol, β-carotene, ascorbic acid, glutathione, uric acid), some hormones (estrogen, angiotensin) and endogenous enzymes (superoxide dismutase, glutathione peroxidase, catalase), all of which are able to remove oxygen free radicals formed in cells and thus protect against oxidative damage[3,5]. Tissue damage resulting from the imbalance between reactive oxygen species generating and scavenging systems (oxidative stress) has been implicated in the pathology of a number of disorders, such as atherosclerosis, ischemia-reperfusion injury, cancer, malaria, diabetes, inflammatory joint disease, asthma, cardiovascular diseases, cataracts, immune system decline and could play a role in neurodegenerative diseases and ageing processes[3,4,6-8].

The family Euphorbiaceae comprises about 750 species, a large portion of which are weeds while others are ornamental plants. They are found in the tropics of Africa, America and Asia. Some of the species are well known in folklore medicine and a few have actually appeared in homeopathic pharmacopoeia[1]. Euphorbiaceae is one of the largest families of plants, with more than 200 genera and 7000 species[9]. *Acalypha* is the

fourth largest genus in the Euphorbiaceae with about 450 species[10]. Several species of the genus *Acalypha* has been studied and it has been demonstrated that they present antioxidant, wound healing, post-coital antifertility, neutralization of venom, antibacterial[11], apoptosis[12], antifungal[13] and antitrypanosomal activities[14] *e.g.,* one of its species *Acalypha monostachya* Cav. (synonyms: *Acalypha hederacea* Torr.) is one of the most important medicinal plants used by the inhabitants of San Rafael and Zapotitlan Salinas, Puebla, Mexico to alleviate illnesses like skin eruptions and use in wound healing and diarrhea[15]. Another species *Acalypha indica* L. is a weed widely distributed throughout the plains of India. It has been reported to be useful in treating pneumoniae, asthma, rheumatism and several other ailments[16]. The dried leaves of *Acalypha indica* was made into a poultice to treat bedsores and wounds and the juice of *Acalypha indica* is added to oil or lime and used to treat a variety of skin disorders. The leaves of *Acalypha grandis* have also been reported to possess contraceptive activity[17]. Several chemical[18] and biological[19] investigations have been carried out on this plant. *Acalypha ornata* Hochst is found in southern Nigeria. Its leaves are pounded together with those of other plants in water and the liquid is given to the children with rabies. In view of the aforesaid facts, the plant *Acalypha brachystachya* Hornem. was selected to evaluate the antioxidant potential of its various parts *i.e.*, seeds, leaves and shoot by the methods such as DPPH radical assay, FRAP assay, total phenolics assay and phosphomolybdenum complex assay.

EXPERIMENTAL

The plant *Acalypha brachystachya* hornem. was collected from Kotli in October 2010 and identified by Mr. Muhammad Ajaib (Taxonomist), Department of Botany, GC University, Lahore. A voucher specimen No. GC. Herb. Bot. 901 has been deposited in the herbarium of the same University.

Extraction and fractionation of antioxidants: The shade dried ground whole plant (1042 g) was firstly separated into shoots (353 g), seeds (207 g) and leaves (332 g) and then exhaustively extracted separately with methanol at room temperature. These fractions were evaporated to yield the residue, which was dissolved in distilled water (500 mL) and partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-butanol, respectively. These organic fractions were concentrated separately on rotary evaporator and the residues thus obtained were used to evaluate their *in vitro* antioxidant potential.

Chemicals and standards: DPPH (1,1-Diphenyl-2-picrylhydrazyl radical), TPTZ (2,4,6-tripyridyl-*s*-triazine), trolox, gallic acid, follin Ciocalteu reagent and BHT (butylated hydroxytoluene) were obtained from Sigma Chemical Company Ltd. (USA) and organic solvents (*n*-hexane, chloroform, ethyl acetate, *n*-butanol), sulphuric acid, conc. HCl, sodium phosphate, ammonium molybdate, ferric chloride, ferrous chloride, Dragon droff's reagent, cerric sulphate solution, lead acetate solution, Fehling's solution and Benedicts reagent from Merck (Pvt.) Ltd. (Germany).

Phytochemical screening: The extracts were subjected to phytochemical analysis using procedures of Harbone and Evans[20].

Test for alkaloids: Spots of all the extracts were applied on the TLC plate and then Dragon droff's reagent was being sprayed on them. Noted the colour of the spots to check the presence of alkaloids.

Test for terpenoids: Same procedure was being followed for the terpenoids but here the spots were being sprayed by the cerric sulphate solution.

Test for tannins: *n*-Butanol- HCl test: *n*-butanol-HCl test was used to check the presence of tannins in which we took 2 mL of fractions, 5 mL of *n*-butanol-HCl solution and then warm it for 1 h at 95 ºC in water bath.

Test for phenols: Phenols can be tested by adding neutral $FeCl_3$ solution in each fraction and then noted the colour of resulted solutions.

Test for carbohydrates: Fehling's solution was added in each fraction, boils it and noted the colour, for the presence of sugar (carbohydrate).

Test for saponins: All the extracts were taken in separate test tubes and then in these added dilute water. Gel formation with adding water results the presence of saponins.

Test for flavonoids: Benedict's solution test: With the help of capillary tube, spots of all the extracts were taken on the TLC plate and then these were sprayed with benedict's solution. I have noted the colour of the spots to check the presence of flavonoids.

Lead acetate solution test: The same procedure was followed for taking the spots on TLC plate, but here the lead acetate solution were sprayed on spots. To check the presence of flavonoids, noted the colour of the spots.

DPPH Radical scavenging activity: The DPPH radical scavenging activities of various fractions of plant were examined by comparison with that of known antioxidant, butylated hydroxytoluene (BHT) using the method of Lee and Shibamoto[21]. Briefly, various amounts of the samples (1000, 500, 250 and 125 µg/mL) were mixed with 3 mL of methanolic solution of DPPH (0.1 mM). The mixture was shaken vigorously and allowed to stand at room temperature for 1 h. Then absorbance was measured at 517 nm against methanol's a blank in the spectrophotometer. Lower absorbance of spectrophotometer indicated high free radical scavenging activity. The percent of DPPH decoloration of the samples was calculated according to the formula:

$$\text{Antiradical activity} = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

Each sample was assayed in triplicate and mean values were calculated.

Total antioxidant activity by phosphomolybdenum method: The total antioxidant activities of various fractions of plant were evaluated by phosphomolybdenum complex formation method[22]. Briefly, 500 µg/mL of each sample was mixed with 4 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) in sample vials. The blank solution contained 4 mL of reagent solution. The vials were capped and incubated. After the samples had been cooled to room temperature, the absorbance of mixture was measured at 695 nm against blank. The antioxidant activity was expressed relative to that of butylated

hydroxytoluene (BHT). All determinations were assayed in triplicate and mean values were calculated[23].

Ferric reducing antioxidant power (FRAP) assay: The FRAP assay was done according to Benzie and Strain[24] with some modifications. The stock solutions included 300 Mm acetate buffer (3.1 g $CH_3COONa \cdot 3H_2O$ and 16 mL CH_3COOH), pH 3.6, 10 mM TPTZ (2,4,6-tripyridyl-*s*-triazine) solution in 40 mM HCl and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 mL acetate buffer, 2.5 mL TPTZ solution and $FeCl_3 \cdot 6H_2O$ solution and then warmed at 370 ºC before using. The solutions of plant samples and that of Trolox were formed in methanol (250 µg/mL). 10 µL of each of sample solution and BHT solution were taken in separate test tubes and 2990 µL of FRAP solution was added in each of to make total volume upto 3 mL the plant samples were allowed to react with FRAP solution in the dark for 0.5 h. Readings of the coloured product [ferrous tripyridyltriazine complex] were then taken at 593 nm. The FRAP values were determined as micromoles of Trolox equivalents per mL of sample by computing with standard calibration curve constructed for different concentrations of Trolox. Results were expressed in TE µM/mL.

Total phenolic content: Total phenolic contents of various fractions of plant were determined by the method of Makkar *et al.*[25]. The 0.1 mL (0.5 mg/mL) of sample was combined with 2.8 mL of 10 % Na_2CO_3 and 0.1 mL of 2N Folin-Ciocalteu reagent. After 40 min absorbance at 725 nm was measured by UV-visible spectrophotometer. Results are expressed as gallic acid equivalents.

RESULTS AND DISCUSSION

The results for the phyto-chemical screening (Table-1) showed that the alkaloids were completely absent in *n*-hexane soluble fraction of seeds and shoot and *n*-butanol soluble fraction of leaves. However, all remaining fractions contained considerable amount of these. The *n*-hexane soluble fractions of seed, shoot and leaves contained considerable amount of terpenoids. The chloroform soluble fraction of seeds and the ethyl acetate soluble fractions of shoot and leaves also showed good amount of terpenoids. However, these were completely absent from the remaining fractions. The ethyl acetate and chloroform soluble fractions of seed and shoot contained good amounts of tannins. The *n*-butanol soluble fraction of shoot and ethyl acetate soluble fraction of leaves also showed considerable amounts of tannins, however, in the remaining fractions these were completely absent. Phenols were present in good amounts in the ethyl acetate and chloroform soluble fractions of seeds, shoot and leaves. In all the remaining fractions these were completely absent. Only ethyl acetate and *n*-butanol soluble fraction of shoot contained good amounts of carbohydrates, while in all the remaining fractions these were absent. Saponins were present in *n*-butanol fractions of all the three parts of this plant. Flavonoids were absent in the *n*-hexane and *n*-butanol soluble fractions of seed. These were also absent in chloroform and *n*-butanol soluble fraction of shoot and leaves. However, all the remaining fractions showed considerable amounts of flavonoids.

$DPPH^{\cdot}$ is a stable free radical and accepts electron or hydrogen radical to become a stable diamagnetic molecule[26]. Radical scavenging activity of plant extracts against stable $DPPH^{\cdot}$ (2,2-diphenyl-2-picrylhydrazyl hydrate) was determined spectrophotometrically. When $DPPH^{\cdot}$ reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep violet to light yellow) were measured at 517 nm on a UV/visible light spectrophotometer[27]. Thus, antioxidant molecules can quench $DPPH^{\cdot}$ by providing hydrogen atom or by electron donation and convert it to a colourless product, resulting in a decrease in absorbance at 517 nm[28]. In brief, the reduction capacity of $DPPH^{\cdot}$ was determined by the decrease in its absorbance at 517 nm, which is reduced by antioxidants[29]. In the present study the organic fractions of *Acalypha brachystachya* hornem. were evaluated for their free radical scavenging activity using the DPPH radical assay. Reduction of DPPH radical was observed by the decrease in absorbance at 517 nm. The values of per cent scavenging of DPPH radical have been shown in Table-2. It was observed that the radical scavenging activity was increased by increasing the concentration of samples. The various concentrations of ethyl acetate soluble fraction of seeds exhibited the highest per cent inhibition of DPPH radical as compared to the other fractions. It showed 80 % ± 0.32 inhibition of DPPH radical at a concentration of 500 µg/mL. The half maximal inhibitory concentration (IC_{50}) is a measure of the effectiveness of a compound in inhibiting biological or biochemical function. This quantitative measure indicates how much of a particular drug or other substance (inhibitor) is

TABLE-1
PHYTOCHEMICAL SCREENING OF VARIOUS FRACTIONS OF *Acalypha brachystachya* Hornem.

Plant part	Fractions	Alkaloids	Terpenoids	Tannins	Phenols	Sugars	Saponins	Flavonoids
Seeds	*n*-Hexane soluble	–	+	–	–	–	–	–
	Chloroform soluble	+	+	+	+	–	–	+
	Ethylacetate soluble	+	–	+	+	–	–	+
	n-Butanol soluble	+	–	–	–	–	+	–
Shoots	*n*-Hexane soluble	–	+	–	–	–	–	+
	Chloroform soluble	+	–	+	+	–	–	–
	Ethylacetate soluble	+	+	+	+	+	–	+
	n-Butanol soluble	+	–	+	–	+	+	+
Leaves	*n*-Hexane soluble	+	+	–	–	–	–	+
	Chloroform Soluble	+	–	–	+	–	–	+
	Ethylacetate Soluble	+	+	+	+	–	–	+
	n-Butanol soluble	–	–	–	–	–	+	–

(+) = Presence of constituents; (–) = Absence of constituents.

TABLE-2
FREE RADICAL SCAVENGING ACTIVITYOF VARIOUS FRACTIONS OF *Acalypha brachystachya* Hornem. USING 1,1-DIPHENYL-2-PICRYLHYDRAZYL RADICAL (DPPH)

Plant part	Sample	Concentration in assay(µg/mL)	Percentage scavenging of DPPHradical ± SEM[a]
Seeds	*n*-Hexane soluble	1000	52.60 ± 0.12
		500	24.78 ± 0.34
		250	17.60 ± 0.11
		125	27.61 ± 0.93
	Chloroform soluble	1000	62.60 ± 0.33
		500	39.35 ± 0.67
		250	29.13 ± 0.22
		125	39.35 ± 0.77
	Ethyl acetate soluble	500	80 ± 0.32
		250	61.30 ± 0.45
		125	39.56 ± 0.65
	n-Butanol soluble	500	21.74 ± 0.78
		250	17.17 ± 0.13
		125	14.56 ± 0.34
Shoot	*n*-Hexane soluble	500	14.13 ± 0.90
		250	12.60 ± 0.12
		125	12.10 ±0.54
	Chloroform soluble	1000	66.30 ± 0.89
		500	28.08 ± 0.78
		250	18.04 ± 0.22
		125	15.65 ± 0.35
	Ethyl acetate soluble	1000	70.86 ± 0.87
		500	44.35 ± 0.99
		250	28.48 ± 0.32
		125	14.35 ± 0.56
	n-Butanol soluble	1000	61.73 ± 0.52
		500	24.35 ± 0.21
		250	11.74 ± 0.39
		125	11.08 ± 0.44
Leaves	*n*-Hexane soluble	1000	62.60 ± 0.77
		500	23.91 ± 0.89
		250	11.52 ± 0.58
		125	39.56 ± 0.10
	Chloroform soluble	1000	61.74 ± 0.47
		500	70.43 ± 0.28
		250	51.74 ± 0.69
		125	18.48 ± 0.99
	Ethyl acetate soluble	1000	35.65 ± 0.98
		500	21.08 ± 0.13
		250	20.87 ± 0.23
		125	14.13 ± 0.44
	n-Butanol soluble	1000	60.43 ± 0.31
		500	18.91 ± 0.71
		250	10.43 ± 0.88
		125	14.13 ± 0.41
–	BHT[b]	60	91.25 ± 0.13
		30	75.56 ± 0.07
		15	42.67 ± 0.04
		08	23.57 ± 0.31

[a]Standard mean error of three assays.[b]Standard antioxidant.

needed to inhibit a given biological process. A lower value would reflect greater antioxidant activity of the fraction. Ethyl acetate soluble fraction of seeds extract revealed lowest IC_{50} value (204.512 ± 0.12 µg/mL) as compared to the other fractions so it had highest antioxidant activity, followed by chloroform soluble fraction of leaves (264.64 ± 0.34 µg/mL) relative to butylated hydroxytoluene (BHT), a reference standard, having IC_{50} of 12.7 ± 0.99 µg/mL (Table-3).

Total antioxidant activity of the studied fractions was determined by phosphomolybdenum complex method (Table-3). The phosphomolybdenum complex method is based on the reduction of Mo(VI) to Mo(V) by an antioxidant and the formation of green phosphate/Mo(V) complex with maximal absorption at 695 nm. The assay was successfully used to quantify vitamin E in seeds[22] and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant polyphenols. A higher absorbance indicates a higher antioxidative activity. The total antioxidant activities of these fractions were compared with the standard antioxidant BHT and the results have been shown in Table-3. The results indicated that chloroform soluble fraction of leaves had highest total antioxidant activity (0.486 ± 0.08). The medium antioxidant activity has been shown by ethyl acetate soluble fraction of seeds (0.185 ± 0.25) and chloroform soluble fraction of seeds (0.152 ± 0.46), however remaining fractions exhibited poor activity. BHT (standard) showed total antioxidant activity 0.83 ± 0.18.

Ferric reducing antioxidant assay (FRAP) is a simple direct test for measuring antioxidative contents. Ferric reducing antioxidant assay, a simple and reliable test that depends upon the reduction of ferric 2,4,6-tripyridyl-S-triazine [Fe(III)-TPTZ] to the ferrous 2,4,6-tripyridyl-S-triazine [Fe(II)-TPTZ] complex by a reductant at low pH, was adopted. This complex has an intense blue colour that can be monitored at 593 nm. Although initially elaborated for estimation of the total antioxidant activity in biological samples, this method was then modified for routine analysis of the antioxidative activity of pure chemical substances and plant extracts[17,18]. The reduction capacity of a compound may serve as a significant indicator of its potential antioxidant activity[19]. A higher absorbance indicates a higher ferric reducing power. In the current study (Table-3), ethyl acetate soluble fraction of seeds, chloroform soluble fraction of leaves and ethyl acetate soluble fraction of shoot exhibited promising antioxidant power with FRAP values 37.3 ± 0.15, 33 ± 0.13 and 29.6 ± 0.12 (µM of Trolox equivalent), respectively and the remaining fractions showed poor activity.

Phenols are very important plant constituents because of their radical scavenging ability due to their hydroxyl groups[29]. The phenolic content may contribute directly to the antioxidative action[30]. It is suggested that polyphenolic compounds have inhibitory effects on mutagenesis and carcinogenesis in humans[31]. Most of the work dealing with phenolic content in natural products uses gallic acid and (±)-catechin as standards (independently of phenolic species detected), the content of phenolic in this work was expressed as gallic acid equivalents[32]. It has been shown that the total phenolic content of plant material is correlated with their antioxidant activity shows the contents of total phenolics in different fractions, which are expressed as milligrams of gallic acid equivalents (GAEs) per gram of fraction. Among these twelve fractions, the ethyl acetate soluble fraction of seeds showed the highest amount of total phenolic compounds (27.7 ± 0.23 mg/g) followed by the ethyl acetate soluble fraction of leaves (20.2 ± 0.23 mg/g), ethyl acetate fraction of shoot (16.2 ± 0.98 mg/g), chloroform soluble fraction of shoot (7.4 ± 0.65 mg/g), chloroform fraction of seeds (7.0 ± 0.34 mg/g) and *n*-butanol fraction of seeds

TABLE-3
IC_{50}, TOTAL ANTIOXIDANT ACTIVITY, FRAP VALUE AND TOTAL PHENOLICS OF DIFFERENT FRACTIONS OF *Acalypha brachystachya* Hornem.

Plant part	Sample	IC_{50} of DPPH assay (μg/mL) ± SEM[a]	Total antioxidant activity ± SEM[a]	FRAP value (μM of trolox equivalent) ± SEM[a]	Total phenolics (mg/g of GAE) ± SEM[a]
Seeds	*n*-Hexane soluble	966.80 ± 0.55	0.125 ± 0.55	12.6 ± 0.22	3.3 ± 0.32
	Chloroform soluble	731.07 ± 0.91	0.152 ± 0.34	28 ± 0.87	7.0 ± 0.34
	Ethylacetate soluble	204.512 ± 0.12	0.185 ± 0.23	37.3 ± 0.15	27.7 ± 0.23
	n-Butanol soluble	1985.52 ± 0.22	0.066 ± 0.97	26 ± 0.23	5.7 ± 0.87
Shoot	*n*-Hexane soluble	–	0.111 ± 0.66	10.3 ± 0.44	2.6 ± 0.11
	Chloroform soluble	772.11 ± 0.56	0.086 ± 0.76	26.3 ± 0.56	7.4 ± 0.65
	Ethylacetate soluble	629.117 ± 0.43	0.079 ± 0.45	29.6 ± 0.12	16.2 ± 0.98
	n-Butanol soluble	845.20 ± 0.34	0.052 ± 0.67	25 ± 0.34	4.8 ± 0.78
Leaves	*n*-Hexane soluble	833.21 ± 0.65	0.083 ± 0.43	11 ± 0.88	2.0 ± 0.43
	Chloroform soluble	264.64 ± 0.34	0.486 ± 0.99	33 ± 0.13	5.2 ± 0.26
	Ethylacetate soluble	–	0.061 ± 0.56	25.6 ± 0.33	20.2 ± 0.23
	n-Butanol soluble	874.35 ± 0.11	0.048 ± 0.67	25 ± 0.45	4.4 ± 0.77
–	BHT[b]	12.1 ± 0.92	1.218 ± 0.07	–	–

[a]Standard mean error of three assays. [b]Standard antioxidant.

(5.7 ± 0.87 mg/g), respectively while the remaining fractions showed poor activity.

Conclusion

It was concluded from the above results that *Acalypha brachystachya* hornem. contained good amounts of flavonoids, terpenoids, tannis and phenols and very less amounts of sugars. From the results of antioxidant activity, it is evident that ethyl acetate soluble fraction of seeds showed highest value of inhibition (%) of DPPH (80 % ± 0.32) at concentration of 500 μg/mL. Its IC_{50} was 204.51 ± 0.12 μg/mL relative to butylated hydroxytoluene (BHT), having IC_{50} of 12.1 ± 0.92 μg/mL. It also showed highest total phenolic contents (27.7 ± 0.43 mg of gallic acid equivalents) as well as the highest FRAP value (37.3 ± 0.15 μM of trolox equivalents) as compared to the other studied fractions. However the chloroform fraction of leaves showed highest antioxidant activity *i.e.*, 0.486 ± 0.98. Ethyl acetate soluble fraction of seeds showed good activity, chloroform soluble fractions of seeds and leaves showed medium activity while all the remaining showed poor activity. This study showed that this plant has different compounds with antioxidant potential and to the best of our knowledge, the detailed antioxidant activity of this plant has not reported earlier to this investigation. As some of the fractions of this plant exhibit good antioxidant potential so these can serve as very valuable sources of therapeutic agents, which would be expected to increase shelf life of foods and protect against peroxidative damage in living systems in relation to aging and carcinogenesis.

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