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RESEARCH ARTICLE

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ANTIOXIDANT ACTIVITY WITH TOTAL PHENOLIC CONSTITUENTS FROM *AERVA TOMENTOSA* FORSK.

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ABSTRACT

In the present study, antioxidant potential of the different extract of the whole plant of *Aerva tomentosa* Forsk. was evaluated by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay and total phenolic contents was analyzed using the Folin-Ciocalteu reagent method. The methanol extract showed significant activity in antioxidant assay compared to other successive extracts such as petether, dichloromethane, ethylacetate and aqueous. In DPPH scavenging assay the RC_{50} value of the methanol extract was found to be 6.0 µg/ml. The highest amount of total phenolic contents was found in methanol soluble fraction (34.5 ± 0.726 mg gallic acid equivalent/g extract). These results suggest that there was a direct correlation between total phenolics and antioxidant activities which could introduce phenols as the main antioxidant of *Aerva tomentosa* extracts and offering effective protection from free radicals.

KEYWORDS

Aerva tomentosa, total phenolics, antioxidant activity, DPPH, % inhibition.

INTRODUCTION

Antioxidant research is an important topic in the medical field as well as in the food industry. Recent research with important bioactive compounds in many plants and food materials has received much attention. Free radicals and reactive oxygen species are byproducts in aerobic organism and have aroused significant interest among scientists in the past decade. It has been proposed that they could induce cellular damage and might be involved in several human diseases including cancer, arteriosclerosis, diabetic mellitus, hypertension and AIDS and in aging processes.Of various kinds of natural antioxidants, flavonoids and phenolic compounds have received much attention. Therefore, antioxidants with free radical scavenging activities may have great relevance in the prevention and therapeutics of diseases in which oxidants or free radicals are implicated[1]. In this respect, polyphenolic compounds, like flavonoids and phenolic acids, commonly found in plants have been reported to have multiple biological effects, including antioxidant activity[2,3,4,5]. Currently, the possible toxicity of synthetic antioxidants has been criticized. Thus interest in natural antioxidant, especially of plant origin, has greatly increased in recent years[6].

Ayurveda, Unani, Chinese and other traditional medicinal systems, provide substantial lead to find active and therapeutically useful antioxidant compounds from plants. Considering the growing interest in assessing the antioxidant capacity of natural products the phyto-chemistry of plants having antioxidant activity has been reported.

Aerva tomentosa Forsk. (syn. *A. javanica* Juss. ex Schult): The plant belongs to the family Amaranthaceae and the genus *Aerva* comprises sixty one species, distributed in the warm parts of Asia and Africa. Out of these, *A. lanata* has been studied for flavonoid-glycosides, β-sitosterol, α-amyrin, compesterol, chrysin, four new alkaloids viz. aervine, methylaervine, aervoside and aervolanine[7,8,9,10,11]. Similarly, from *A. persica* alkaloids, leucoanthocyanidins, flavonoids, triterpenoids, cardiac-glycosides, coumarins and saponins are reported[12].

Aerva tomentosa is a deciduous shrub, widely distributed in Punjab, Rajasthan and Gujarat. It is diuretic, demulcent, purgative and emetic. Flowers and seeds are used against swelling, headache and rheumatism[13,14,15].Phytochemically, ecdysteroids (20-hydroxyecdysone and 5, 20 dihydroxyecdysone) and alkaloids from the whole plant and aervanone, chrysin-7-O-galactoside, β-sitosterol, α-amyrin and fatty acids from the roots have been reported[16,17]. Antimicrobial and hepato-protective activities have also been demonstrated from its perianth lobes[18].

MATERIALS AND METHODS

Plant material

The plant material of *Aerva tomentosa* Forsk. were obtained from the shrub growing in the University of Rajasthan campus, in June 2008 and identified by Professor Dr. R. A. Sharma, Department of botany, University of Rajasthan, Jaipur, India where a voucher specimen has been deposited.

Chemicals

2, 2-diphenyl-1-picrylhydrazyl (DPPH) , quercetin, trichloroacetic acid (TCA), gallic acid

(GA) and ferric chloride were obtained from Sigma Chemical Co. (USA). All other chemicals were of analytical grade.

Extraction

The shade-dried leaves were coarsely powdered and successively extracted with pet-ether, dichloromethane, ethylacetate, methanol and aqueous by a Soxhlet apparatus at 45°C. The solvent was completely removed by rotary evaporator and obtained greenish gummy exudates. This crude extract was used for further investigation for potential antioxidant properties.

Antioxidant potentialities

The method used by[19] was adopted with suitable modifications to our particular circumstances. Methanolic solution of DPPH (20 mg/ 10 ml) was used.

Qualitative analysis

The methanol extract was applied on a TLC plate as a spot (100µg/ml) for chromatographic separation of the extract using the mobile phase methanol: chloroform (95:5, v/v). It was allowed to develop the chromatogram for 30 minutes. After completion of the chromatogram the whole plate was sprayed with DPPH (0.15 % w/v) solution using an atomizer. The color changes (yellowish color development on pinkish background on the TLC plate) were noted as an indicator of the presence of antioxidant substances.

Quantitative analysis

For quantitative assay, each of the extract (8 mg) was dissolved separately in 10 ml of methanol and various concentrations (80, 60, 40, 20 and 10 µg) were prepared. Each 2.5 ml of test extract was mixed with DPPH (20 mg/ 10 ml) and allowed 30 minutes for any reaction to occur. The absorbance of the colour developed was measured at 517 nm by UV spectrophotometer (Varian type Cary PCB 150 Water Peltier System with Standard Cuvettes). The negative control and standard quercetin as positive control was subjected to the same procedure. Three replicates were used and the average absorption was noted for each concentration. Data was processed using EXCEL and concentration, that causes 50% reduction in absorbance (RC_{50}), was calculated. Percent inhibition of DPPH was calculated by following equation[20]

$$\% \text{ Inhibition} = 1 - (A_1 / A_2) \times 100$$

Where, A_1 is the absorbance of the test sample and A_2 as the absorbance of control reaction.

Total Phenolic Contents Analysis

Total phenolic content was analyzed using the Folin-Ciocalteu reagent method. Gallic acid was used as the standard for the calibration curve and the total phenolic contents were expressed as mg gallic acid equivalents per gram of tested extracts.

A stock solution of the standard phenol (Gallic acid) was prepared in 80 % ethanol (400 mg/ml), out of which 0.1 to 0.9 ml was taken into separate test tubes and the volume of each was raised to 1 ml with 80 % ethanol. To each tube, 1 ml Folin-Ciocalteu reagent (diluted with distilled water in 1:2 ratios, just before use) was added followed by 2 ml of 20 % Na_2CO_3 solution and this mixture was shaken vigrously. Such samples were placed in a boiling water bath for exactly 1 min and later, cooled under a running tap. Each of the reaction mixture was diluted to 25 ml with distilled water and to OD was taken at 750 nm against a blank using a spectrophotometer. Three replicates were taken for each concentration and the average absorbance was plotted against the respective concentration to compute a regression curve which followed the Beer's Law.

Similarly, various extracts of plant were processed and the absorbance measured. From the mean values, total levels of phenolics were calculated (with reference to Gallic acid) by referring the absorbance of the experimental samples with the standard regression curve.

RESULT

Life on earth survives only due to presence of oxygen; Oxygen gives us energy by oxidation of food which is essential for living. During this process highly reactive and harmful oxygen species are also generated which can damage living organisms. Organisms contain a complex network of antioxidant molecules and enzymes that work together to prevent oxidative damage of cellular components such as DNA, proteins and lipids[21, 22,23,24,25]. In traditional societies nutrition and health care are strongly interconnected and many plants have been consumed both as food and for medicinal purposes. The consumption of non-cultivated botanicals plays a central role in the diet, but very few ethnopharmacological and phyto-pharmacological studies have dealt exhaustively with the potential health benefits of such diets. In the past few years, there has been growing interest in the involvement of reactive oxygen species (ROS) in several pathological situations. ROS produced in vivo include superoxide radical (O2 . •-), hydrogen peroxide . •- can interact in the presence of certain transition metal ions (H_2O_2) and hypochlorous acid (HOCl). H_2O_2 and O_2 to yield a highly-reactive oxidizing species, the hydroxyl radical (• OH)[26]. Phenolic compounds and flavonoids have been reported to be associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals[27,28]. Literature reviews confirm that phytochemically, ecdysteroids (20-hydroxyecdysone and 5, 20 dihydroxyecdysone) and alkaloids from the whole plant and aervanone, chrysin-7-O-galactoside, β-sitosterol, α-amyrin and fatty acids from the roots have been reported in this species. Many naturally occurring triterpinoids exhibited a good anti-inflammatory activity have been isolated from various plants[29,30]. Pentacyclic triterpinoids have a wide spectrum of biological activities and some of them may be useful in medicine. There is growing interest in natural triterpinoids caused as much by the scientific aspects extraction and structural analysis of these compounds, as by the fact of their wide spectrum of biological activities, they are bactericidal, fungicidal, antiviral, cytotoxic, analgesic, antiinflammatory, anti-cancer and antiallergic[31].

Graph 1 shows the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the different successive extracts of the plant *A. tomentosa* Forsk. This activity was increased by increasing the concentration of the sample extract. DPPH antioxidant assay is based on the ability of 2, 2- Diphenyl-1-picrylhydrazyl (DPPH), a stable free radical, to decolorize in the presence of antioxidants. The DPPH radical contains an odd electron, which is responsible for the absorbance at 517 nm and also for a visible deep purple color. When DPPH accepts an electron donated by an antioxidant compound, the DPPH is decolorized, which can be quantitatively measured from the changes in absorbance. The RC_{50} value of the methanol extract was found to be 6.0 µg/ml. Total phenolic contents (mg gallic acid equivalent/g extract), as affected by the extracting solvents were ranked from high to low: methanol extract > dichloromethane extract > petether extract > ethylacetate extract > aqueous extract (Table 1). Graph 2 shows the total phenolics of the methanol extract of *Aerva tomentosa* compared to gallic acid. The total phenolic contents were found remarkable in methanol soluble fraction to be 34.5 mg gallic acid equivalent/g extract. The total phenolics contents of methanol extract were ten times and five times of those of aqueous extract and ethylacetate extract respectively. The phenolic contents of the extract were observed to rise as the concentration of the extract gradually increased.

Table 1

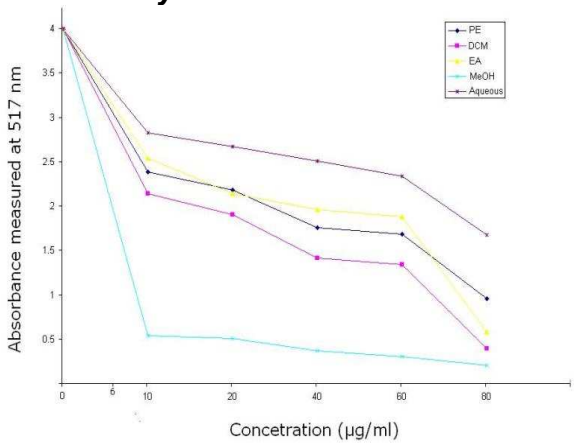
Antioxidant activity RC_{50} (µg/ml) and total phenolic contents of the Aerva tomentosa extracts

Extract	Yield %	RC_{50} µg/ml	% inhibition of DPPH µg/ml concentrations					Total phenolics (mg GAE/g)
			10	20	40	60	80	
PE	0.35	28	40.23	45.30	56.00	57.83	75.87	9.5 ± 0
DCM	0.65	16	46.23	52.17	64.45	66.25	89.95	24.3 ± 0.173
EA	0.50	36	36.20	46.30	50.70	52.82	85.30	7.3 ± 0.168
Methanol	2.3	6	86.30	87.08	90.70	92.40	94.65	34.5 ± 0.726
Aqueous	2.09	70	29.18	33.08	37.18	41.43	57.93	3.5 ± 0.288

***Abbreviation**: PE = Pet-ether, DCM = Dichloromethane, EA = Ethyl-acetate, MeOH = Methanol, NA= not applicable, RC_{50} = Concentration of the extract (µg/ml) at which the absorbance (at 517 nm) decreases to half of its intial value*

Graph 1

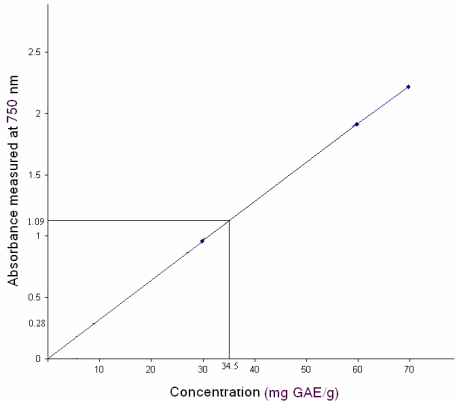
Antioxidant activity of the different successive extracts



Antioxidant activity of the different extracts of the whole plant of Aerva tomentosa values are the average of triplicate experiments and represented as mean ± standard deviation.

Graph 2

Total phenolics of the methanol extracts



Graph 2

Total phenolics of the methanol extracts of the whole plant of Aerva tomentosa. Values are the average of triplicate experiments and represented as mean ± standard deviation.

DISCUSSION

The antioxidative effect is mainly due to phenolic components, such as phenolic acids, and phenolic diterpenes[32]. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides[33]. For the measurements of the reductive ability, it has been found that the Fe^{3+} – Fe^{2+} transformation occurred in the presence of extract samples which were postulated previously by[34]. Earlier authors[35] have observed a direct correlation between antioxidant activity and total phenolic contents of certain plant extracts.

CONCLUSION

In conclusion, the extracting solvent significantly affected the yield, total phenolic content and antioxidant activity of *Aerva tomentosa* Forsk. Methanol extract had the highest extract yield and total phenolic recovery, as well as the highest antioxidant activity when determined by the DPPH assay. Thus our results indicate that selective extraction from natural materials, by an appropriate solvent, is important for obtaining fractions with high antioxidant activity. The results also showed that different solvent extracts contained different antioxidant capacities in terms of reducing and radical-scavenging power. From the above results and discussion it can be concluded that the methanol extract could be the optimal solvent for extraction of *Aerva tomentosa* Forsk. which possesses the potent antioxidant substances and responsible for chemoprotective mechanism, as well as justify the basis of using this plant's extract as folkloric remedies.

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