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Full Length Research Paper

Screening of medicinal plant extracts for antioxidant activity

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The methanolic crude extracts of *Desmodium gangeticum* (Linn.), *Eclipta alba* (Linn.) *Ocimum sanctum* (Linn.), *Piper longum* (Linn.), *Solanum nigrum* (Linn.) and *Amaranthus caudatus* (Linn.) were screened for their free radical scavenging properties using ascorbic acid as standard antioxidant. Free radical scavenging activity was evaluated using 1,1-diphenyl-2-picryl hydrazyl (DPPH) free radical. The overall antioxidant activity of *D. gangeticum* was found to be the strongest, followed in descending order by *A. caudatus*, *S. nigrum*, *P. longum*, *E. alba* and *O. sanctum*. The IC_{50} values of the extracts ranged between 0.05 ± 0 and 0.19 ± 0 mg/l. The ascorbic acid levels varied from 3.86 ± 0.20 to 21.33 ± 1.49 mg/100g and the carotenoids content were observed between 9.0 ± 0.24 to 24 ± 1.16 mg/100g in plant extracts. The highest total phenols content were found to be in *O. sanctum* (Linn.) with the value 48.93 ± 0.24 mg/g. The present study reveals that the selected plants would exert several beneficial effects by virtue of their antioxidant activity and could be harnessed as drug formulation.

Key words: 1,1-diphenyl-2-picryl hydrazyl, antioxidant, phenol, radical scavenger.

INTRODUCTION

It has been established that oxidative stress is among the major causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others (Young and Woodside, 2001). A great number of aromatic, medicinal, spice and other plants contain chemical compounds exhibiting antioxidant properties. Oxidative process is one of the most important routes for producing free radicals in foods, drugs and even in living systems (Halliwell, 1994). The most effective path to eliminate and diminish the action of free radicals which cause the oxidative stress is antioxidative defense mechanisms. Antioxidants are those substances which possess free radical chain reaction breaking properties. Recently there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidants in reducing oxidative stress-induced tissue injury (Pourmorad et al., 2006). Among the numerous naturally occurring antioxidants; ascorbic acid, carotenoids and phenolic compounds are more effective (Duh et al., 1999). They are known to inhibit lipid peroxidation (by inactivating lipoxygenase), to scavenge free radicals and active oxygen species by propagating a reaction cycle and to chelate heavy metal ions (Sudarajan et al., 2006).

The study done on medicinal plants and vegetables strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Cao et al., 1996). On continuation of our experimental work for the search of antioxidant activity of medicinal plants, we studied extracts of six medicinal plants. The free radical scavenging activity against 1,1-diphenyl-2-picryl hydrazyl (DPPH) was evaluated during the course of work. The ascorbic acid, carotenoids and total phenol contents with antioxidant activity were also determined. The assessment of such properties remains an interesting and useful task, particularly for finding new sources for natural antioxidants.

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Table 1. Characteristics of the used medicinal plants.

Botanical name	English name	Part used	Family name	*Medicinal use*
Desmodium gangeticum L.	Desmodieae	Leaf	Fabaceae	Asthma, typhoid, piles
Eclipta alba L.	False daisy	Leaf	Asteraceae	Antihepatotoxic
Ocimum sanctum L.	Tulsi	Leaf	Lamiaceae	Antirheumatic, anticarcinogenic
Piper longum L.	Pipli	Fruit	Piperaceae	Gonorrhea, hepatitis
Solanum nigrum L.	Duscle	Fruit	Solanaceae	Antiseptic, antidysenteric
Amaranthus caudatus L.	Quilete	Leaf	Amaranthaceae	Anthelmintic, astringen

MATERIALS AND METHODS

Plant materials

The six medicinal plants studied were collected by a botanist from Auraon Research Center (an authenticated research center recognized by Central Scientific and Industrial Research, India), Botanical garden of National Botanical Research Institute, Lucknow. The plant materials were cleaned and powdered. The botanical names, family names, English names and parts used are presented in Table 1.

Extraction

The plant materials presented in Table 1 were air-dried in shed at room temperature (26°C) for 2 weeks, after which it was grinded to a uniform powder. Methanol extracts were prepared by soaking 100 g each of the dry powdered plant materials in 1 litre of methanol at room temperature for 48 h. The extracts were filtered first through a Whatmann filter paper No. 42 (125 mm) and then through cotton wool. The extracts were concentrated using a rotary evaporator with the hot water bath set at 40°C. The percentage yield of extracts ranged from 5 - 20% (w/w).

Antioxidant activity (DPPH free radical scavenging activity) determination

The antioxidant activity of the plant extracts was examined on the basis of the scavenging effect on the stable DPPH free radical activity (Braca et al., 2002). Ethanolic solution of DPPH (0.05 mM) (300 µl) was added to 40 µl of extract solution with different concentrations (0.02 - 2 mg/ml). DPPH solution was freshly prepared and kept in the dark at 4°C. Ethanol 96% (2.7 ml) was added and the mixture was shaken vigorously. The mixture was left to stand for 5 min and absorbance was measured spectrophotometrically at 517 nm. Ethanol was used to set the absorbance zero. A blank sample containing the same amount of ethanol and DPPH was also prepared. All determinations were performed in triplicate. The radical scavenging activities of the tested samples, expressed as percentage of inhibition were calculated according to the following equation (Yen and Duh, 1994).

$$\text{Percent (\%) inhibition of DPPH activity} = [(A_B - A_A) / A_B] \times 100$$

Where A_A and A_B are the absorbance values of the test and of the blank sample, respectively. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and represented as IC_{50} value for each of the test solutions.

Determination of ascorbic acid

The ascorbic acid was determined according to Cakmak and Marschner (1992) with some modification. Each plant extract (0.5 ml of 1:10 g/ml) in methanol was separately mixed with 5 ml of 5% meta-phosphoric acid, and centrifuged at 4000 rpm for 30 min. The reaction mixture contained 0.2 ml aliquot of the 4000 rpm supernatant, 0.5 ml 150 mM phosphate buffer (pH 7.4) containing 5 mM EDTA, 0.1 ml (10 mM) DTT (1,4-dithiothreitol) and 0.1 ml (0.5%, w/v) N-ethylmaleimide (NEM) to remove excess DTT. The color was developed after addition of the following reagents in the reaction mixture: 0.4 ml (10%) trichloroacetic acid (TCA), 0.4 ml (44%) ortho-phosphoric acid, 0.4 ml (4%) 2,2'-bipyridine in 70% ethyl alcohol, and 0.2 ml (3%) $FeCl_3$. The mixture was then incubated at 40°C for 40 min, and the absorbance was measured at 525 nm. Ascorbic acid was used as a standard in the range of 0 to 100 µg/ml.

Determination of carotenoids

Total carotenoids were determined by the method of Jensen (1978). One gramme sample was extracted with 100 ml of 80% methanol solution and centrifuged at 4000 rpm for 30 min. The supernatant was concentrated to dryness. The residue was dissolved in 15 ml of diethyl ether and after addition of 15 ml of 10% methanolic KOH the mixture was washed with 5% ice-cold saline water to remove alkali. The free ether extract was dried over anhydrous sodium sulphate for 2 h. The ether extracts were filtered and its absorbance was measured at 450 nm by using ether as blank.

Determination of total phenol

Total phenols were recorded by Folin Ciocalteu reagent (McDonald et al., 2001). A dilute extract of each plant extract (0.5 ml of 1:10 g/ml) or gallic acid used as standard was mixed with Folin Ciocalteu reagent (5 ml, 1:10 diluted with distilled water) and aqueous Na_2CO_3 (4 ml, 1M). The mixture was allowed to stand for 10 min and the absorbance was measured by colorimetry at 765 nm. The standard curve was prepared using 0, 50, 100, 150, 200, 250 mg/l solutions of gallic acid in methanol: water (50:50, v/v). Total phenol contents were expressed in terms of gallic acid equivalent (mg/g of dry mass), which is used as a reference compound.

Statistical analysis

The statistical significance between free radical scavenging activity values of the extracts was analyzed with a Mann-Whitney U test. P values less than 0.05 were considered to be statistically significant.

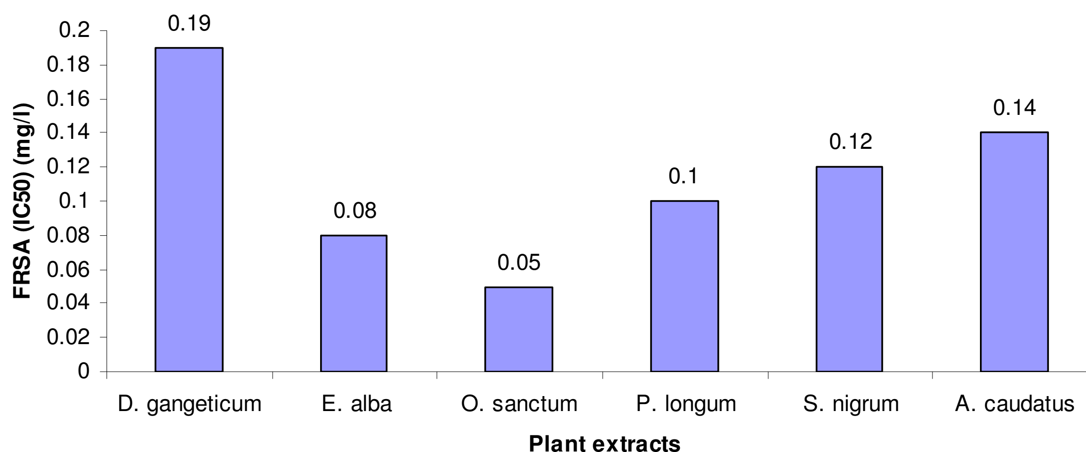

Figure 1. Free radical scavenging activity (IC_{50}) in different methanolic plant extracts.

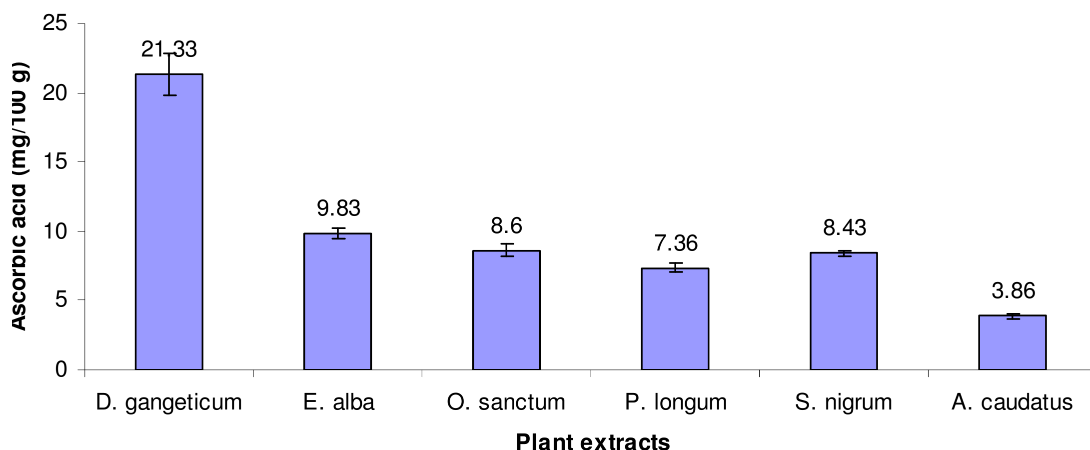

Figure 2. Ascorbic acid content in different methanolic plant extracts.

RESULTS AND DISCUSSION

In the present study several biochemical constituents and free radical scavenging activities of six medicinal plants were evaluated. Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS. Antioxidants due to their scavenging activity are useful for the management of those diseases. DPPH stable free radical method is a sensitive way to determine the antioxidant activity of plant extracts (Koleva et al., 2002; Suresh et al., 2008). Figure 1 shows the amount of each extract required for 50% inhibition of DPPH activity (IC_{50}). The free radical scavenging action of methanol extracts of plant are in the order as *Desmodium gangeticum* > *Amaranthus caudatus* > *Solanum nigrum* > *Piper longum* > *Eclipta alba* > *Ocimum sanctum*. The extracts, which showed the strongest DPPH radical scavenging activity, are *D. gangeticum* and *A. caudatus*, while the others show moderate antioxidant properties. The therapeutic potential of natural medicinal plants as an antioxidant in reducing such free radical induced tissue injury, suggests that many plants have antioxidant activities that can be therapeutically useful (Kanatt et al., 2007).

Ascorbic acid acting as a chain breaking antioxidant impairs with the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine (Beyer, 1994; Aqil et al., 2006). The quantitative determination of ascorbic acid in plant extracts shows that they are good source of ascorbic acid. High quantity of ascorbic acid was found to be 21.33 ± 1.49 mg/100g in *D. gangeticum* whereas in *A. caudatus* was recorded to have the least value of 3.86 ± 0.2 mg/100g (Figure 2). A striking pathological change resulting from ascorbic acid deficiency is the weakening of the endothelial wall of the capillaries due to a reduction in the amount of intercellular substance. Therefore, a clinical manifestation of

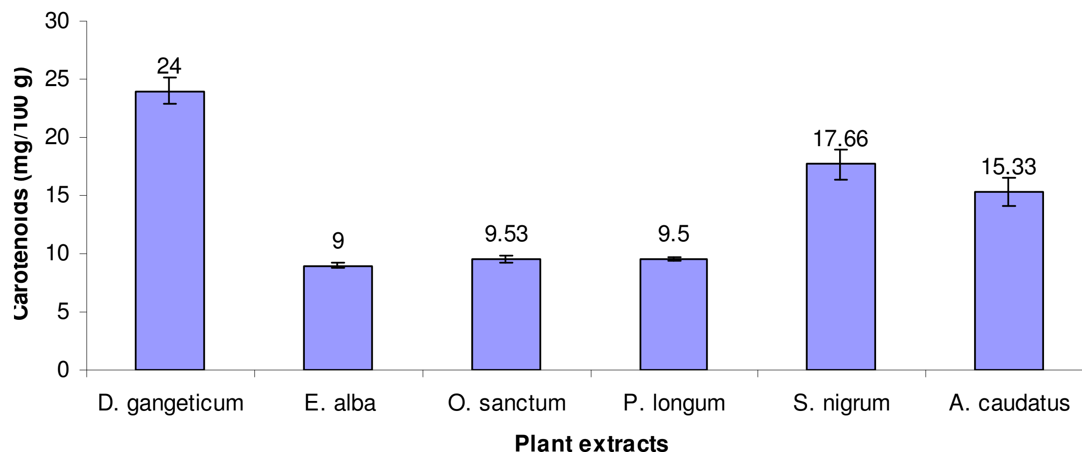

Figure 3. Carotenoids in different methanolic plant extracts.

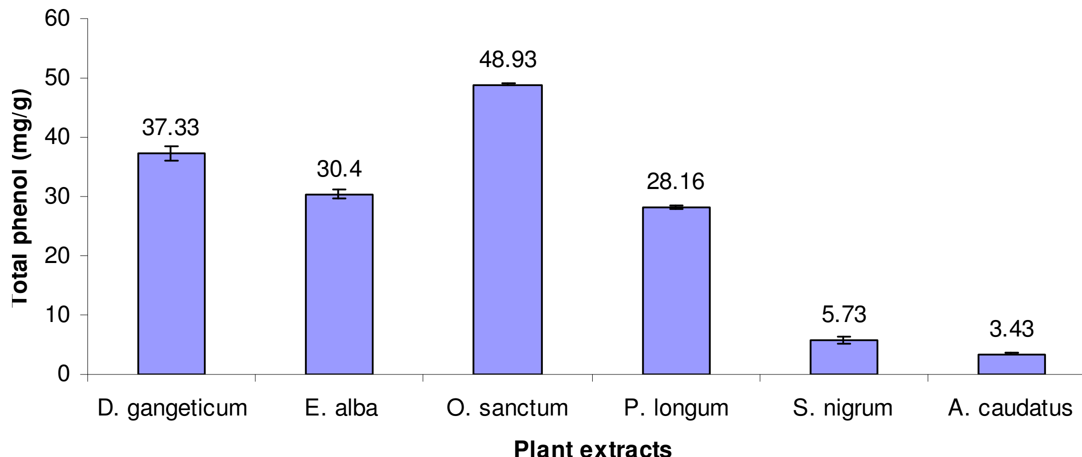

Figure 4. Total phenol content in different methanolic plant extracts.

of scurvy hemorrhage from mucous membrane of the mouth and gastrointestinal tract, anemia, pains in the joints can be related to the association of ascorbic acid and normal connective tissue metabolism (Malencic et al., 2000).

Figure 3 demonstrate the analysis of carotenoid contents in the six investigated medicinal plants. The carotenoid content was more in *D. gangeticum* (24.0 ± 1.16 mg/100g) while *E. alba*, *O. sanctum* and *P. longum* had significantly lower values as 9.0 ± 0.24 mg/100g, 9.53 ± 0.28 mg/100g and 9.5 ± 0.16 mg/100g respectively. It was proved that carotenoids have a positive role on the epithelisation process and influence the cell cycle progression of the fibroblasts (Stivala et al., 1996). Carotenoids act as photoprotective agents and may reduce the risk of sunburns, photo-allergy and even some types of skin cancer (Lee et al., 2000). The examined result shows that *D. gangeticum* is a strong source of carotenoids and it can be a promising plant for use in pharmacological products designed for antioxidant activity.

So far as plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators, it was reasonable to determine their total amount in the selected plant extracts (Cook and Samman, 1996). The content of total phenols in methanolic extracts expressed in gallic acid equivalents (GAE) varied between 3.43 ± 0.12 and 48.93 ± 0.24 mg/g (Figure 4). The phenols contain hydroxyls that are responsible for the radical scavenging effect mainly due to redox properties (Rice-Evans et al., 1997). According to our study, the high phenolic content in *O. sanctum* can explain its high free radical scavenging activity.

This study reveals that tested plant materials have moderate to significant antioxidant activity and free radical scavenging activity. The result of the present study suggests that selected plants can be used as a source of antioxidants for pharmacological preparations which is

which is very well evidenced by the present work.

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