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Antioxidant and Free Radical Scavenging Properties of Twelve Traditionally Used Indian Medicinal Plants

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Abstract: The methanolic crude extracts of 12 traditionally used Indian medicinal plants were screened for their antioxidant and free radical scavenging properties using α-tocopherol and butylated hydroxy toluene (BHT) as standard antioxidants. Antioxidant activity was measured by ferric thiocyanate (FTC) assay and compared with the thiobarbituric acid (TBA) method. Free radical scavenging activity was evaluated using diphenyl picryl hydrazyl (DPPH) radicals. The overall antioxidant activity of *Lawsonia inermis* was the strongest, followed in descending order by *Ocimum sanctum, Cichorium intybus, Piper cubeba, Punica granatum, Allium sativum, Delonix regia, Terminalia chebula, Terminalia bellerica, Mangifera indica, Camellia sinensis, and Trigonella foenum-graecum. Seven plants, namely Terminalia chebula, Mangifera indica, Terminalia bellerica, Punica granatum, Ocimum sanctum, Cichorium intybus,* and *Camellia sinensis*, showed strong free radical scavenging activity with the DPPH method. Phytochemical analysis of plant extracts indicated the presence of major phytocompounds, including phenolics, alkaloids, glycosides, flavonoids, and tannins. The phenolic concentrations in the above plants ranged from 28.66 to 169.67 mg/g of dry plant extract. A fair correlation between antioxidant/free radical scavenging activity and phenolic content was observed among 9 plants; however, in 3 plants (*Piper cubeba, Lawsonia inermis* and *Trigonella foenum-graecum*), no such relationship was observed. The tested plant extracts showed promising antioxidant and free radical scavenging activity, thus justifying their traditional use.

Key Words: Medicinal plant, antioxidant activity, free radical scavenging activity, phytocompounds, phenolics

Introduction

Reactive oxygen species (ROS) are an entire class of highly reactive molecules derived from the metabolism of oxygen. ROS, including superoxide radicals, hydroxyl radicals, and hydrogen peroxide, are often generated as byproducts of biological reactions or from exogenous factors. In vivo, some of these ROS play positive roles in cell physiology; however, they may also cause great damage to cell membranes and DNA, inducing oxidation that causes membrane lipid peroxidation, decreased membrane fluidity, and DNA mutations leading to cancer, degenerative, and other diseases (1-4).

Mammalian cells possess elaborate defense mechanisms for radical detoxification. Key metabolic steps are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX), which destroy toxic peroxides. In addition to antioxidant enzymes, non-enzymatic molecules, including thioredoxin, thiols, and disulfide-bonding play important roles in antioxidant defense systems. Some of the compounds are of an exogenous nature and are obtained from food, such as α-tocopherol, β-carotene, and ascorbic acid, and such micronutrient elements as zinc and selenium (5). If cellular constituents do not effectively scavenge free radicals, they lead to disease conditions as described above.

Antioxidant-based drugs/formulations for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease, and cancer have appeared during the last 3 decades (6). This has attracted a great deal of research interest in natural antioxidants. Subsequently, a worldwide trend towards the use of natural phytochemicals present in berry crops, tea, herbs, oilseeds, beans, fruits, and vegetables has increased (7-9).

Several herbs and spices have been reported to exhibit antioxidant activity, including rosemary, sage, thyme, nutmeg, turmeric, white pepper, chili pepper, ginger, and several Chinese medicinal plant extracts (10-13). The majority of the active antioxidant compounds are

flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, catechins, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, β-carotene, and α-tocopherol are known to possess antioxidant potential (14-16). A direct relationship between antioxidant activity and phenolic content of plant extracts has been reported (16,17). Epidemiological studies have shown that the consumption of foods and beverages rich in phenolic content can reduce the risk of heart disease (18).

Many Indian medicinal plants are considered potential sources of antioxidant compounds. In some cases, their active constituents are known. *Terminalia chebula, T. bellerica, T. muelleri*, and *Phyllanthus emblica*, all of which have antioxidant activity, showed high content of phenolics like gallic acid (19,20). Conversely, the antioxidant activity of *Hemidesmus indicus, Cichorium intybus*, *Withania somnifera*, *Ocimum sanctum, Mangifera indica*, and *Punica granatum*, as determined by several methods, has been poorly documented (19-23).

There is an increased quest to obtained natural antioxidants with broad-spectrum actions. The majority of the rich diversity of Indian medicinal plants are yet to be scientifically evaluated for such properties. Furthermore, the relationship between phenolic content and antioxidant activity is largely not examined in Indian medicinal plants. Among the above 12 medicinal plants that are commonly used in the Indian system of medicine, Ayurveda, Siddha, and Unani were selected for study. We have previously evaluated these plants for their broad-spectrum antimicrobial activity (24,25). In this report, we investigated the crude methanolic extracts of these plants for their potential antioxidant activity with the thiobarbituric acid (TBA), ferric thiocyanate (FTC), and 1, 1, diphenyl picryl hydrazyl (DPPH) radical scavenging methods.

Materials and Methods

Preparation of crude plant extracts

Test plants were collected locally or obtained from the Himalaya Drug Company, New Delhi-15. All the plant materials were further identified by the Department of Botany, Aligarh Muslim University (AMU), Aligarh. Voucher specimens of these plants were deposited in the department of Agricultural Microbiology, AMU, Aligarh. About 800 g of dried, ground plant material was soaked in 2.5 l of 98% methanol for 8-10 days, stirring every 18 h using a sterile glass rod. The final extract was passed through No.1 Whatman filter paper (Whatman Ltd., England). The filtrate obtained was concentrated under vacuum on a rotary evaporator at 40 ºC and stored at 4 °C for further use. The crude extract was obtained by dissolving a known amount of the dry extract in 98% methanol to obtain a stock solution of 40 mg/ml concentrations.

Antioxidant assay

The antioxidant activity of each plant extract was tested using the ferric thiocyanate (FTC) and thiobarbituric acid (TBA) methods. The FTC method was used to measure the amount of peroxide at the beginning of lipid peroxidation, in which peroxide will react with ferrous chloride and form ferric ions. Ferric ions will then unite with ammonium thiocyanate and produce ferric thiocyanate. The substance is red, and denser color is indicative of higher absorbance. The TBA method measures free radicals present after peroxide oxidation.

(a) Ferric thiocyanate (FTC) method

The standard method as described by Kikuzaki and Nakatani (12) was used. A mixture of 4.0 mg of plant extract in 4 ml of absolute ethanol, 4.1 ml of 2.52% linolenic acid in absolute ethanol, 8.0 ml of 0.05 M phosphate buffer (pH 7.0), and 3.9 ml of water was placed in a vial with a screw cap and then placed in a dark oven at 40 °C. To 0.1 ml of this solution were added 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate. Precisely 3 min after the addition of 0.1 ml of 0.02 M ferrous chloride in 3.5% HCl to the reaction mixture, the absorbance of red color was measured at 500 nm every 24 h until one day after the absorbance of control reached its maximum. Butylated hydroxy toluene (BHT) and α-tocopherol were used as positive controls, while a mixture without a plant sample was used as the negative control.

(b) Thiobarbituric acid (TBA) method

The method of Ottolenghi (26) was followed. Two milliliters of 20% trichloroacetic acid and 2 ml of 0.67% 2-thiobarbituric acid were added to 1 ml of sample solution, prepared with the FTC method. The mixture was placed in a boiling water bath and after cooling was centrifuged at 3000 rpm for 20 min. Absorbance of supernatant was measured at 552 nm. Antioxidant activity was based on the absorbance on the final day of the FTC method.

Free radical scavenging assay

The scavenging activity of DPPH free radicals by different plant extracts was determined according to the method reported by Gyamfi (27). Fifty microliters of the plant extract in methanol, yielding 100 µg/ml in each reaction, was mixed with 1 ml of 0.1 mM DPPH in methanol solution and 450 µl of 50 mM Tris-HCl buffer (pH 7.4). Methanol (50 µl) only was used as the experimental control. After 30 min of incubation at room temperature, the reduction in the number of DPPH free radicals was measured, reading the absorbance at 517 nm. BHT and α-tocopherol were used as controls. The percent inhibition was calculated from the following equation:

% Inhibition = [Absorbance of control – Absorbance of test sample/Absorbance of control] × 100

Phytochemical analysis

Phytochemical analysis of the major phytoconstituents of the plant extracts was undertaken using standard qualitative methods (color tests and/or TLC) as described earlier (24).

Total phenolic compound analysis

The amounts of phenolics in the selected Indian medicinal plant extracts were determined with Folin-Ciocalteu reagent using the method of Spanos and Wrolstad (28), as modified by Lister and Wilson (29). To 50 ml of each sample (3 replicates), 2.5 ml of 10% dilution of Folin-Ciocalteu reagent and 2 ml of Na_2CO_3 (7.5%, w/v) were added and the resulting mixture was incubated at 45 °C for 15 min. The absorbance of all samples was measured at 765 nm using a Spectronic 20D$^+$. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

Results and Discussion

Reactive oxygen species (ROS), from both endogenous and exogenous sources, may be involved in the etiologies of such diverse human diseases as arteriosclerosis, ischemic injury, cancer, and neurodegenerative diseases, as well as in processes like inflammation and ageing (5,30,31). There is evidence that indigenous antioxidants may be useful in preventing the deleterious consequences of oxidative stress and there is increasing interest in the protective biochemical functions of natural antioxidants contained in spices, herbs, and medicinal plants (32,33). Our attention has been focused, in particular, on the parts of 12 commonly used Indian medicinal plants.

The antioxidant activities of the plant extracts were measured by the FTC method and compared with the TBA method at a concentration of 0.02% in methanolic solutions. The plant extracts tested showed low absorbance values, which indicated a high level of antioxidant activity. None of the plant extracts showed absorbance values greater than the negative controls (without plant extracts) at the end point of both methods, indicating the presence of antioxidant activity. However, all the plant extracts exhibited strong antioxidant activity as determined by both the FTC and TBA methods, surpassing the activity of the standard commercial antioxidants, alpha-tocopherol, and butylated hydroxy toluene (Figures 1-4).

The extracts of *Delonix regia* (flowers), *Terminalia bellerica* (fruits), *Terminalia chebula* (fruits), *Ocimum sanctum* (leaves), and *Camellia sinensis* (leaves) and some other plants exerted good antioxidant activity by various methods. The antioxidant activity detected with the TBA method was higher than that detected with the FTC method. This might suggest that the amount of peroxide in the initial stage of lipid peroxidation was less than the amount of peroxide in the secondary stage. Furthermore, the secondary product was much more stable for a period of time.

Free radicals have been implicated in many disease conditions, the important ones being superoxide radicals, hydroxy radicals, peroxyl radicals, and single oxygen. Herbal drugs containing free radical scavengers are gaining importance in treating such diseases. Many plant extracts exhibit efficient antioxidant properties due to their phytoconstituents, including phenolics (34). In the present experiment, methanolic extracts of 12 plants were evaluated for their free radical scavenging activity using the DPPH radical assay. Reduction of DPPH radicals can be observed by the decrease in absorbance at 516 nm. Different plant extracts reduced DPPH radicals significantly. Values of percent decolorization of DPPH radicals are shown in Table 1. Of the 12 plants tested, 7 plants, namely *Mangifera indica, Punica granatum, Terminalia bellerica, Terminalia chebula, Cichorium intybus, Ocimum sanctum*, and *Camellia sinensis* showed more than 70% decolorization. The activity of *Trigonella foenum graecum* (57%) was at par when compared with

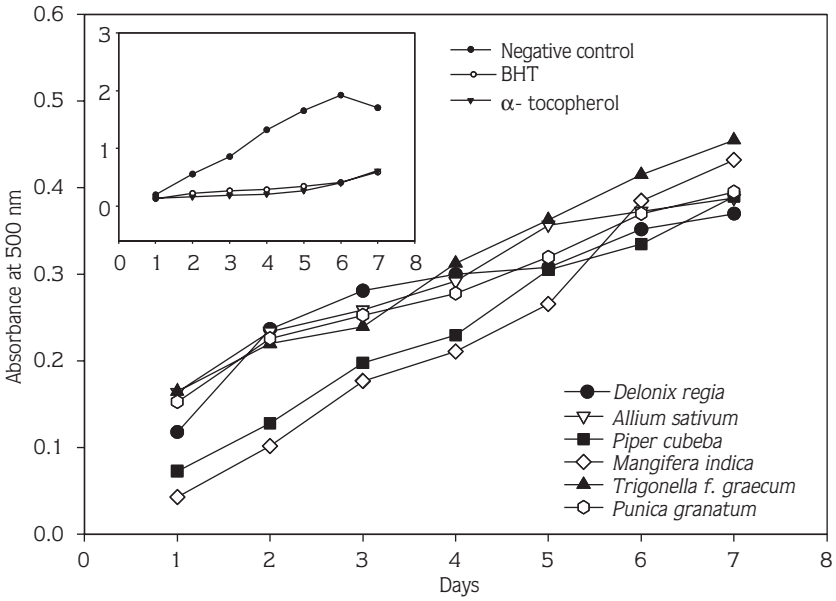

Figure 1. Antioxidant properties of plant extracts determined with the FTC method.

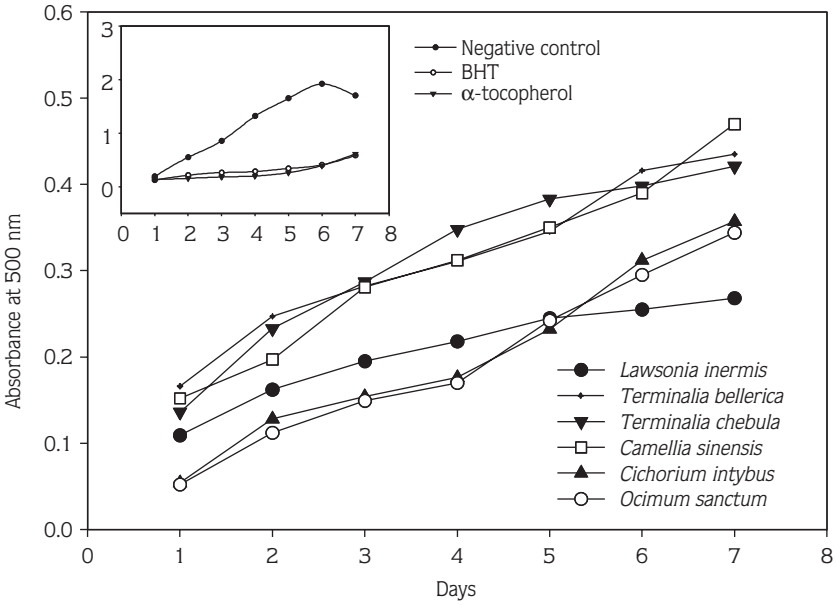

Figure 2. Antioxidant properties of plant extracts determined with the FTC method.

the commercial antioxidants α-tocopherol (58%) and butylated hydroxy toluene (49%), while the extract of *Allium sativum* showed negligible activity.

Some variations in the extent of extract antioxidant activity were observed for each type of assay used in this study. The extracts of *Terminalia bellerica*, *Terminalia chebula*, and *Mangifera indica* had good DPPH radical scavenging activity, but low lipid peroxidation, while the extracts of *Camellia sinensis*, *Cichorium intybus*, and *Ocimum sanctum* had relatively high antioxidant potential according to the FTC method. These differences may be due to their different antioxidant mechanisms; however, in some of the above plant extracts, the active constituents are known, such as gallic acid (*T. chebula*)

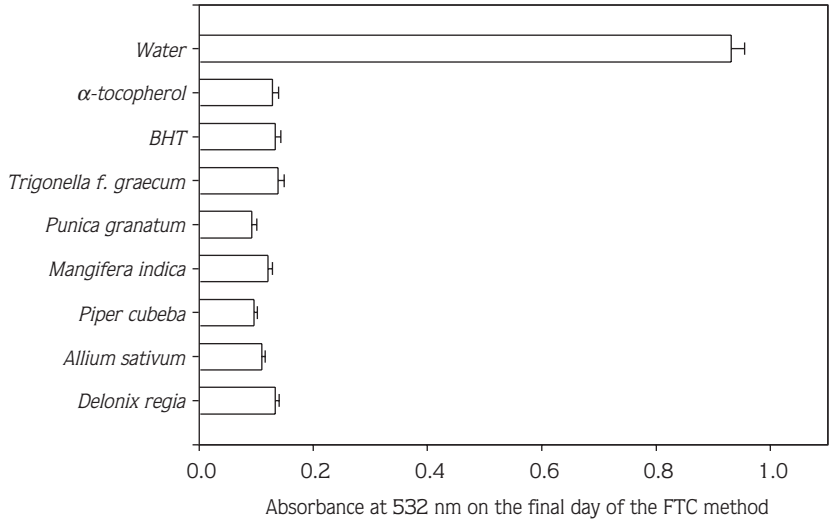

Figure 3. Antioxidant activities of plant extracts determined with the TBA method.

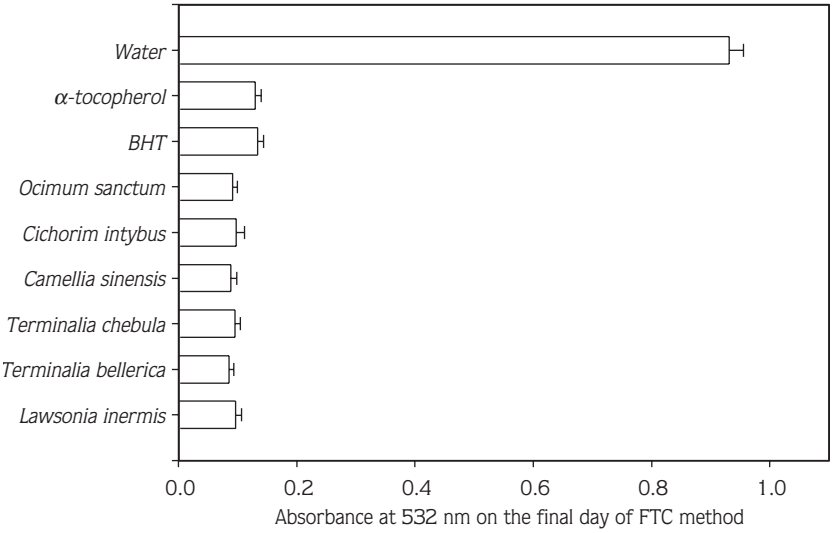

Figure 4. Antioxidant activities of plant extracts determined with the TBA method.

and catechins (*Camellia sinensis*). Our findings on the antioxidant activity of medicinal plants correlated with the reports of others (19-23).

A fair correlation between total phenolic content and antioxidant activity was observed in 9 of the 12 plants, whereas no direct relationship could be detected in *Piper cubeba, Lawsonia inermis*, and *Trigonella foenum-graecum* extracts. These observations clearly indicated a close linkage between phenolics and antioxidant activity; however, due to the phytochemical diversity in the antioxidant phytocompounds, the above variation is expected.

The phytochemical analysis of the crude extracts indicated the presence of major phytocompounds, including phenolics, alkaloids, glycosides, flavonoids, and tannins (Table 1), which may have been responsible for the observed antioxidant activity. Our results further support the view that some traditionally used Indian

Table 1. Free radical scavenging activity of Indian medicinal plants determined with the DPPH method, and total phenolic contents.

S. No.	Name of the plant/ Family	Common Name	Part used	% Decolorization ± SD	*Phytocompounds detected	**Total phenolic contents ± SD
1.	*Allium sativum* L. (Alliaceae)	Lesan	Bulb	7.97 ± 1.09	P, T	28.66 ± 2.52
2.	*Camellia sinensis* L. (Theaceae)	Tea	Leaves	69.95 ± 3.62	P, G, S	163.33 ± 7.37
3.	*Cichorium intybus* L. (Compositae)	Chicory	Roots	73.34 ± 4.87	A, P, F, G, T	76.83 ± 8.75
4.	*Delonix regia* Gamble. (Leguminosae)	Gulmohar	Flowers	71.93 ± 3.86	A, P, F, G	169.67 ± 11.23
5.	*Lawsonia inermis* L. (Lythraceae)	Heena	Leaves	67.67 ± 5.48	A, P, G, S	38.67 ± 4.51
6.	*Mangifera indica* L. (Anacardiaceae)	Aam	Leaves	78.14 ± 3.63	F, G	135.00 ± 9.54
7.	*Ocimum sanctum* L. (Labiatae)	Tulsi	Leaves	73.70 ± 5.87	P, G, S	80.00 ± 8.18
8.	*Piper cubeba* L. (Piperaceae)	Kabab chini	Seeds	66.20 ± 3.20	P, F	42.83 ± 3.75
9.	*Punica granatum* L. (Punicaceae)	Anar	Rind	75.50 ± 3.90	A, P, F, G	122.00 ± 6.24
10.	*Terminalia bellerica* Roxb. (Combretaceae)	Bahera	Fruit	76.29 ± 3.06	P, F, G, S, T	111.67 ± 19.29
11.	*Terminalia chebula* Retz. (Combretaceae)	Harir	Fruit	85.36 ± 2.44	P, G, S	166.33 ± 18.01
12.	*Trigonella foenum-graecum* L. (Leguminosae)	Methi	Leaves	57.45 ± 2.44	A, F, G	74.33 ± 5.13
13.	BHT	----	----	49.28 ± 2.10		
14.	α-tocopherol	----	----	58.16 ± 4.13		

*Phytocompounds Key: A, Alkaloids; P, Phenols; F, Flavonoids; G, Glycosides, S, Saponins; T, Tannins
**mg/g of dry plant extracts

medicinal plants are promising sources of potential antioxidants. Further study will be aimed at isolating and identifying the substances responsible for the antioxidant activity of plant extracts, which may be further exploited in herbal formulations.

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