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Antioxidant Activity of Some Common Plants

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Abstract: The methanolic crude extracts of some commonly used medicinal plants 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 green tea (*Camellia sinensis* Linn.) was the strongest, followed in descending order by black tea (*Camellia sinensis* Linn.), *Eugenia caryophyllus* (Spreng.) Bullock and Harrison, *Piper cubeba* Linn., *Zingiber officinale* Roscoe and *Piper nigrum* Linn. *Trigonella foenum graecum* Linn. and *Elettaria cardamomum* (Linn.) Maton showed weak free radical scavenging activity with the DPPH method. All the methanolic extracts exhibited antioxidant activity significantly. The IC_{50} of the methanolic extracts ranged between 6.7 ± 0.1 and 681.5 ± 8.4 µg/ml and that of ascorbic acid was 8.9 ± 0.1 µg/ml. The study reveals that the consumption of these spices would exert several beneficial effects by virtue of their antioxidant activity.

Key Words: *Camellia sinensis* Linn., *Zingiber officinale* Roscoe, *Trigonella foenum-graecum* Linn., *Eugenia caryophyllus* (Spreng.) Bullock and Harrison, *Piper nigrum* Linn., *Elettaria cardamomum* (Linn.) Maton, *Piper cubeba* Linn., antioxidant, DPPH, free radical scavenging activity

Bazı Bitkilerin Antioksidant Aktiviteleri

Özet: Çok sık kullanılan bazı tıbbi bitkilerin metanolik ham özütleri ile serbest radikal süpürme aktivitelerii standart antioksidant olarak askorbik asit kullanılarak incelenmiştir. Serbest radikal süpürücü aktivite için 1,1-difenil-2-pikril-hidrazil serbest radikali kullanılmıştır. Antioksidant aktivitesi en yüksek, yeşil çayda (*Camellia sinensis* Linn.) gözlenirken, siyah çay (*Camellia sinensis* Linn.), *Eugenia caryophyllus* (Spreng.) Bullock ve Harrison, *Piper cubeba* Linn., *Zingiber officinale* Roscoe ve *Piper nigrum* Linn, onu azalarak takip etmişlerdir. DPPH yöntemi ile, *Trigonella foenum graecum* Linn. ve *Elettaria cardamomum* (Linn.) Maton, zayıf serbest radikal süpürücü aktivite göstermiştir. Bütün metanolik özütler önemli ölçüde antioksidant aktivite göstermiştir. Metanolik özütün IC_{50} değeri 6.7 ± 0.1 ile 681.5 ± 8.4 µg/ml arasında değişirken, askorbik asitin değeri 8.9 ± 0.1 µg/ml bulunmuştur. Bu çalışma ile incelenen bitkilerin verimli antioksidant aktivite özelliği gösterdikleri açığa çıkarılmıştır.

Anahtar Sözcükler: *Camellia sinensis* Linn., *Zingiber officinale* Roscoe, *Trigonella foenum-graecum* Linn., *Eugenia caryophyllus* (Spreng.) Bullock and Harrison, *Piper nigrum* Linn., *Elettaria cardamomum* (Linn.) Maton, *Piper cubeba* Linn., antioxidant, DPPH, serbest radikal süpürücü aktivite

Introduction

In response to the increased popularity and greater demand for medicinal plants, a number of conservation groups are recommending that wild medicinal plants be brought into cultivation. Ethnopharmacological surveys conducted among herbal practitioners of traditional Arab medicine in Palestine and the Middle East have revealed that a large number of indigenous plant species are being used as a source of herbal therapies.

A large number of medicinal plants and their purified constituents have shown beneficial therapeutic potentials. Various herbs and spices have been reported to exhibit antioxidant activity, including *Ocimum sanctum, Piper cubeba* Linn., *Allium sativum* Linn., *Terminalia bellerica, Camellia sinensis* Linn., *Zingiber officinale* Roscoe and several Indian and Chinese plants. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins (1).

Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer (2).

Green tea and black tea leaves are obtained from dried leaves of *Camellia sinensis* Linn., belonging to the family Theaceae. Steaming or drying fresh tea leaves at elevated temperature makes the commercial green tea. Its chemical composition is similar to that of fresh tea leaves. Green tea contains polyphenols, which include flavonols, flavandiols, flavonoid and phenolic acids; these compounds may account for up to 30% of the dry weight. Most of the green tea catechins are (-) epicatechin-3-gallate, (-)-epigallocatechin, (-)-epigallocatechin-3-gallate, (-)-epicatechin, and (+) catechin. Caffeine, theobromine and theophylline, the principle alkaloids, account for about 4% of the dry weight. In addition, there are phenolic acids such as gallic acids and characteristic amino acids such as theanine (3).

Piper species, commonly used in diet and traditional medicine, were assessed for their antioxidant potential. Catalase activity predominated in *Piper longum* Linn., followed by *Piper cubeba* Linn., green pepper, *Piper brachystachyum* Linn. and *Piper nigrum* Linn. Black pepper (*P. nigrum* Linn.) was richest in glutathione peroxidase and glucose-6-phosphate dehydrogenase, green pepper was richest in peroxidase and vitamin C, while vitamin E was greater in *P. longum* Linn. and *P. nigrum* Linn. *P. brachystachyum* Linn. and *P. longum* Linn. were rich sources of vitamin A (4).

The antioxidant and radical scavenging activities of black pepper (*Piper nigrum* Linn.) seeds have been well reported (5). Both water extract and ethanol extract of black pepper exhibited strong antioxidant activity. Antimicrobial (6), larvicidal (7) and anti-cancer (8) activities of *Piper nigrum* Linn. have been reported.

The essential oil of clove flower buds (*Eugenia caryophyllus* (Spreng.) Bullock and Harrison) has been used as local anesthetic for ages. The antimicrobial, anticonvulsant, ovicidal and adulticidal effects against *Pediculus capitis* have been reported. The cloves are a commonly used household spice. Antioxidant, superoxide, dismutase, and catalase activities of *Piper cubeba* Linn. have been reported (1,4,10).

Cardamom spice consists of whole or ground dried fruit of *Elettaria cardamomum* (Linn.) Maton, a herbaceous perennial of the ginger family (Zingiberaceae) containing essential oil. It has been traditionally used to treat skin condition and in digestion. Cardamon oil is also used in cosmetics because of its cooling properties and because its pale to colorless liquid can be easily incorporated into different solutions (11).

Spices and herbs are recognized as sources of natural antioxidants and thus play an important role in the chemoprevention of diseases and aging. The ethnobotanical review of the uses of plants in Jordan led us to investigate and compare the antioxidant activity of these medicinally important plants like *Camellia sinensis* Linn. *Zingiber officinale* Roscoe, *Trigonella foenum-graecum* Linn., *Eugenia caryophyllus* (Spreng.) Bullock and Harrison, *Piper nigrum* Linn., *Elettaria cardamomum* (Linn.) Maton, and *Piper cubeba* Linn., which are available and commonly used by natives as herbal drink and for herbal therapies. Free radical scavenging activity was evaluated in vitro using 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical.

Materials and Methods

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. All other chemicals used were of analytical grade.

Preparation of crude plant extract

Test plants were collected locally or obtained from the local market. Plant material consisting of mature leaves of green tea and black tea (*Camellia sinensis* Linn.) and fruits of *Elettaria cardamomum* (Linn.) Maton (cardamom), *Piper nigrum* Linn. (black pepper), *Piper cubeba* Linn. (sweet black pepper), *Trigonella foenum-graecum* Linn. (fenugreek) and *Eugenia caryophyllus* (Spreng.) Bullock and Harrison (cloves) were collected and dried. Fresh rhizomes of ginger (*Zingiber officinale* Roscoe) were also collected and dried. The dried plant materials were powdered using a grinder. The extraction was done at room temperature. About 100 g of dried, ground plant materials were soaked in methanol (1 L of 98%) for 5-7 days separately. The soaked material was stirred every 18 h using a sterilized glass rod. The final extracts were passed through Whatman filter paper No.1 (Whatman Ltd., England). The filtrates obtained were concentrated under vacuum on a rotary evaporator at 40 ^{o}C and stored at 4 ^{o}C for further use. The stock solution of crude extracts (5 mg/ml) was prepared by dissolving a known amount of dry extract in 98% methanol. The working solutions (1, 2, 4, 6, 8, 10, 15, 25, 50, 75, 100, 250, 500 and 750 µg/ml) of the extracts were prepared from the stock solution using suitable dilution.

Preliminary phytochemical screening of extract

The methanolic extract was tested by applying general chemical tests (12) for alkaloids, glycosides, reducing sugars, tannins, fixed oils and fats, proteins and free amino acids. Green tea and black tea gave positive test with Murexide reagent, and negative test with Dragendorff's reagent. Cardamom and clove gave positive test with Dragendorff's reagent. Fenugreek, ginger and black pepper gave positive test with Meyer's reagent.

Antioxidant activity (DPPH free radical scavenging activity) of methanolic extract

The antioxidant activity of the plant extracts and the standard was assessed on the basis of the radical scavenging effect of the stable 1, 1-diphenyl-2-picryl-hydrazyl (DPPH)-free radical activity by modified method (13). The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1-100 µg/ml solution. 0.002% of DPPH was prepared in methanol and 1 ml of this solution was mixed with 1 ml of sample solution and standard solution separately. These solution mixtures were kept in dark for 30 min and optical density was measured at 517 nm using Cecil-Elect Spectrophotometer. Methanol (1 ml) with DPPH solution (0.002%, 1 ml) was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below (14):

$$\text{Percent (\%) inhibition of DPPH activity} = \frac{A - B}{A} \times 100$$

Where A = optical density of the blank and B = optical density of the sample.

Results and Discussion

Natural antioxidants that are present in herbs and spices are responsible for inhibiting or preventing the deleterious consequences of oxidative stress. Spices and herbs contain free radical scavengers like polyphenols, flavonoids and phenolic compounds. In the present paper, we have evaluated the free radical scavenger activity of methanolic extract of green and black tea leaves *(Camellia sinensis* Linn.), powdered rhizomes of ginger (*Zingiber officinale* Roscoe), seeds of fenugreek (*Trigonella foenum-graecum* Linn.), cloves buds (*Eugenia caryophyllus* (Spreng.) Bullock and Harrison), black pepper (*Piper nigrum* Linn.), cardamom (*Elettaria cardamomum* (Linn.) Maton and sweet pepper (*Piper cubeba* Linn.).

Among the eight extracts and standard tested for the in vitro antioxidant activity using the DPPH method, the crude methanolic extracts of green tea, black tea (*Camellia sinensis* Linn.), *Eugenia caryophyllus* (Spreng.) Bullock and Harrison, *Piper cubeba* Linn., *Zingiber officinale* Roscoe and *Piper nigrum* Linn. showed antioxidant activity, with IC_{50} values of 6.7 ± 0.1, 9.7 ± 0.1, 9.9 ± 0.2, 11.3 ± 0.3, 65.1 ± 1.7, 144.1 ± 2.2 µg/ml, respectively (Table). *Trigonella foenum graecum* Linn. and *Elettaria cardamomum* (Linn.) Maton showed weak antioxidant activity, with IC_{50} values of 444.1 ±

Table. In vitro antioxidant activity of methanolic extract of spices.

Test compound (methanolic extract)	IC_{50} (µg/ml) (Mean ± SD)	Weight of extract (g %, w/w)
Green tea (*Camellia sinensis* Linn.)	6.7 ± 0.1	22.4
Eugenia caryophyllus (Spreng.) Bullock and Harrison	9.9 ± 0.2	23.9
Zingiber officinale Roscoe	65.1 ± 1.7	9.2
Trigonella foenum graecum Linn.	444.1 ± 5.5	9.7
Black tea (*Camellia sinensis* Linn.)	9.7 ± 0.1	13.3
Elettaria cardamomum (Linn.) Maton	681.5 ± 8.4	8.1
Piper nigrum Linn.	144.1 ± 2.2	9.2
Piper cubeba Linn.	11.3 ± 0.3	9.5
Ascorbic acid	8.9 ± 0.1	-

5.5 and 681.5 ± 8.4 µg/ml, respectively. The IC_{50} value for ascorbic acid was 8.6 ± 0.1 µg/ml. The results indicate that the antioxidant activity of the crude extract of green tea is higher than that of ascorbic acid. The antioxidant activity of black tea (*Camellia sinensis* Linn.), *Eugenia caryophyllus* (Spreng.) Bullock and Harrison, *Piper cubeba* Linn., and *Zingiber officinale* Roscoe was nearly the same when compared to ascorbic acid. However, the other extracts from *Trigonella foenum graecum* Linn. and *Elettaria cardamomum* (Linn.) Maton were found to be less active than ascorbic acid since their IC_{50} values were found to be higher (Table). The antioxidant activity is presented in the Figure, which showed that the percentage inhibition of 10 µg/ml of green tea (*Camellia sinensis* Linn.) extract was 69.4%, which is comparable with the standard antioxidant activity of ascorbic acid (55.8%). The free radical scavenging activity of methanolic extract was confirmed in the present investigation.

However, the chemical constituents present in the extract, which are responsible for this activity, need to be investigated, and it is obvious that the constituents like tannins, reducing sugars and proteins present in the extract may be responsible for such activity. The phytochemical tests indicated the presence of alkaloids, glycosides, tannins, and flavonoids in the crude methanolic extract. Several of such compounds are known to possess potent antioxidant activity (15). Some of these constituents have already been isolated from this plant. Hence, the observed antioxidant activity may be due to the presence of any of these constituents. The plant exhibited strong anticancer, hepato-protective, anti-viral and several other activities. These properties may be due to its antioxidant activity. The crude methanolic extract merits further experiments in vivo.

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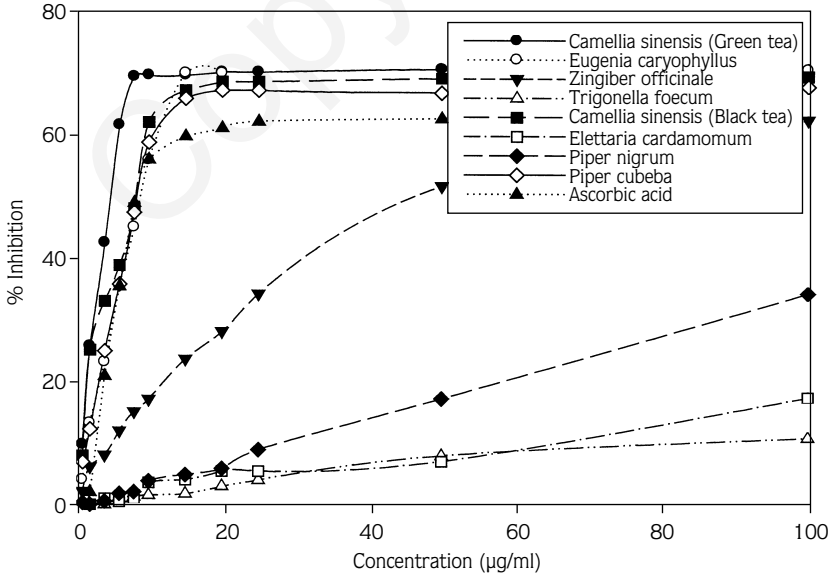

Figure. DPPH free radical scavenging activity of standard ascorbic acid and methanolic plant extracts.

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