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Comparative Antioxidant Activity of Some Edible Plants

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Abstract: *Terminalia chebula, Salvia officinalis* L., *Calamintha incana, Thymus vulgaris* L., *Matricaria chamomilla*, and *Nigella sativa* are commonly used spices. In the present study the methanolic extracts of these spices were evaluated for their antioxidant activity by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging method. All the methanolic extracts showed significant antioxidant activity. The IC_{50} of the methanolic extracts ranged between 3.8 ± 0.1 and 168.8 ± 2.1 µg/ml, and that of ascorbic acid was 8.6 ± 0.1 µg/ml. The study revealed that the consumption of these spices could exert several beneficial effects by virtue of their antioxidant activity.

Key Words: *Terminalia chebula, Salvia officinalis* L., *Calamintha incana, Thymus vulgaris* L., *Matricaria chamomilla, Nigella sativa*, antioxidant activity, DPPH, ascorbic acid, methanolic extract

Bazı Yenilebilen Bitkilerin Antioksidant Aktivitelerinin Karşılaştırılması

Özet: *Terminalia chebula, Salvia officinalis* L., *Calamintha incana, Thymus vulgaris* L., *Matricaria chamomilla* ve *Nigella sativa* baharat olarak sık kullanılan bitkilerdir. Bu çalışmamızda bu baharatların metanolik özütleri 1,1-difenil-2-pikrill-hidrazil serbest radikal süpürücü etkisi yöntemi ile antioksidant etkileri incelenmiştir. Metanolik özütlerin tamamı önemli ölçüde antioksidant aktivite göstermişlerdir. Metanolik özütlerin IC_{50} değerleri 3,8 ± 0,1 ile 168,8 ± 2,1 µg/ml arasında değişirken askorbik asidin değeri ise 8,6 ± 0,1 µg/ml olarak ölçülmüştür. Bu baharatların kullanımı antioksidant aktiviteleri nedeniyle insanlara bir çok faydaları olabilir.

Anahtar Sözcükler: *Terminalia chebula, Salvia officinalis* L., *Calamintha incana, Thymus vulgaris* L., *Matricaria chamomilla, Nigella sativa*, antioksidan aktivite, DPPH, askorbik asit, methanolic özüt

Introduction

A large number of medicinal plants and their purified constituents have been shown to have beneficial therapeutic potential. Natural antioxidants may function as (a) reducing agents, (b) free radical scavengers, (c) complexes of pro-oxidant metals, and (d) quenchers of singlet oxygen. They can be used in the food industry and there is evidence that they may exert antioxidant effects within the human body (1). The majority of their antioxidant activity is due to flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins, and isocatechins (2). The name salvia comes from the lain work meaning 'to heal'. Some species of *Salvia* have been cultivated worldwide for use in folk medicine and for culinary purposes (3). The dried root of *Salvia miltiorrhiza* (danshen or red sage root) has been used extensively for the treatment of coronary diseases, hepatitis, chronic renal failure, and ulcers (4). In Turkish folk medicine, an infusion of *Salvia* spp. herbs was investigated for its antioxidant properties and for treating the common cold, abdominal pain, and stomach disorders (5,6). On the other hand, *Salvia officinalis* (sage), a well-known herbal plant, is used for the treatment of various ailments, in addition to having antiseptic and antispasmodic properties (7). In Jordan and the Middle East, sage is used for fever, digestive disorders, and stomach ache. Oral administration of a *Salvia officinalis* infusion improved the liver antioxidant status in mice and rats (8). The methanolic extract of *Salvia officinalis* L. leaves showed significant inhibitory effects on serum triglyceride elevation in olive oil loaded mice and rats (9). Caffeic acid, rosmarinic acid, and oligomers of caffeic acid, with multiple catechol groups, are all constituents of *Salvia officinalis*. Antioxidant activity of the chemical components of sage (*Salvia officinalis* L.) and thyme

(*Thymus vulgaris* L.) has been reported (10,11). In addition to immunomodulatory properties, the seeds of *Nigella sativa*, commonly known as black seed, black cumin, or "Habatul-Barakah", are used all over the world for the treatment of asthma, headache, obesity, back pain, hypertension, gastrointestinal problems, diarrhea, and dyslipidemia (12). *Matricaria recutita* L., or *Matricaria chamomilla* (chamomile), is one of the most popular single-ingredient herbal teas (13). Essential oils obtained from fresh or dried flower heads have an aromatic flavor, coloring properties, and anti-streptococcal and antioxidant activity (14). Commercial chamomile products, including soaps, perfumes, ointments, and herbal teas are readily available.

Powdered triphala (three fruits) is a widely used herbal formulation that contains equal proportions of dried fruit powder of *Emblica officinalis, Terminalia chebula*, and *Terminalia bellirica*. In the Indian system of medicine it is used for cleaning wounds, urinary disorders, diabetes mellitus, leprosy, constipation, eyesight promotion, piles, and as a rejuvenator.

There is emerging public interest in using crude plant extracts, such as green tea infusion for self-medication. In a normal diet the intake of herbs may contribute significantly to the total intake of plant antioxidants, which may even exceed the dietary intake of antioxidants from many other food groups. As such, the use of what we know as traditional medicine has generated a demand for therapeutic alternatives in Asia, Latin America, the Caribbean, and industrialized countries. The consumption of foodstuffs rich in antioxidants provides protection against aging, cancer, and cardiovascular and cerebrovascular diseases. This protection can be explained by the capacity of these antioxidants to scavenge free radicals, which are responsible for oxidative damage to lipids, proteins, and nucleic acids. An ethnobotanical review of the uses of plants in Jordan led us to investigate some edible and wound-healing herbs that are available and used by natives as herbal drinks and for herbal therapies. Free radical scavenging activity was evaluated in vitro using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) free radical scavenging method.

Materials and Methods

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 a local market. Plant material consisted of mature leaves of *Salvia officinalis, Thymus vulgaris, Calamintha incana,* and *Matricaria chamomilla*, and seeds of *Nigella sativa* and *Teminalia chebula*, which were collected and dried. Extraction was performed at room temperature. About 200 g of dried, ground plant material were soaked in 2 l of 98% methanol for 5-7 days, stirring every 18 h using a sterilized glass rod, separately. The final extracts were passed through No. 1 Whatman filter paper (Whatman Ltd., UK). The filtrates obtained were concentrated under vacuum in a rotary evaporator at 40 °C and stored at 4 °C for further use. The crude extracts were obtained by dissolving a known amount of dry extract in 98% methanol to obtain a stock solution of 5 mg/ml. The stock solutions were serially diluted with the respective solvents to obtain lower dilutions (1, 2, 4, 6, 8, 10, 15, 25, 40, 50, 75, 100, 250, and 500 µg/ml).

Preliminary Phytochemical Screening of the Extracts

Methanolic extracts were screened by applying general chemical tests (15) for alkaloids, glycosides, reducing sugars, tannins, fixed oils and fats, proteins, and free amino acids.

Antioxidant Activity (DPPH Free Radical Scavenging Activity) of Methanolic Extract

Antioxidant activity of the plant extracts and the standards was assessed on the basis of the radical scavenging effect of the stable DPPH free radical, using a modified method (16). The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as the standard in solutions ranging from 1 to 100 µg/ml. We prepared 0.002% DPPH in methanol. Then 1 ml of this solution was mixed with 1 ml of sample solution and the standard solution to be tested separately. These solution mixtures were kept in the dark for 20 min and optical density was measured at 517 nm using a Cecil spectrophotometer against methanol.

The blank was used as 1 ml of methanol with 1 ml of DPPH solution (0.002%). The optical density was recorded and percent of inhibition was calculated using the formula given below (17):

% of inhibition of DPPH activity = A–B/A × 100 where A is optical density of the blank and B is optical density of the sample.

Statistics and IC_{50}

Decolorization was plotted against the sample extract concentration and a linear regression curve was established in order to calculate IC_{50} (µg/ml), which is the amount of sample required to decrease the absorbance of the DPPH free radical by 50%. All the analyses were carried out in triplicate and the results are expressed as mean ± SD. Statistical analyses were performed using GraphPad InStat™ v.2.02.

Results and Discussion

Polyphenols, phenolic compounds, flavonoids, and terpenes are well known for their antioxidant activity. Preliminary phytochemical screening indicated the presence of phenolic compounds and flavonoids in the methanolic extracts of *Salvia officinalis, Calamintha incana, Thymus vulgaris, Matricaria chamomilla*, *Terminalia chebula*, and *Nigella sativa*. Among the 6 extracts and the standard tested for in vitro antioxidant activity using the DPPH method, the crude methanolic extracts of *Terminalia chebula, Salvia officinalis, Calamintha incana, Thymus vulgaris, Matricaria chamomilla*, and *Nigella sativa* showed antioxidant activity with IC_{50} values of 3.8 ± 0.1, 17.7 ± 1.5, 23.1 ± 1.1, 54.2 ± 1.8, 65.8 ± 1.4, and 168.8 ± 2.1 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 *Terminalia chebula* was higher than that of ascorbic acid; however, the other extracts were less active than ascorbic acid, as their IC_{50} values were higher (Table 1). Antioxidant activity is presented in the Figure and shows that the percent inhibition of 10 µg/ml of *Terminalia chebula* extract was 71.56%, which is comparable with the standard antioxidant activity of ascorbic acid (57.0%). The free radical scavenging activity of the methanolic extract was confirmed in the present investigation. It was observed

Table 1. In vitro antioxidant activity of the methanolic extracts.

Test compound (methanolic extract)	IC_{50} (µg/ml) (Mean ± SD)	Weight of extract (g %, w/w)
Salvia officinalis	17.7 ± 1.5	2.26
Nigella sativa	168.8 ± 2.1	3.25
Matricaria chamomilla	65.8 ± 1.4	3.25
Terminalia chebula	3.8 ± 0.1	22.05
Thymus vulgaris	54.2 ± 1.8	5.11
Calamintha incana	23.1 ± 1.1	4.04
Ascorbic acid (*aq.*)	8.6 ± 0.1	-

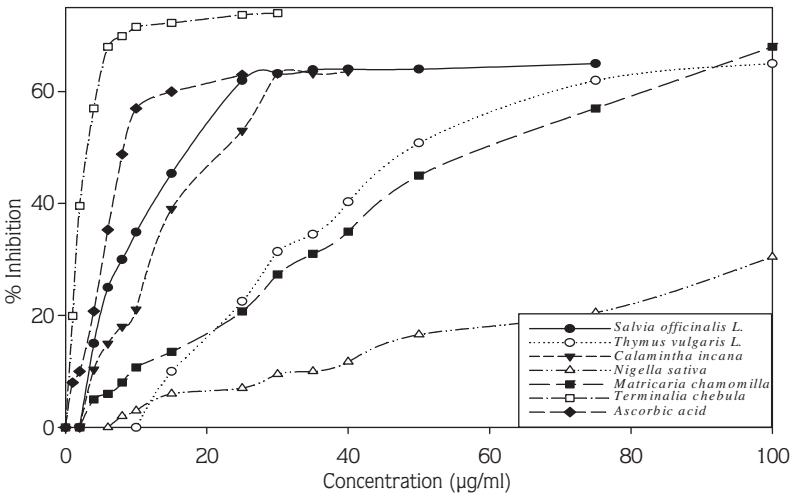

Figure. DPPH free radical scavenging activity of the standard, ascorbic acid, and methanolic plant extracts.

that a very small amount of *Teminalia chebula* seed extract was able to produce 71.56% inhibition. The antioxidant activity of the methanolic extracts of *Salvia officinalis* and *Calamintha incana* were comparable to ascorbic acid. Earlier studies attributed the antioxidant activity of *Salvia officinalis* to the presence of polyphenolic compounds (18-20). The methanolic extract of *Nigella sativa* had the lowest antioxidant activity when compared to the other extracts. Thymoquinone, carvacrol, and 4-terpineol are the main constituents of *Nigella sativa* that are responsible for its antioxidant activity (21).

Identification of all the chemicals in leaf and seed extracts that are responsible for antioxidant activity requires further investigation, although it is obvious that constituents like polyphenols, tannins, reducing sugars, and proteins, which are present in the extracts, may be responsible for such activity. Currently there is considerable interest in new natural antioxidants to replace the synthetic ones that are used in foods and cosmetics.

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References

1. Ramadan MF, Kroh LW, Morsel JT. Radical scavenging activity of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.) and niger (*Guizotia abyssinica* Cass.) crude seed oils and oil fractions. J Agric Food Chem 51: 6961-6969, 2003.
2. Aqil F, Ahmed I, Mehmood Z. Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. Turk J Biol 30: 177-183, 2006.
3. Imanshahidi M, Hossinzadeh H. The pharmacological effects of Salvia species on central nervous system. Phytother Res 20: 427-437, 2006.
4. Du G, Zhang J. The general situation and progress of the modern research of red sage root (*Radix Salviae miltiorrhizae*). Yiyao Daobao 23: 435-440, 2004.
5. Weng XC, Wang W. Antioxidant activity of compounds isolated from *Salvia plebeia*. Food Chem 71: 489-493, 2000.
6. Honda G, Yesilada E, Tabata M et al. Traditional medicine in Turkey VI. Folk medicine in West Anatolia: Afyon, Kutahya, Denizli, Mugla, Aydin provinces. J Ethnopharmacol 53: 75-87, 1996.
7. Bruneton J. Pharmacognosy, Phytochemistry, Medicinal Plants. Lavoisier Publishing: Paris. 1995.
8. Lima CF, Andrade PB, Seabra RM et al. The drinking of a *Salvia officinalis* infusion improves liver antioxidant status in mice and rats. J Ethnopharmacol 97: 383-389, 2005.
9. Ninomiya K, Matsuda H, Shimoda H et al. Carnosic acid, a new class of lipid absorption inhibitor from sage. Bioorg Med Chem Lett 14: 1943-1946, 2004.
10. Lee KG, Shibamoto T. Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. J Agric Food Chem 50: 4947-4952, 2002.
11. Miura K, Kikuzaki H, Nakatani N. Antioxidant activity of chemical components from Sage (*Salvia officinalis* L.) and Thyme (*Thymus vulgaris* L.) measured by the oil stability index method. J Agric Food Chem 50: 1845-1851, 2002.
12. Salem ML. Immunomodulatory and therapeutic properties of *Negella sativa* L. seeds. Int Immunopharmacol 5: 1749-1770, 2005.
13. McKay DL, Blumberg JB. A review of the bioactivity and potential health benefits of chamomile tea (*Matricaria recutita* L.). Phytother Res 20: 519-530, 2006.
14. Owlia P, Rasooli I, Saderi H. Antistreptococcal and antioxidant activity of essential oil from *Matricaria chamomilla* L. Research J Bio Sci 2: 155-160, 2007.
15. Zafar R, Mujeeb M. Retinoid and rutin in callus culture of *Tephrosia purpurea* pers. Indian J Pharm Sci 3 (Suppl. 2): 58-61, 2002.
16. Braca A, Sortino C, Politi M et al. Anti-oxidant activity of flavonoids from *Licania licaniae* flora. J Ethanopharmacol 79: 379-381, 2002.
17. Bors W, Saran M, Eltsner EF. Screening for plant anti-oxidants. Modern methods Plant Anal 13: 277-295, 1992.
18. Bors W, Michel C, Stettmaier K et al. Antioxidant mechanisms of polyphenolic caffeic acid oligomers, constituents of *Salvia officinalis*. Biol Res 37: 301-311, 2004.
19. Cuvelier ME, Richard H, Berset C. Antioxidative activity and phenolic composition of pilot-plant and commercial extracts of sage and rosemary. J Am Oil Chem Soc 73: 645-652, 1996.
20. Lu Y, Foo Y. Salvianolic acid, a potent phenolic antioxidant from *Salvia officinalis*. Tetrahedron Lett 42: 8223-8225, 2001.
21. Burits M, Bucar F. Antioxidant activity of *Nigella sativa* essential oil. Phytother Res 14: 323-328, 2000.