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Antioxidant activity and total phenolic compound content of certain medicinal plants

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Abstract

Aqueous extracts were prepared from eight medicinal plants and other plants were prepared as essential oils. The radical-scavenging ability of each plant extract was determined by the 1,1-diphenyl-2-picryl hydrazyl (DPPH) radical scavenging assay. The total phenolic content of plants was determined by the Folin–Ciocalteu reagent in terms of gallic acid equivalents. The DPPH scavenging potential of the aqueous extracts ranged from 17 to 79%, whereas the essential oils showed inhibition of the DPPH activity in the 12–88% range. The highest inhibition of DPPH radicals was observed for Pinus halepensis extract. Meanwhile, amongst the essential oils, the greatest antioxidant potential was exhibited by Nigella sativa. The Bauhinia variegate extract had the highest phenolic content (149.18 mg/g gallic acid equivalents), followed by Albizzia lebbeck (148.00 mg/g) and Pinus halepensis (145.67 mg/g), whereas, amongst the essential oils, the highest phenolic content (98.57 mg/g) was found for Thymus vulgaris. The lowest contents were observed for Kigelia africana and Rosmarinus officinalis. The antioxidant activity had a positive correlation (R=0.654) with the phenolic content of most aqueous extracts, whereas it had a weak correlation using the essential oils (R=0.335). This confirms that the phenolic content of aqueous extracts may contribute towards their antioxidant properties.

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Introduction

Plant-derived products have proven to be an excellent source for the discovery and development of novel drugs. Currently, there are more than 120 chemotherapeutic agents of plant origin (Fransworthe, 1988), many of which are still acquired directly from the plants in which they are synthesised (Craig, 1999). Several anti-inflammatory, digestive, anti-necrotic, neuroprotective and hepatoprotective drugs have recently been shown to have an antioxidant and/or anti-radical scavenging mechanism as part of their activity (Lin and Huang, 2002; Repetto and Llesuy, 2002; Karamova *et al.*, 2011).

The generation of free radicals is associated with several normal metabolic processes as well as environmental pollution, UV radiation and so forth. The term "free radicals" mostly refers to reactive oxygen species (ROS) and are oxygen-centred. Major ROS include the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and the hydroxyl radical (·OH). In addition to ROS, reactive nitrogen species (RNS), including nitric oxide (NO), peroxynitrite (NO_3^-) and S-nitrosothiols, also contribute to the generation of free radicals (Kumar *et al.*, 2012). Free radicals are responsible for oxidative stress, which can initiate physiopathological processes such as age-related and chronic diseases like diabetes, neurodegenerative and cardiovascular diseases, inflammation, Alzheimer's and Parkinson's disease and carcinogenesis. (Ames *et al.*, 1993; Gutteridge, 1995; Martinez-Cayuela, 1995).
Although higher organisms including humans have developed efficient antioxidant networks, many clinical and epidemiological studies have suggested that the intake of dietary antioxidants has an important role in the prevention of the oxidative-stress-related diseases. Natural antioxidants are being extensively studied for their capacity to protect organisms and cells against the deleterious effects of oxidative stress (Cazzi *et al.*, 1997). The use of essential oils, which contain several antioxidant constituents, as functional ingredients in foods, drinks, toiletries and cosmetics is becoming popular (Reische *et al.*, 1998; McClements and Decker, 2000; Ormancey *et al.*, 2001; Sawamura, 2000). Phenolic compounds from medicinal plants possess strong antioxidant activity and may help to protect the cells against the oxidative damage caused by free radicals (Kahkonen *et al.*, 1997). They are known as radical scavengers, metal chelators, reducing agents, hydrogen donors and singlet oxygen quenchers (Proestos *et al.*, 2006).

This study was carried out to determine whether the content of phenolic compounds in the aqueous extracts and essential oils from of medicinal plants correlates with their antioxidant activity.

Materials and methods

Plant materials

Plant materials were obtained from the botanical garden in the Faculty of Agriculture, Assiut University, Assiut, Egypt. The plant materials were collected during 2013 and authenticated by botanists in the Faculty of Science, Assiut University. Once the plants from Egypt were harvested, they were cleaned and chopped into small pieces, shade dried and ground into a powdered form and stored under dark refrigerated conditions. The current study was conducted during 2013 and 2014 years at Fundamental Medicine and Biology Institute, Kazan (Volga region) Federal University, Russia. The medicinal plants used for the study are shown in Table 1.

Extraction

Aqueous extract

Aqueous extracts of *Brachychiton opulneus, Ceiba pentandra, Bombax malabaricum, Chorisia speciosa, Albizzia lebbeck, Bauhinia variegate, Kigelia africana* and *Pinus halepensis* were prepared by maceration of the powdered plant material in distilled water at a ratio of 1 g/10 mL, which was put on a shaker for 2 days at room temperature. The macerate was first filtered through double-layer muslin cloth and then filtered through a Whatman No. 1 filter paper. Subsequently, each extract was sterilised using 0.22 µm filters. Each sterile extract was stored at –20°C.

Essential-oil extract

Plant samples (100 g) of *Zingiber officinale, Pimpinella anisum, Piper nigrum, Origanum majorana, Rosmarinus officinalis, Ocimum basilicum, Thymus vulgaris, Mentha piperita, Simmondsia chinensis, Nigella sativa, Linum usitatissimum* and *Eruca sativa* were subjected to hydro-distillation for 2 h using the Clevenger apparatus for essential oils (Clevenger, 1928). Currently, the most popular method of extraction is steam distillation, in which water is heated to produce steam, which removes the most volatile chemicals and aromatic materials. Essential oils usually float on the surface hydrosol (a component of distilled water). Extracted essential oils are stored in a dark, clean glass bottle and stored at 4°C. For the antioxidant assay, 100 µL of each essential oil was diluted with 1 mL of 80% ethanol and stored at –20°C.

Antioxidant activity (DPPH radical scavenging assay)

The antioxidant activity of the plant materials was assayed by employing the 1, 1-diphenyl-2-picryl hydrazyl (DPPH, CalBiochem, Germany) radical scavenging assay (Mensor *et al*., 2001). The DPPH assay method is based on the reduction of DPPH, a stable free radical. The free radical of DPPH has an odd electron, which gives a maximum absorption at 517 nm (purple colour). The plant extract (10 µL) was added to DPPH solution (100 µL of 0.2 mM DPPH in ethanol) on a microtitre plate. The reaction mixture was incubated at 25°C for 5 min, after which the absorbance was measured at 517 nm. When the antioxidants react with DPPH, the DPPH is reduced to DPPH-H and, as a consequence, the absorbance decreased. DPPH-H formation results in decolourisation (yellow colour) with respect to the number of electrons captured. The DPPH solution with corresponding solvents (i.e., without plant material) served as the control. Ethanol with the respective plant extracts served as the blank. The DPPH radical scavenging activity of each plant extract was calculated as the percentage inhibition. % Inhibition of DPPH radical activity = [(A control - A sample) x 100/A control].

Determination of total phenolic compound content

Total phenol content of aqueous extract

The content of total phenolic compounds was determined spectrophotometrically according to the Folin–Ciocalteu method (Ainsworth and Gillespie, 2007) with slight modification. Briefly, each aqueous extract sample (0.1 mL) was pipetted into a tube and the 10% Folin–Ciocalteu reagent (0.2 mL) (Panreac, Barcelona, Spain) in water (v/v) was added to each standard and sample tube; this was then vortexed for 10 s, covered and incubated for exactly 30 min at room temperature. Aqueous 700 mM sodium carbonate (Na_2CO_3) solution (0.8 mL) was added and the mixture was again vortexed, covered and incubated at room temperature for 2 h. Exactly 0.25 mL of the assayed sample was transferred (in triplicate) into a 96-well plate. During the oxidation of phenolic compounds, phosphomolybdic and phosphotungstic acids, contained in the Folin–Ciocalteu reagent, were reduced to blue-coloured molybdenum and tungsten oxides. The absorbance of the solutions was measured at λ = 735 nm against a blank sample (in triplicate). The measurements were compared to a standard curve of gallic acid solutions (25, 50, 75, 100, 125, 150, 175 and 200 mg/L) and expressed as milligrams of gallic acid equivalents per 100 g ± standard deviation (SD).

Total phenol content of essential oils

The content of total phenolic compounds in the studied essential oils was determined according to the method described by Taga *et al*. (1984). Briefly, each pure (100%) essential oil (100 µL) was dissolved in 80% ethanol (1 mL); 0.2 mL of this solution was made up with 0.3% HCl to 0.5 ml. An aliquot (100 µL) of the resulting solution was added to 7% Na_2CO_3 (2 mL) and, after 2 min, the Folin-Ciocalteau reagent diluted with methanol 1:1 (100 µL) was added and mixed well. After 30 min incubation, exactly 0.25 mL of the assayed sample was transferred (in triplicate) into a 96-well plate and the absorbance of mixtures was recorded at 735 nm.

Calculation of the total phenolic content

The total phenolic content was calculated in terms of

gallic acid equivalents (GAEs) from a calibration curve of gallic acid standard solutions (Fig. 1), and the results were expressed as milligrams of gallic acid per 100 μL of essential oil. The total phenol value was obtained from the regression equation: $y = 0.00095x + 0.0499$ and expressed as milligrams per gram GAE using the formula, $T = CV/M$, where T = total content of phenolic compounds (mg/g GAE), C = concentration of gallic acid (mg/mL), established from the calibration curve, V = volume of extract (0.25 mL) and m = the weight of plant extract (0.029 g).

Statistical analysis

The obtained antioxidant and antimicrobial results were stated in mean ± SD for three replicates. Statistical analysis was performed and graphs were obtained using Microsoft Excel (Microsoft Inc. 2007).

Results and discussion

Determination of total antioxidant activity

Free radicals cause many deleterious effects. ROS react with nucleic acids, proteins and membrane lipids in a largely non-specific manner, which may result in gene mutations, impairments, loss of enzyme activity or altered cell-membrane permeability, whereas RNS directly or indirectly lead to protein S-nitrosylation (Valko *et al.*, 2006; Finkel, 2003). For protection from the oxidative stress caused by free radicals, cells have developed effective and adaptive systems in order to maintain cellular homeostasis through a series of antioxidant molecules and detoxifying enzymes, which can provide control through quick removal or detoxification (Kumar, 2012). To date, several investigations have confirmed the potential benefit of antioxidant supplements as a tool to prevent the consequences of oxidative damages, especially in the progression of certain diseases (Bahramikia *et al.*, 2009). Medicinal plants are considered as an alternative and effective source of medicine to mitigate diseases associated with oxidative stress (Abdul-Hafeez *et al.*, 2014; Roja and Rao, 2000). The free-radical scavenging activity the plant extracts is related to their hydrogen- or electron-donation abilities and the conformations of the antioxidant compounds of the extracts.

Table 1. Plant material.

No.	Botanical name	Common name	Family	Part used	Extract form
1	*Brachychiton populneus* Schott & Endl.	kurrajong	Malvaceae	Stem bark	Aqueous extract
2	*Ceiba pentandra* L.	Kapok	Malvaceae	Stem bark	Aqueous extract
3	*Bombax malabaricum* DC	Bombax	Malvaceae	Stem bark	Aqueous extract
4	*Chorisia speciosa* A.St.-Hil.	Drunken tree	Malvaceae	Stem bark	Aqueous extract
5	*Albizzia lebbeck* (L.) Benth.	Lebbeck	Fabaceae	Stem bark	Aqueous extract
6	*Bauhinia variegate* L.	Camel's foot	Fabaceae	Stem bark	Aqueous extract
7	*Kigelia Africana* (Lam.) Benth.	Kigelia	Bignoniaceae	Stem bark	Aqueous extract
8	*Pinus halepensis* Miller	Aleppo pine	Pinaceae	Stem bark	Aqueous extract
9	*Zingiber officinale* Roscoe	Ginger	Zingiberaceae	Rhizome	Essential oil
10	*Pimpinella anisum* L.	Anise	Apiaceae	Fruit	Essential oil
11	*Piper nigrum* L.	Black pepper	Piperaceae	Fruit	Essential oil
12	*Origanum majorana* L.	Marjoram	Lamiaceae	Leaf	Essential oil
13	*Rosmarinus officinalis* L.	Rosemary	Lamiaceae	Leaf	Essential oil
14	*Ocimum basilicum* L.	Basil	Lamiaceae	Leaf+stem	Essential oil
15	*Thymus vulgaris* L.	Thyme	Lamiaceae	Leaf+flower	Essential oil
16	*Mentha piperita* L.	Peppermint	Lamiaceae	Leaf	Essential oil
17	*Simmondsia chinensis* (Link) C. K. Schneid.	Jojoba	Simmondsiaceae	Seed	Essential oil
18	*Nigella sativa* L.	Black cumin	Ranunculaceae	Seed	Essential oil
19	*Linum usitatissimum* L.	Flax	Linaceae	Seed	Essential oil
20	*Eruca sativa* Mill.	Rocket	Brassicaceae	Seed	Essential oil

Owing to the complexity of the antioxidant materials and their mechanism of actions, it is clear that no single testing method is capable of providing a comprehensive picture of the antioxidant profile of a studied sample, and a combination of different methods is necessary. Despite such limitations, the DPPH free radical scavenging assay can be helpful for primary screening in order to find novel antioxidants (Molyneux, 2004).

Table 2. DPPH free radical scavenging activity of different medicinal plants.

Extract №	Extract form	Plant	Absorbance±SD	Inhibition %
1	Aqueous extract	*Brachychiton populneus*	0.519±0.062	32.65
2		*Ceiba pentandra*	0.483±0.027	37.32
3		*Bombax malabaricum*	0.638±0.031	17.28
4		*Chorisia speciosa*	0.542±0.019	29.73
5		*Albizzia lebbeck*	0.215±0.005	72.08
6		*Bauhinia variegate*	0.280±0.004	63.72
7		*Kigelia africana*	0.296±0.029	61.64
8		*Pinus halepensis*	0.155±0.002	79.93
9	Essential oil	*Zingiber officinale*	0.532±0.102	31.03
10		*Pimpinella anisum*	0.651±0.044	15.63
11		*Piper nigrum*	0.668±0.088	13.33
12		*Origanum majorana*	0.677±0.016	12.26
13		*Rosmarinus officinalis*	0.520±0.027	32.62
14		*Ocimum basilicum*	0.280±0.022	63.68
15		*Thymus vulgaris*	0.312±0.047	59.60
16		*Mentha piperita*	0.660±0.035	14.40
17		*Simmondsia chinensis*	0.711±0.015	7.81
18		*Nigella sativa*	0.087±0.005	88.78
19		*Linum usitatissimum*	0.520±0.020	32.56
20		*Eruca sativa*	0.524±0.053	32.07

In this work, the DPPH scavenging ability of 20 medicinal plants was screened in both aqueous extracts and essential oils, as shown in Table 2. In aqueous extracts of stem bark, *Pinus halepensis* showed the strongest inhibition of DPPH radical activity (79.93%) followed by *Albizzia lebbeck* (72.08% inhibition) and *Bauhinia variegate* (63.72% inhibition). *Brachychiton opulneus, Ceiba pentandra, Bombax malabaricum* and *Chorisia speciosa* exhibited DPPH radical scavenging capacities that ranged from 17 to 37% in aqueous solvents. Twelve medicinal plants were screened as essential oils; *Nigella sativa* ranked first with a percentage inhibition of DPPH radical of 88.78%. This was followed by *Ocimum basilicum* with 63.68% inhibition. The essential oils of *Zingiber officinale, Rosmarinus officinalis, Linum usitatissimum* and *Eruca sativa* exhibited similar anti-radical effects. The essential oil that showed the weakest inhibition of DPPH radicals was *Simmondsia chinensis* (7.81%). The *in vitro* study carried out on DPPH radicals was based on the measurement of the scavenging ability of antioxidants towards this stable free radical. The DPPH radical reacts with suitable reducing agents, the electrons become paired off and the solution loses colour stoichiometrically, depending on the number of electrons taken up (Kaleem and Asif, 2006). From the present results, it may be concluded that the extracts reduce the radical to the corresponding hydrazine when they react with hydrogen donors in the principle antioxidant.

Total phenolic compound content

The free-radical scavenging activity of plant extracts may partly be attributed to the wide variety of antioxidant constituents such as phenolic

compounds, ascorbate and carotenoids. Also, two types of antioxidants, inhibitors of free radicals, which initiate oxidation, and inhibitors of the free-radical chain propagation reactions, are known (Pandey and Rizvi , 2009; Dimitrios, 2006). Total phenolic compounds possess a broad spectrum of chemical and biological activities including radical scavenging properties.

Table 3. Total phenolic compounds of some medicinal plants.

Extract №	Extract form	Plant	Absorbance±SD	Total phenolic content (mg/g Gallic acid equivalent)
1	Aqueous extract	*Brachychiton populneus*	1.62±0.062	136.53
2		*Ceiba pentandra*	1.57±0.004	131.88
3		*Bombax malabaricum*	1.41±0.004	118.26
4		*Chorisia speciosa*	1.60±0.012	134.87
5		*Albizzia lebbeck*	1.76±0.019	148.00
6		*Bauhinia variegate*	1.77±0.012	149.18
7		*Kigelia africana*	1.45±0.043	121.82
8		*Pinus halepensis*	1.73±0.008	145.67
9	Essential oil	*Zingiber officinale*	0.29±0.095	21.97
10		*Pimpinella anisum*	0.27±0.005	19.61
11		*Piper nigrum*	0.20±0.007	14.12
12		*Origanum majorana*	0.14±0.029	8.40
13		*Rosmarinus officinalis*	0.10±0.014	5.39
14		*Ocimum basilicum*	0.21±0.016	14.64
15		*Thymus vulgaris*	1.18±0.009	98.57
16		*Mentha piperita*	0.29±0.025	21.36
17		*Simmondsia chinensis*	0.21±0.007	14.90
18		*Nigella sativa*	0.27±0.003	20.21
19		*Linum usitatissimum*	0.25±0.007	17.86
20		*Eruca sativa*	0.17±0.012	11.13

Table 3 shows the contents of total phenolic compounds of both aqueous extracts and essential oils from some medicinal plants, which were measured using the Folin-Ciocalteu reagent in terms of GAEs (standard curve equation: $y = 0.0001x \pm 0.0382$, $R^2 = 0.7863$; see Fig. 1). The total phenolic contents of the plant extracts are shown in Table 2. Among all aqueous plant extracts, *Bauhinia variegate* had the highest phenolic content (149.18 mg/g GAE), followed by *Albizzia lebbeck* (148.00 mg/g) and *Pinus halepensis* (145.67 mg/g). The results indicated that *Kigelia africana* had the lowest phenolic content. However, the essential oil of *Thymus vulgaris* possessed the highest essential oil phenolic content (98.57 mg/g), whereas the lowest phenolic contents were observed in *Rosmarinus officinalis* and *Origanum majorana* plants (5.39 and 8.40 mg/g, respectively). *Zingiber officinale, Mentha piperita, Nigella sativa* and *Pimpinella anisum* had similar phenolic contents (21.97, 21.36, 20.21 and 19.61 mg/g, respectively).

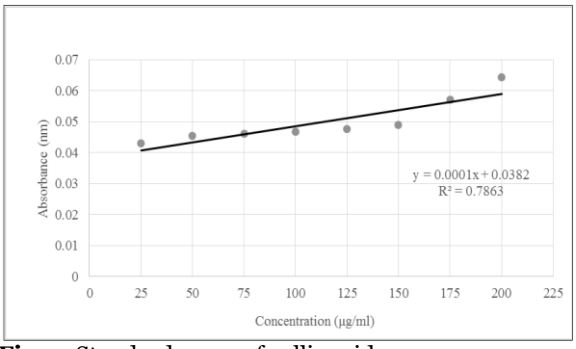

Fig. 1. Standard curve of gallic acid.

The phenolic hydroxyl groups present in plant antioxidants have redox properties (Pietta, 2000), allowing them to act as reducing agents and hydrogen donors in the DPPH assay. Thus, the difference in composition of the extracts and essential oils might result in their different antioxidant activity. Epidemiological studies have suggested a positive association between the consumption of phenol-rich

foods or beverages and the prevention of disease, owing to the presence of antioxidant components such as phenolic compounds (Rice-Evans *et al.*, 1997).

The antioxidant activity of the plants extracts tested had a positive correlation (R=0.654) with the phenolic content of most aqueous extracts and had a weak correlation when using the essential oils (R=0.335). This confirms the assertion that the phenolic content of aqueous extracts may contribute to their antioxidant properties (Fig. 2).

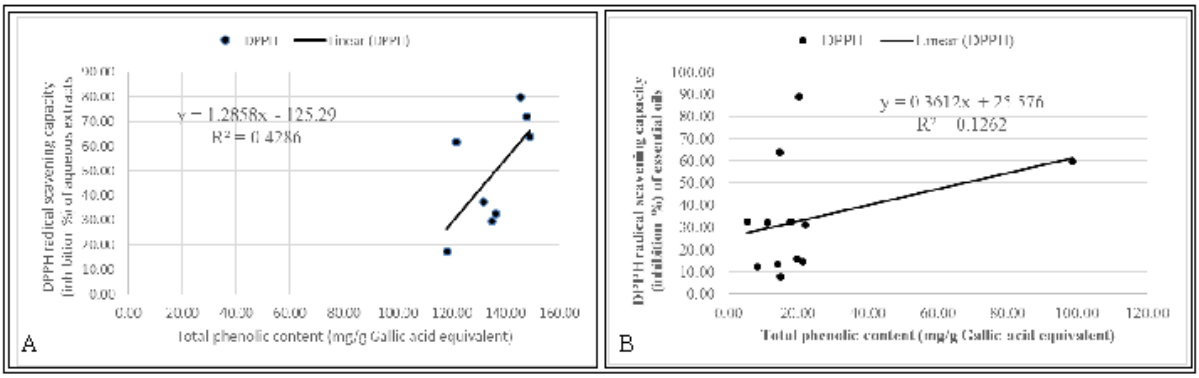

Fig. 2. Correlation between the DPPH radical scavenging capacity assays (A: aqueous extracts and B: essential oils) and the total phenolic content.

(A): Correlation between the DPPH radical scavenging capacity assays of aqueous extracts and the total phenolic content. Correlation coefficient R=0.654.

(B): Correlation between the DPPH radical scavenging capacity assays of essential oils and the total phenolic content. Correlation coefficient R=0.355.

The weak correlation in the volatile oils between the antioxidant activity and the total content of phenols reflects the fact that the high percentage of antioxidant activity is not only attributable to the volatile oil content of phenols, but it can also be attributed to the volatile oil content of other active components. Many investigations have been carried out to isolate the possible active components of *Nigella sativa* seeds oil (Ghosheh *et al.*, 1999). The essential oil of black cumin seeds, *N. satia* L., was tested for its possible antioxidant activity by Burits and Bucar (2000); they found that thymoquinone and the components carvacrol, t-anethole and 4-terpineol demonstrated respectable radical scavenging properties. These four constituents of the essential oil possessed varying antioxidant activities.

Conclusions

Among all of the aqueous extracts of the plants analysed, *Pinus halepensis* showed the highest percentage of antioxidant activity (79.93%). This extract had a total phenolic content of 145.67 mg/g GAE. The *Nigella sativa* essential oil ranked first in terms of percentage inhibition of DPPH radicals (88.78%). *Bauhinia variegate* extract had the highest phenolic content (149.18 mg/g GAE) amongst the aqueous extracts tested. The essential oil of *Thymus vulgaris* demonstrated the highest phenolic content (98.57 mg/g) of the essential oils. Correlation analysis showed that the total phenolic content of the plants tested correlated positively with the antioxidant capacity of most of the aqueous extracts (R=0.654). This suggests that the aqueous extracts of these plants may be potent sources of natural antioxidants and can be used to prevent the deleterious consequences of oxidative stress.

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References

Abdul-Hafeez EY, Nguyen TN, Nazira SK, Olga NI. 2014. Antibacterial activity of certain medicinal plants on different bacterial strains associated with colorectral cancer. International Journal of Biosciences **5,** 219-229. http://dx.doi.org/10.12692/ijb/5.7.219-229.

Ainsworth EA, Gillespie KM. 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature Protocols **2**, 875-877. http://dx.doi.org/10.1038/nprot.2007.102.

Ames BN, Shigenaga MK, Hagen TM. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proceedings of the National Academy of Sciences of the United States of America **90,** 7915–7922.

Boskou D. 2006. Sources of natural phenolic antioxidants. Trends in Food Science & Technology **17,** 505–512. http://dx.doi.org/10.1016/j.tifs.2006.04.004.

Burits M, Bucar F. 2000. Antioxidant activity of *Nigella sativa* essential oil. Phototherapy Research **14,** 323-328. http://dx.doi.org/10.1002/10991573(200008)14:5<323::AID-PTR621>3.0.CO;2-Q.

Cazzi R, Ricardy R, Aglitti T, Gatta V, Petricone P, De Salvia R. 1997. Ascorbic acid and bcarotene as modulators of oxidative damage. Carcinogenesis **18,** 223–228. http://dx.doi.org/10.1093/carcin/18.1.223.

Clevenger JH. 1928. Apparatus for the determination of volatile oil. Journal of American Pharmaceutical Association **17**, 346. http://dx.doi.org/10.1002/jps.3080170407.

Craig WJ. 1999. Health-promoting properties of common herbs. The American Journal of Clinical Nutrition **70,** 491–499.

Duthie GG, Duthie SJ, Kyle JAM. 2000. Plant polyphenols in cancer and heart disease: Implications as nutritional antioxidants. Nutrition Research Reviews **13,** 79–106. http://dx.doi.org/10.1079/095442200108729016.

Finkel T. 2003. Oxidant signals and oxidative stress. Current Opinion Cell Biology **15**, 247–254. http://dx.doi.org/10.1016/S0955-0674(03)00002-4.

Fransworthe NR, Wilson EO, Peters FM. 1988. Screening plants for new medicines. Biodiversity. Academic Press: New York, 61-73.

Ghosheh OA, Houdi AA, Crooks PA. 1999. High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed (*Nigella sativa* L.). Journal of Pharmaceutical and Biomedical Analysis **19,** 757-762. http://dx.doi.org/10.1016/S0731-7085(98)00300-8.

Gutteridge JMC. 1995. Free radicals in Disease Processes: A Compilation of cause and consequence. Free radical Research Communications **19,** 141.

Huang DJ, Ou BX, Prior RL. 2005. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry **53,** 1841-1856. http://dx.doi.org/10.1021/jf030723c.

Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry **47,** 3954-3962. http://dx.doi.org/10.1021/jf990146l.

Kaleem M, Asif MA. 2006. Antidiabetic and

antioxidant activity of *Annona squamosa* extract in streptozotocin induced diabetic rats. Journal of Singapore Medicine **47,** 670 - 676.

Kanti BP, Syed IR. 2009. Plant polyphenols as dietary antioxidants in human health and disease. Oxidative Medicine and Cellular Longevity **2**, 270–278. http://dx.doi.org/10.4161/oxim.2.5.9498.

Karamova NS, Fatykhova DG, Abdrakhimova JR, Ilinskaya ON. 2011. An Investigation of Antigenotoxic Properties of Plant Extracts of *Chelidonium majus* L., *Plantago major* L. and *Tussilago farfara* L. Russian Journal of Genetics **1**, 371-378. http://dx.doi.org/10.1134/S207905971105008X.

Kumar H, Lim H, More SV, Kim B, Koppula S, Kim I, Choi D. 2012. The Role of Free Radicals in the Aging Brain and Parkinson's Disease: Convergence and Parallelism. International Journal of Molecular Sciences **13**, 10478–10504. http://dx.doi.org/10.3390/ijms130810478.

Lin CC, Huang PC. 2002. Antioxidant and hepatoprotective effects of *Acathopanax senticosus*. Phototherapy Research **14,** 489-494. http://dx.doi.org/10.1002/10991573(200011)14:7<489::AID-PTR656>3.0.CO;2-G.

Martinez-Cayuela M. 1995. Oxygen free radicals and human disease. Biochimie **77,** 147–161.

McClements J, Decker EA. 2000. Lipid oxidation in oil-water emulsions: impact of molecular environment on chemical reactions in heterogeneous food system. Journal of Food Sciences **65**, 1270–1282. http://dx.doi.org/10.1111/j.13652621.2000.tb10596.x

Mensor LL, Meneze FS, Leitao L. 2001. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phototherapy Research **15,** 127-130. http://dx.doi.org/10.1002/ptr.687.

Ormancey X, Sisalli S, Coutiere P. 2001. Formulation of essential oils in functional parfumery. Parfums, Cosmetiques, Actualites **157,** 30–40.

Philip M. 2004. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin Journal of Science and Technology **26,** 211-219.

Pietta PG. 2000. Flavonoids as antioxidants. Journal of Natural Product **63,** 1035-1042. http://dx.doi.org/10.1021/np9904509.

Proestos C, Boziaris IS, Nychas GJE, Komaitis M. 2006. Analysis of f lavonoids and phenolic acids in Greek aromatic plants: investigation of their antioxidant capacity and antimicrobial activity. Food Chemistry **95,** 664-67. http://dx.doi.org/10.1016/j.foodchem.2005.01.049.

Reische DW, Lillard DA, Eitenmiller RR. 1998. Antioxidants in Food Lipids. In: Ahoh CC, Min DB, eds. Chemistry Nutrition and Biotechnology. New York: Marcel Dekker, 423–448.

Repetto MG, Llesuy SF. 2002. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Brazilian Journal of Medicinal and Biological Researches **35,** 523-534. http://dx.doi.org/10.1590/S0100879X2002000500003.

Rice-Evans CA, Miller NJ, Paganga G. 1997. Antioxidant properties of phenolic compounds. Trends in Plant Science **2,** 152-159. http://dx.doi.org/10.1016/S1360-1385(97)010.18-2.

Roja G, Rao PS. 2000. Anticancer compounds from tissue cultures of medicinal plant. Journal of Herbs, Spices & Medicinal Plants **7**, 71-102. http://dx.doi.org/10.1300/J044v07n02_08.

Sawamura M. 2000. Aroma and functional

properties of Japanese yuzu (Citrus junos Tanaka) essential oil. Aroma Research **1,** 14–19.

Seifollah B, Amin A, Razieh Y. 2009. Protective effects of four Iranian medicinal plants against free radical-mediated protein oxidation. Food Chemistry **115**, 37–42. http://dx.doi.org/10.1016/j.foodchem.2008.11.054.

Taga MS, Miller EE, Pratt DE. 1984. Chia seeds as a source of natural lipid antioxidants. Journal of the American Oil Chemists' Society **61,** 928-931. http://dx.doi.org/10.1007/BF02542169.

Valko M, Rhodes CJ, Moncol J, Izakovic M, Mazur M. 2006. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactactions **160,** 1–40. http://dx.doi.org/10.1016/j.cbi.2005.12.009.

Yen W, Chang L, Duh P. 2005. Antioxidant Activity of Peanut Seed Testa and Its Antioxidative Component, Ethyl Protocatechuate. LWT-Food Science Technology **38,** 193. http://dx.doi.org/10.1016/j.lwt.2004.06.004.