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Antioxidant activity and volatile components of Egyptian *Artemisia judaica* L.

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Abstract

The volatile components of the aerial parts of *Artemisia judaica* L., grown on the north coast of Egypt, were isolated via hydrodistillation and analysed by GC–MS. The oil was found to contain 25 components. Piperitone (45.0%), *trans*-ethyl cinnamate (20.8%) and ethyl-3-phenyl propionate (11.0%) were the predominant components, followed by spathulenol (6.27%), *cis*-ethyl cinnamate (5.64%), 2,6-dimethyl phenole (1.39%) and methyl cinnamate (1.06%). *A. judaica* volatile oil showed antioxidative activity, determined by thiocyanate and scavenging effect on 1,2 diphenyl picrylhydrazyl (DPPH) methods. Its activity may be due to the presence of 2,6-dimethyl phenol (1.39%) and camphor (0.38%). The *Artemisia* oil has a characteristic flavour, due to the presence of many components with strong sensory properties at a low threshold, such as *trans*-ethyl cinnamate (20.8%) and thus could be suitable for using as antioxidant and flavouring agent in the food industry.

Keywords: A*rtemisia judaica* L.; Volatile oil; Chemical composition; Natural antioxidant; Thiocyanate method; DPPH

1. Introduction

Plant volatile oils have been well known since antiquity as possessing biological activities. Chief amongst these are their antibacterial, antifungal and antioxidant properties (Deans & Waterman, 1993). With a growing interest in the use of essential oils in both the food and the pharmaceutical industries, a systematic examination of plant extracts for these properties has become increasingly important (El-Ghorab, El-Massry, Marx, & Fadel, 1999).

Autoxidation of lipids has long been recognized as a major deterioration process affecting both the sensory and nutritional quality of foods. The high oxidative stability of lipids, which can be ensured by the addition of synthetic antioxidants, is important for health protection and for economic reasons. Some toxicological studies have implicated the widely used synthetic inhibitors, tertiary butyl hydroquinone (TBHQ) and butylated hydroxyanisole (BHA), in promoting the development of cancerous cells in rats (Gordon, 1996). These findings, together with consumer interest in natural food additives, have reinforced interest in natural antioxidants. Natural antioxidants are extensively studied for their capacity to protect organisms and cells from damage induced by oxidative stress, the latter being considered a cause of ageing, degenerative diseases and cancer (Cozzi, Ricordy, Aglitti, Gatta, Petricone, & De Salvia, 1997).

Herbs and spices are harmless sources for obtaining natural antioxidants. Many spices of the genus *Artemisia* are known as aromatic plants. One of them is *Artemisia judica* L. which is a desert chemotype belonging to the tribe anthemideae. Many *Artemisia* species have a characteristic scent or taste, caused by monoterpenes and sesquiterpenes, which in many cases, are the reason for their application in folk medicine. These herbs are used worldwide in tonic, stomachic and stimulant beverages and as antiseptic oils or tinctures applied for the relief of rheumatic pains (Paris & Moise, 1971).

Recently, many attempts have been made to better characterize therapeutic properties and to obtain enhanced production of these useful compounds from selected chemotypes growing in soil or in vitro, e.g. artemisinin, a sesquiterpene lactone peroxide (with anti-

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malarial activity) present in the aerial parts of *A. annua* L (Elhag, El-Domiaty, El-Feraly, Mossa, & El-Olemy, 1992). For many years, *A. judaica* L., Arabic name "Shih Balady" has enjoyed a reputation among herb experts in Egypt as a medicinal herb. Two main chemotypes were found in *A. judaica,* one type characterized by the existence of artemisyl skeleton-type compounds in the volatile oil and the other by the absence of these compounds and the presence of relatively high percentages of piperitone and camphor (Ravid, Putievsky, Katzir, Carmali, Eshel, & Schenk, 1991).

Distinct subspecies of *A. judaica* L. have been reported from the Sinai peninsula (Fleisher & Fleisher, 1990; Putievsky, Ravid, Dudai, Ktazir, Carmeli, & Eshel, 1992); however, no studies have been carried out on Artemisia species on the north coast of Egypt, concerning either their antioxidant activity or the chemical composition of their volatile oil. Thus, the aim of this work was to evaluate the antioxidant properties and the chemical composition of the *A. judaica* L. volatile oil.

2. Materials and methods

2.1. Plant materials

Samples of mature fresh green leaves of *A. judaica* L. were collected from the north coast, Egypt. The plant was identified and authenticated by a botanist at the Plant Protection Department, National Research Center, Cairo, Egypt.

2.2. Extraction method

Batches of 500 g of plant material were submitted to hydrodistillation for 3 h using a Clevenger-type apparatus (Clevenger, 1928). The oil obtained after extraction by diethyl ether was dried over anhydrous sodium sulphate, evaporated and concentrated under a gentle stream of N_2.

2.3. Identification of oil components

GC–MS analysis of the volatile oil was performed on a Varian gas chromatograph interfaced to a Finnigan SSQ 7000 mass selective detector (MSD) with ICIS V2.0 data system for MS identification of the GC components. The column used was a DB-5 (J&W Scientific, Folsom, CA) cross-linked fused silica capillary column (30 m, 0.25 mm i.d.), coated with polydimethylsiloxane (0.5 μm film thickness). The oven temperature was programmed from 50 °C for 3 min, isothermal, then heating by 7 °C/min to 250 °C, and isothermally for 10 min at 250 °C. Injector temperature was 200 °C and the volume injected was 0.5 μl. Transition-line and ion source temperatures were 250 and 150 °C, respectively. The mass spectrometer had a delay of 3 min to avoid the solvent peak and then scanned from m/z 40 to m/z 350. Ionization energy was set at 70 eV. Identifications were based on comparison with the MS computer library (NIST—Software package, Finnigan), and on the respective retention indices. The separated components were identified by matching them with the National institute of Standards and Technology (NIST) mass spectral library data, comparison of the Kovat's indices with those of authentic components and with published data (Adams, 1995). The quantitative determination was carried out by peak area integration.

2.4. Antioxidative assays

2.4.1. Determination of the antioxidant activity in linoleic acid system

Antioxidant activity was determined by using the linoleic system (Osawa & Namiki, 1981). *A. judaica* L. volatile oil (5 mg) was added to a solution mixture of linoleic acid (0.13 ml), 99.8% ethanol (10 ml), and 0.2 M phosphate buffer (pH 7.0, 10 ml). The total volume was adjusted to 25 ml with distilled water. The solution was incubated at 40 °C and the degree of oxidation was measured by the thiocyanate method (Mitsuda, Yasumoto, & Twaki, 1966), with 10 ml of ethanol (75%), 0.2 ml of an aqueous solutions of ammonium thiocyanate (30%), a 0.2 ml sample solution and 0.2 mL ferrous chloride solution (20 mM in 3.5% HCl) being added sequentially. After stirring for 3 min, the absorbance values of the mixtures measured at 500 nm [SHIMADZU UV-1601PC, JAPAN] were taken as the peroxide contents. The percent inhibition of linoleic peroxidation, 100−(Abs increase of sample/Abs increase of control×100), was calculated to express antioxidative activity, which was compared to commercial antioxidants such as butylated hydroxytoluene (BHT) and tertiary butylhydroquinone (TBHQ). All test data are the average of triplicate analyses.

2.5. Scavenging effect on DPPH radical

The effect of *A. judaica* volatile oil on the DPPH radical was estimated according to the method of Hatano, Kagawa, Yasuhara, and Okuda (1988). *A. judaica* volatile oil (500 μg) was added to a methanolic solution (1 ml) of DPPH radical (final concentration of DPPH was 0.2 mM). The mixture was shaken vigorously and left to stand at room temperature for 30 min; the absorbance of the resulting solution was measured spectrophotometrically at 517 nm. In this test, the percentage of DPPH reduction by *A. judaica* volatile oil was compared to that of BHT, TBHQ and cinnamic acid (negative control; 500 μg).

3. Results and discussion

The volatile oil obtained from the leaves of *A. judaica* L. picked from the north coast, Egypt was pale yellow with a pleasant and distinct odour (1.4±0.05 g/100 g fresh leaves). The constituent percentage composition of the volatile oil is shown in Table 1. Twenty-five compounds could be identified in the oil, accounting for about 99% of it. The major compounds, which were identified by GC–MS, were piperitone (45.0%), *trans*-ethyl cinnamate (20.8%), ethyl-3-phenyl propionate (11.0%), *cis*-ethyl cinnamate (5.64%) and methyl cinnamate (1.06%).

The quality of the investigated oil was somewhat similar to that of the Sinai chemotype. It is reported that the volatile oil composition of samples collected from the Sinai Peninsula, is rich in piperitone and dispersed over a wide area of southern Sinai; it contains piperitone (27–46%), *cis*-ethyl cinnamate (5–6%), *trans*-ethyl cinnamate (8–13%), camphor (16–23%), chrysanthenone (5–6%) and ethyl-3-phenyl propionate (0.2–0.5%). In contrast, camphor and chrysanthenol were detected in low concentrations (0.38 and 0.14%, respectively), while *trans*-ethyl cinnamate and ethyl-3-phenyl propionate were found in high concentrations (20.81 and 11%, respectively) in the present investigated oil of the north coast type (Carmali, Ravid, Putievsky, Shenk, & Eshel, 1991; Putievsky et al., 1992). Also our results are in close agreement with those of Saleh (1985), who reported that the volatile oil of *A. judaica*, grown in the desert of Egypt, was a mixture of esters, ketones and aldehydes in which piperitone was the major component.

The variations in the volatile oil content and composition of *A. judaica* plants were related to a variety of factors, such as season, plant age and different plant parts. Differences were found also among samples collected at various locations. The content fluctuated with the season, reaching the highest level during late summer. The content of the leaves was higher than that of branches and flowers. It is therefore concluded that these differences reflect environmental differences between the populations (Karawya, Hifnawy, & El-Hawary, 1979; Ravid, Putievsky, Katzir, Carmeli, Eshel, & Schenk, 1992; Saleh, El-Negoumy, & Abou-Zaid, 1987). Also, there is evidence that the high oil content is correlated with warm and dry conditions (Piperk, Graven, & Whitfield, 1982; Putievsky, Ravid, & Dudai 1988).

The results for linoleic acid peroxidation, determined by the thiocyanate method, at 40 °C after the addition

Table 1
Chemical constitution of the volatile oil of *Artemisia judaica* L. using gas chromatography–mass spectrometry (GC–MS)

No.	Rt.[a] (min)	KI[b]	Conc.	Type[c]	Compound	Identification method
1	4.12	930.3	0.36	M	Tricyclene	MS[d] & KI
2	5.5	943.5	0.85	M	α-Pinene	MS & KI & ST[e]
3	7.12	972.2	0.12	M	Verbenene	MS & KI
4	7.56	979.5	0.88	M	Sabinene	MS & KI
5	9.41	996.4	0.38	M	Mesitylene	MS & KI
6	10.23	1026	0.84	M	p-Cymene	MS & KI & ST
7	12.55	1110.3	0.18	LOC	Thujone (*cis*)	MS & KI
8	13.28	1150.2	0.38	LOC	Camphor	MS & KI & ST
9	14.29	1155.1	1.39	LOC	2,6-Dimethyl phenol	MS
10	14.50	1163	0.14	LOC	Chrysanthenol	MS & KI
11	15.09	1170	0.05	LOC	Benzyl acetate	MS & KI
12	17.23	1243.1	0.50	LOC	Cuminaldehyde	MS & KI
13	18.15	1253	0.46	LOC	Carvenone	MS & KI
14	19.34	1260.4	45.0	LOC	Piperitone	MS & KI
15	19.45	1296	1.10	LOC	2-(4-Phenoxyl) ethanol	MS
16	20.37	1360.6	5.64	LOC	Ethyl cinnamate (*cis*)	MS &KI
17	20.48	1366.3	1.25	LOC	Benzylmethanol-α-Bentyl	MS
18	21.07	1370.5	1.06	LOC	Methyl cinnamate	MS & KI
19	22.00	1390.4	11.0	HOC	Ethyl-3-phenyl propionate	MS
20	23.14	1451.5	20.8	LOC	Ethyl cinnamate (*trans*)	MS & KI
21	23.49	1571.3	6.27	HOC	Spathulenol	MS & KI
22	24.48	1588	0.42	HOC	Guaiol	MS & KI
23	25.45	1620	0.50	HOC	Arteannuic alcohol (*cis*)	MS & KI
24	27.56	1642	0.18	HOC	γ-Eudesmol	MS & KI
25	28.50	1686.2	0.20	HOC	α-Caryophylene acetate	MS & KI

[a] Retention time (min) Conc.%: the percent of concentrations based on peak area integration.
[b] Confirmed by comparison with Kovat's index on DB5 column (Adams, 1995).
[c] M, monoterpene; LOC, light oxygenated compounds; HOC, heavy oxygenated compounds.
[d] Tentative identification by comparison with data obtained from NIST mass spectra library.
[e] Confirmed by comparison with mass spectrum of authentic compound.

of *A. judaica* essential oil, BHT and TBHQ, respectively, are plotted in Figs. 1 and 2. In the early stages, the autoxidation of linoleic acid without added volatile oil was accompanied by a rapid increase of peroxide value at 12 days of testing. Significant differences ($P < 0.5$) were found between the control and the linoleic acid-containing *A. judaica* volatile oil, which slowed the rate of peroxide formation more or less as did BHT and TBHQ. The antioxidative activity of the oil may be attributed to the presence of 2,6-dimethyl phenol (1.39%) and camphor (0.38%). Our results are in accordance with those of Farag, Badei, and El-Baroty (1989) who reported that there is a relationship between inhibition of the hydroperoxide formation and the presence of some phenolic nucleus in some essential oils (carvenone, camphor, borneol, eugenol, thymol). The antioxidative effectivness in natural sources was reported to be mostly due to phenolic compounds (Hayase &

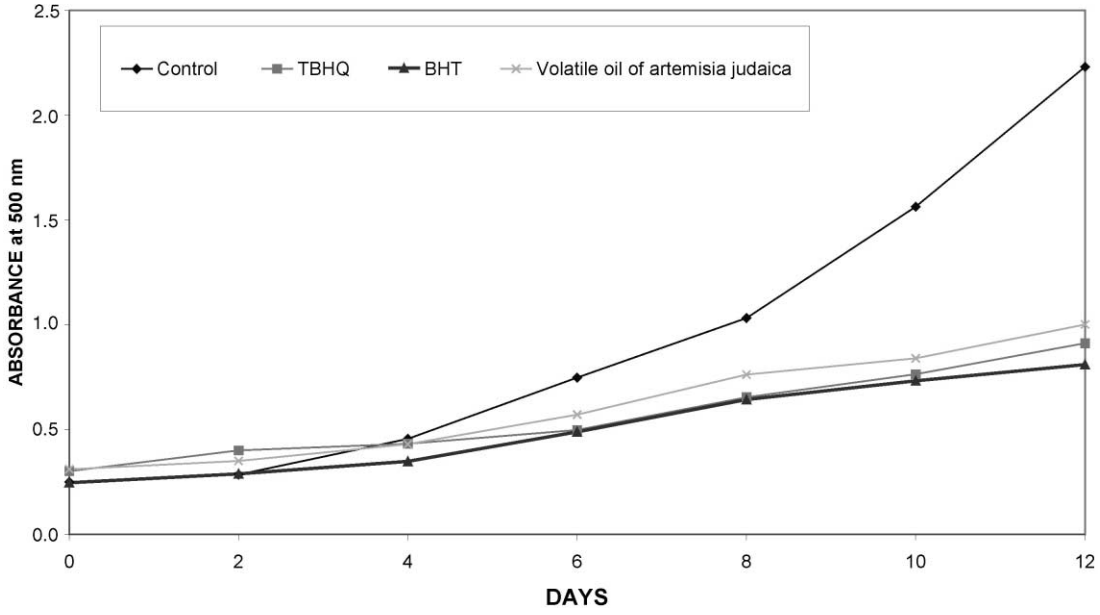

Fig. 1. Determination of the antioxidant activity of volatile oil of *Artemisia judaica* L. by ferric thiocyanate method.

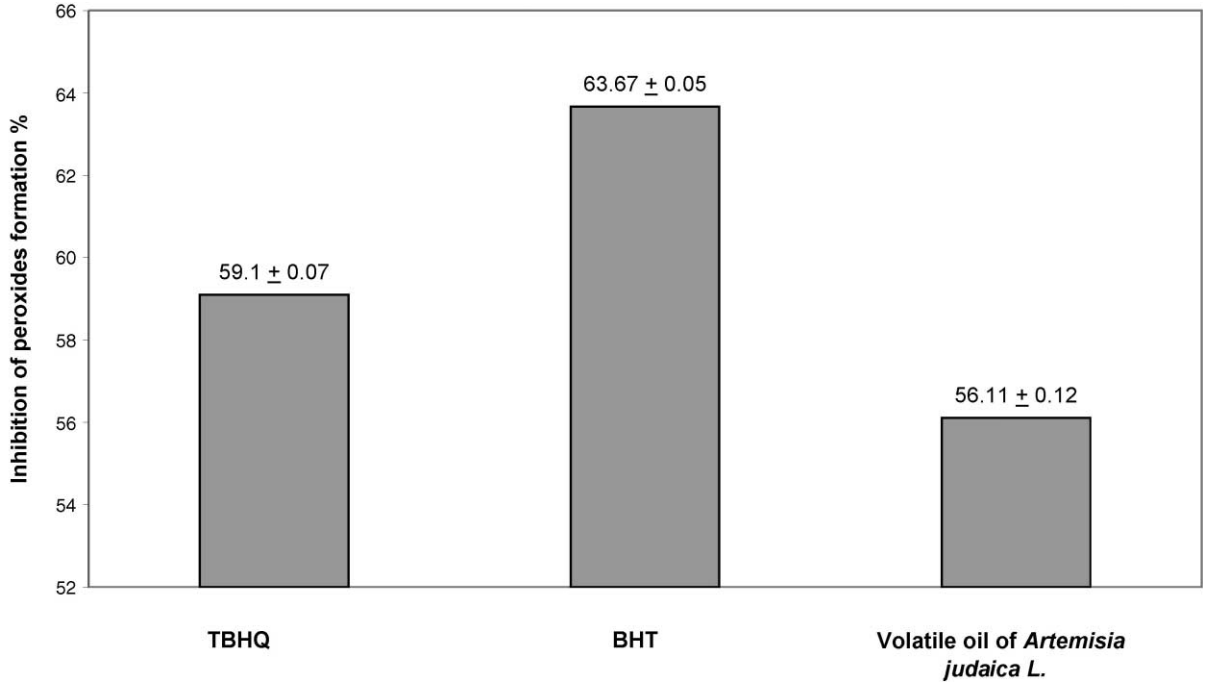

Fig. 2. The inhibition percentage of peroxides formation in the presence of *Artemisia judaica* L. volatile oil as compared to BHA and TBHQ after 12 days.

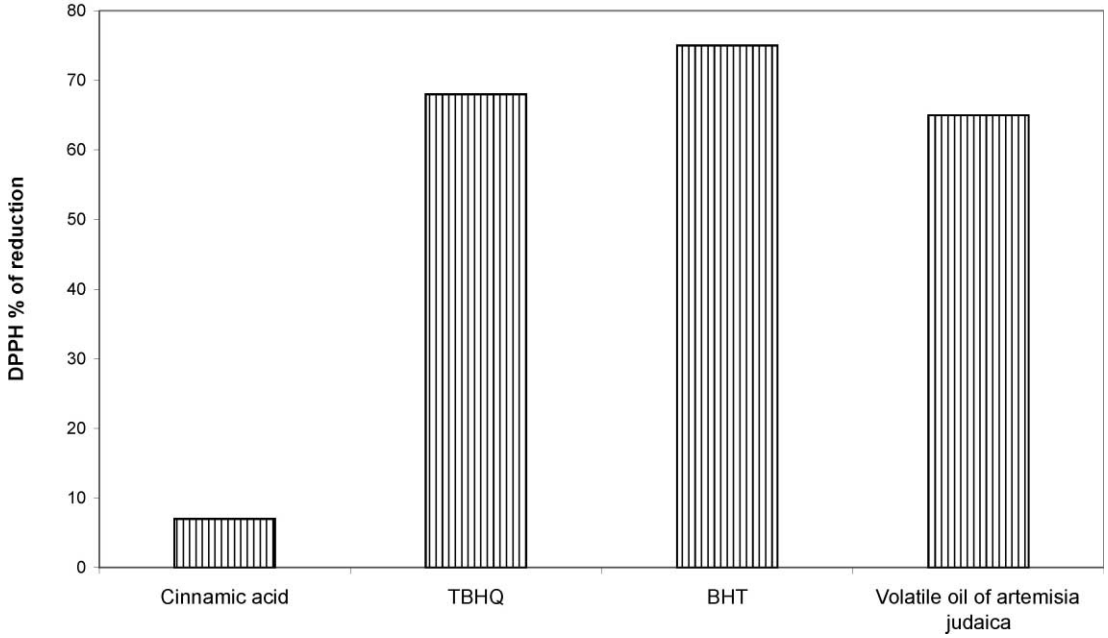

Fig. 3. Comparison of the free radical-scavenging activity of *Artemisia judaica* L. volatile oil with reference compounds.

Kato, 1984). Ramarathnam, Osawa, Namiki, and Tashiro (1986) discovered that phenolic compounds play an important role in inhibiting autoxidation of the oils.

The radical scavenging activity of *A. judaica* volatile oil (Fig. 3) was found to be slightly lower than that of BHT, known as a very efficient synthetic antioxidant agent and widely used in food technology (Potterat, 1997), but equipotent to that of TBHQ, which could be assigned to the presence of some phenolic compounds (Table 1). It is well known that free radicals play an important role in autoxidation of unsaturated lipids in foodstuffs (Kaur & Perkins, 1991). On the other hand, antioxidants are believed to intercept the free radical chain of oxidations, and to contribute hydrogen from the phenolic hydroxyl groups themselves, thereby forming stable free radicals which do not intiate or propagate further oxidation of lipids (Dziezak, 1986). These results demonstrated that the *A. judaica* essential oil has effective activity as a hydrogen donor and as a primary antioxidant by reacting with the lipid radical. This may be responsible for the main cause of suppression of autoxidation, both in linoleic acid and DPPH assays.

The *Artemisia* oil has a characteristic flavour, due to the presence of many components with strong sensory properties at a low threshold. Synthetic *trans*-cinnamyl acetate is used as a flavouring agent in non-alcoholic beverages (2.7 ppm), ice cream (6.5 ppm), baked goods (11 ppm), chewing gum (8.7 ppm) and in condiments (2 ppm) (Ash & Ash, 1995). It is also used in perfumery because of its excellent sensory and fixative properties. In addition it is utilized as a modifier in berry, nut and spice flavour systems (Grant & Othmer, 1993). Obviously, natural *trans*-cinnamyl acetate from this plant could be used for these purposes as a food flavouring. Opdyke (1978) reported the safety of some essential oil constituents and found that piperitone was safe, non-irritant and non-sensitizing, which shows the safety of *Artemisia* volatile oil to some extent.

4. Conclusion

The present results strongly underline that the *A. judaica* L. volatile oil has a remarkable antioxidant activity and is a radical scavenger, which indicates its effectiveness against diseases caused by over production of radicals. Further studies are needed to evaluate the in vivo potential of these oil in animal models.

References

Adams, R. P. (1995). *Identification of volatile oilcomponents by gas chromatography/mass spectroscopy*. USA: Allured Publishing Corporation.

Ash, M., & Ash, I. (1995). *Handbook of additives*. London: Gower Puplishing.

Carmali, D., Ravid, U., Putievsky, U., Shenk, H. P., & Eshel, A. (1991). The volatile oil of *Artemisia judaica* chemotype. *Israel Journal of Botany*, *40*(3), 252–253.

Clevenger, J. F. (1928). Apparatus for the determination of volatile oil. *Journal of the American Pharmaceutical Association*, *17*, 346–349.

Cozzi, R., Ricordy, R., Aglitti, T., Gatta, V., Petricone, P., & De Salvia, R. (1997). Ascorbic acid and B- carotene as modulators of oxidative damage. *Carcinogenesis*, *18*, 223–228.

Deans, S. G., & Waterman, P. G. (1993). In: R. K. M. Hay, & P. G. Waterman (Eds.), *Volatile oil crops: their biology, biochemistry and production* (pp. 113). London: Longman.

Dziezak, J. D. (1986). Antioxidants. *Food Technology*, *40*, 94–102.

El-Ghorab, A.H., El-Massry, K.F., Marx, F., & Fadel, H.M. (1999). Effect of different extraction methods on the chemical composition and antioxidant activity of Egyptian *Eucalyptus camaldulensis* var. *Berviorstris*. The 15th Egypt Chemical Conference 20–25 November, p. 129.

Elhag, H. M., El-Domiaty, M. M., El-Feraly, F. S., Mossa, J. S., & El-Olemy, M. M. (1992). Selection and micropropagation of high artemisinin producing clones of *Artemisia annua*. *Phytotherapy Research*, *6*, 20.

Farag, R. S., Badei, A. Z. M. A., & El-Baroty, G. S. A. (1989). Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. *Journal of the American Oil Chemists' Society*, *66*, 800–804.

Fleisher, Z., & Fleisher, A. (1990). The volatile oil of *Artemisia judaica* L. from Sini and Negev deserts. Aromatic plants of the Holy land and the Sini, part II. *Journal of Essential Oil Research*, *2*(5), 271–273.

Gordon, M. H. (1996). Dietary antioxidants in disease prevention. *Nat. Prod. Rep. Bioorgan. Chem.*, *14*, 265–273.

Grant, M. H., & Othmer, K. (1993). *Encyclopedia of chemical technology* (4th ed., Vol. 6). New York: Wiley.

Hatano, T., Kagawa, H., Yasuhara, T., & Okuda, T. (1988). Two new flavonoids and other constituents in licorice root; their relative astringency and radical scavenging effects. *Chemical and Pharmaceutical Bulletin*, *36*, 2090–2097.

Hayase, F., & Kato, H. (1984). Antioxidative components of sweet potatoes. *Journal of Natural Science Vitaminology*, *30*, 37–46.

Karawya, M. S., Hifnawy, M. S., & El-Hawary, S. S. (1979). A contribution to the study of the volatile oil of *Artemisia judaica* L growing in Egypt. *Egyptian Journal of Pharmaceutical Science*, *20* (1–4), 147–152.

Kaur, H., & Perkins, J. (1991). The free radical chemistry of food additives. In O. I. Aruoma, & B. Halliwell (Eds.), *Free radicals and food additives* (pp. 17–35). London: Taylor and Francis.

Mitsuda, H., Yasumoto, K., & Twaki, K. (1966). Antioxidative action of indole compounds during the autoxidation of linoleic acid. *Eiyo Shokuryo*, *19*, 210–216.

Opdyke, D. J. L. (1978). Monographs on fragrance raw materials. *Food and Cosmetics Toxicology*, *16*.

Osawa, T., & Namiki, M. (1981). A novel type of antioxidant isolated from leaf wax of Eucalyptus leaves. *Agricultural and Biological Chemistry*, *45*, 735–739.

Paris, R. R., & Moise, H. (1971). *Matiere medicale (Vol. 3). Pharmacognice Speciale-Dicotyldones-Gamopetales*. Paris: Masson.

Piperk, S. R. K., Graven, E. H., & Whitfield, P.. In M. N. Koedman, & D. Vokou (Eds.), *Aromatic plants, basic and applied aspects*. The Hague: Martinus Nijhof.

Potterat, O. (1997). Antioxidants and free radical scavengers of natural origin. *Current Organic Chemistry*, *1*, 415.

Putievsky, E., Ravid, U., Dudai, N., Katzir, I., Carmeli, D., & Eshel, A. (1992). Variations in the volatile oil *Artemisia judaica* L. Chemotypes related to phonological and environmental factors. *Flavour and Fragrance Journal*, *7*(5), 253–257.

Putievsky, E., Ravid, U., & Dudai, N. (1988). *Journal of the Science of Food Agriculture*, *43*, 225.

Ramarathnam, N., Osawa, T., Namiki, M., & Tashiro, T. (1986). Studies on the relationship between antioxidative activity of rice hull and germination ability of rice seeds. *Journal of the Science of Food Agriculture*, *37*, 719–726.

Ravid, U., Putievsky, E., Katzir, I., Carmeli, D., Eshel, A., & Schenk, H. P. (1992). The volatile oil *Artemisia judaica* L. *Chemotypes, Flavour and Fragrance Journal*, *7*, 2 69–72.

Ravid, U., Putievsky, E., Katzir, I., Carmali, D., Eshel, A., & Schenk, H. B. (1991). *Flavour and Fragrance Journal*, *7*, 69.

Saleh, M. A. (1985). Volatile components of *Artemisia judaica* growing in the Egyptian deserts. *Biochemical-systematics and Ecology*, *13*, 3 265–269.

Saleh, N. A. M., El-Negoumy, S. I., & Abou-Zaid, M. M. (1987). Flavonoids from *Artemisia judaica*, *Artemisia monosperma* and *Artemisia herba-alba*. *Phytochemistry*, *26*(11), 3059–3064.