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Full Length Research Paper

Chemical constituents and antioxidant properties of *Rosmarinus officinalis L.* essential oil cultivated from South-Western Tunisia

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Essential oil extracted from the aerial parts of *Rosmarinus officinalis* by hydrodistillation was analyzed by gas chromatography/mass spectrometry and the components identified were 15. The major constituents of the essential oil were 1,8-cineole (35.32%), trans-caryophyllene (14.47%), borneol (9.37%), camphor (8.97%), α-pinene (7.9%) and α-thujone (6.42%). *R. officinalis* essential oil was screened for its *in vitro* antioxidant activities using three different and complementary assays: 1,1-diphenyl-2-picrylhydrazyl (DPPH), β-carotene bleaching test and reducing power. The results of the DPPH assay showed an IC_{50} inhibitory concentration of 110.20 µg/ml. In the β-carotene bleaching test, the IC_{50} value was 20.00 µg/ml. At a higher concentration (70 µg/ml), this essential oil exhibited a lower reducing power with an absorbance value of 0.72 ± 0.02. The corresponding value EC_{50} was evaluated as 38.68 µg/ml. Comparison of the antioxidant properties of the investigated essential oil with those expressed by BHT (used as a positive control), showed that this oil exhibited a slightly weak antioxidant potential than BHT and therefore acts as a natural antioxidant agent.

Key words: *Rosmarinus officinalis,* chemical composition, essential oil, antioxidant activity.

INTRODUCTION

Recently, the importance of aromatic plants is being considered due to their applications in folk medicine and their potential for commercial exploitations as aroma and flavour enhancers, cosmetics and in pharmaceuticals (Boussaada et al., 2008). Among them, Rosemary (*Rosmarinus officinalis* L.), a member of the Lamiaceae family, is widely cultivated all over the world as an ornamental and aromatic plant. This herbal spice (native to the Mediterranean Basin) is used in cuisines as dried leaves not only to improve or modify the flavor of food, but also to prevent its deterioration because of its antimicrobial and antioxidant activities (Fernandez et al., 2005). It is used as food flavoring and is also known Medicinally for its powerful antibacterial and antimutagenic properties, and as a chemopreventive agent (Oluwatuyi et al., 2004). It is used in a number of therapeutic applications as treatments in curing or managing a wide range of diseases, such as respiratory disorders, stomach problems and inflammatory diseases (Erenmemişoğlu et al., 1997; Al-Sereiti et al., 1999; Kültür, 2007).

At present, the demand for *R. officinalis* is increasing due to its use in traditional medicine, pharmaceutical industries, cosmetic fields and agribusiness, and for the quality of their essential oil. Due to their biological activities, the essential oils have also been reported to be useful in aromatherapy (Buttner et al., 1996), food preservation (Faid et al., 1995), and fragrance industries (Van de Braak et al., 1999). Today, the interest towards its cultivation is strongly arising due to the well

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recognized antioxidant action exerted by the essential oil extracted from its leaves and flowers. However, the compounds that confer on Rosemary's essential oil its antioxidant properties are still unclear, but it seems likely that these effects must be attributed to a mixture of different components, acting contemporarily and synergically. The diverse composition of the essential oils of Rosemary's ecotypes coming from different geographical areas could be the reason for their varying level of antioxidant activity.

The role of free radicals and active oxygen is becoming increasingly recognized in the pathogenesis of many human diseases, including cancer, aging and atherosclerosis (Perry et al., 2000). Almost all organisms are well protected against free radical damage by oxidative enzymes, such as superoxide dismutase (SOD) and catalase (CAT), or by chemicals such as a-tocopherol, ascorbic acid, carotenoids, polyphenols and glutathione (Gulcin et al., 2002). Thus, to increase antioxidant intake in human diet is one important way to minimize such oxidative damage. Therefore, research works concerning essential oils as alternative potential antioxidant for the treatment of human diseases, prevention and treatment of free radical-related disorders, and for food preservation are important. Concomitantly, public attention to natural antioxidants has been increasing during the last years, and there is need to find natural sources of antioxidants that could replace synthetic antioxidants or at least reduce their use as food additives (Shahidi, 2000). The aim of this work is to investigate the chemical composition of the essential oil from *R. officinalis* and to evaluate its antioxidant activity by using DPPH assay, β-carotene bleaching test and reducing power assay.

MATERIALS AND METHODS

Chemicals, reagents and plant materials

Chemicals and reagents were supplied by Prolabo (Paris, France) and Pharmacia (Uppsala, Swedeen). Plant materials (aerial parts of *R. officinalis*) were collected from the Local Area of the Mount of Sidi Aich, during the Government of Gafsa (South-west of Tunisia), in February to March 2009.

Distillation method

Dried aerial parts were ground prior to the operation and then 300 g of ground rosemary were placed under water distillation for 4 h using a Clevenger apparatus. The distilled essential oils were dried over anhydrous sodium sulfate, after which they were filtered and stored at +4°C.

GC/MS analysis conditions

The essential oil was analyzed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionization detector and HP-5MS capillary column (30 m × 0.25 mm, film thickness of 0.25 µm; Agilent-Technologies, Little Falls, CA, USA). The injector and detector temperatures were set at 250 and 280°C, respectively. The column temperature was programmed from 35 to 250°C at a rate of 5°C/min, with the lower and upper temperatures being held for 3 and 10 min, respectively. The flow rate of the carrier gas (helium) was 1.0 ml/min. A sample of 1.0 µl was injected, using split mode (split ratio, 1:100). All quantifications were carried out using a built-in data-handling programme provided by the manufacturer of the gas chromatograph. The composition was reported as a relative percentage of the total peak area. The identification of the essential oil constituents was based on a comparison of their retention times to *n*-alkanes, as compared to the published data and spectra of authentic compounds. Compounds were further identified and authenticated using their mass spectra when compared to the Wiley Version 7.0 library.

Identification of essential oil compounds

The components of essential oil were identified by comparing the mass spectra data with the spectra available from the Wiley 275 Mass Spectra Libraries (Software, D.03.00) and those in the literature (Adams, 2001), as well as by comparing the retention indices with the literature data (Adams, 2001; Sibanda et al., 2004). Further identification confirmations were made referring to retention indices (RI) data generated from a series of known standards of n-alkanes mixture (C9-C28) (Kovàts, 1958) on the HP5 and HP-20M columns and to those previously reported in the literature (Adams, 2001; Vagionas et al., 2007; Lopes-Lutz et al., 2008).

Antioxidant activity

DPPH radical scavenging assay

The ability of oil to scavenge free radicals of *R. officinalis* essential oil was assayed with the use of a synthetic free radical compound 1,1-diphenyl-2-picrylhydrazyl (DPPH), according to the method employed by Bersuder et al. (1998). Briefly, a volume of 500 µl of each sample was mixed with 500 µl ethanol and 125 µl DPPH (0.02%) in 99.5% ethanol. The mixture was shaken vigorously and incubated in the dark. After 60 min, the absorbance was measured at 517 nm using a spectrophotometer.

The DPPH radical-scavenging activity is calculated as follows:

$$\text{Radical-scavenging activity} = [(A_{blank} - A_{sample})/A_{blank}] \times 100$$

Where A_{blank} and A_{sample} are the absorbance of the control (blank) and the sample, respectively. The IC_{50} value is defined as the amount of the antioxidant necessary to inhibit DPPH radical formation by 50%. As such, the synthetic antioxidant reagent BHT was used as a positive control.

β-carotene bleaching assay

The antioxidant assay using β-carotene bleaching was determined according to the protocol previously described by Koleva et al. (2002). β-Carotene (0.5 mg) was dissolved in 1 ml of chloroform and mixed with 25 µl linoleic acid and 200 µl tween 40. The chloroform was evaporated under vacuum at 40°C and then 100 ml of distilled water was added and the resulting mixture was vigorously stirred. About 2.5 ml of the obtained emulsion was transferred into different tubes containing 500 µl of essential oil dissolved in ethanol at different final concentrations. The tubes were immediately incubated at 50°C for 120 min and the absorbance was measured at 470 nm before and after heat treatment. A control containing 0.5 ml of ethanol instead of the

Table 1. Chemical composition, retention indices and percentage composition of the *R. officinalis* essential oil.

S/N	Compound	Retention index	Percentage	Identification
1	α-pinene	930	7.90	MS, RI
2	Camphene	947	1.53	MS, RI
3	β-pinene	980	3.35	MS, RI
4	1,8-Cineole	1046	35.32	MS, RI
5	α-thujone	1135	6.42	MS, RI
6	β–thujone	1148	2.57	MS, RI
7	Chrysanthenone	1158	0.97	MS, RI
8	Camphor	1180	8.97	MS, RI
9	Borneol	1205	9.37	MS, RI
10	Bornyl acetate	1338	0.92	MS, RI
11	α-copaene	1433	1.61	MS, RI
12	trans-caryophyllene	1477	14.47	MS, RI
13	α-Humulene	1511	2.01	MS, RI
14	Germacrene-D	1569	1.68	MS, RI
15	δ-cadinene	1577	2.33	MS,RI

The components and their percentages are listed in order of their elution on apolar column (HP-5).

sample solution was carried out parallelly. However, BHT was used as a positive control.

Reducing power antioxidant

The ability of oil to reduce iron (III) was determined according to Yildirim et al. (2001) with some modifications. An aliquot of 500 µl of each sample at different final concentrations was dissolved in ethanol and mixed with 1.25 ml of 0.2 M phosphate buffer reagent (pH 6.6) and 1.25 ml of 1% potassium ferracyanide.

The mixture was incubated for 30 min at 50°C followed by the addition of 1.25 ml of 10% (w/v) trichloroacetic acid. The mixture was then centrifuged at 1500 g for 10 min. Finally, 1.25 ml of the supernatant solution was mixed with 1.25 ml of distilled water and 250 µl of 0.1% (w/v) ferric chloride. After 10 min, the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

RESULTS AND DISCUSSION

Chemical composition

Essential oil obtained by hydrodistillation of the aerial parts of *R. officinalis* had a light yellow color and a strong odor. The percentages of the identified components are listed in Table 1 in the order of their elution on the HP-5MS column. Fifteen components representing 99.42% of the total oil could be identified. The major compounds that were identified by Gas chromatography–mass spectrometry **(**GC–MS) were 1,8-cineole (35.32%), trans-caryophyllene (14.47%), α-pinene (7.90%), borneol (9.37%), camphor (8.97%) and α-thujene (6.42%). The oil contains a complex mixture of 77.32% of monoterpene and 22.10% of sesquiterpene. The essential oil composition was dominated by oxygenated monoterpenes (64.54%) followed by sesquiterpene hydrocarbons (22.10%) and monoterpene hydrocarbons (12.78%). The major constituents of oxygenated monoterpenes were 1,8-cineole (35.32%), borneol (9.37%), camphor (8.97%) and α-thujone (6.42%). However, the principal components of monoterpene hydrocarbons were α-pinene (7.90%) and camphene (3.35%). The oil was poor in oxygenated sesquiterpene but those hydrocarbons were mainly composed of trans-caryophyllene (14.47%) with other minor constituent at less than 3% (α-humulène, germacrene-D and δ-cadinene).

The results obtained in this study differed from those reported by other studies in the literature for this species with the specificity of the presence of trans-caryophyllene (14.47%) and a sesquiterpene hydrocarbon in higher amount. Tomei et al. (1995) investigated the essential oil from flowers and leaves of *R. officinalis* (collected from the wild in Southern Spain) and found the main components to be camphor (32.33%), 1,8-cineole (14.41%) and α-pinene (11.56%). Angioni et al. (2004) reported that the major components were α-pinene, borneol, camphene, camphor, verbenone and bornyl-acetate present in Sardinian *R. officinalis* L. oil, whereas Santoyo et al. (2005) found that α-pinene, 1,8-cineole, camphor, verbenone and borneol constituted and represented about 80% of the total *R. officinalis* oil. Also, Sacchetti et al. (2005) reported that the major compounds of *R. officinalis* essential oil were verbenone (21.76%), camphor (14.6%) and bornyl-acetate (12.3%). Gachkar et al. (2007) found that the chemical composition of *R. officinalis* essential oil from Iran was dominated by piperitone (23.7%), α-pinene (14.9%), linalool (14.9%) and 1,8-cineole (7.43%), while Bozin

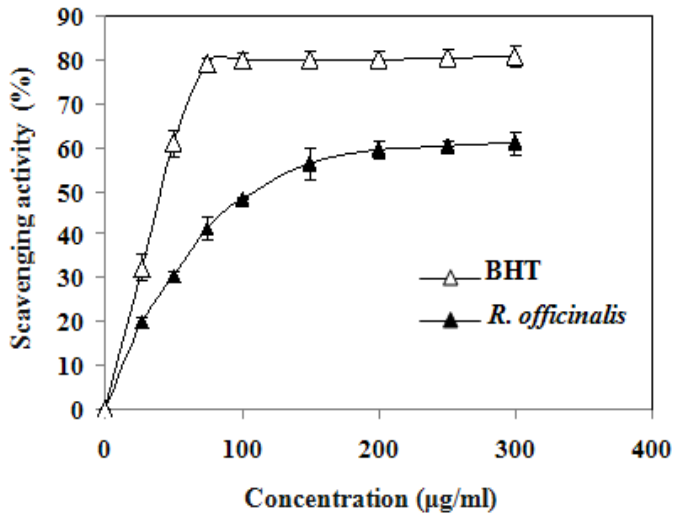

Figure 1. Free radical scavenging activity of *R. officinalis* essential oil and positive control (BHT). Scavenging activity was measured using the DPPH radical assay.

et al. (2007) found that the main compounds of *R. officinalis*
oil were limonene (21.7%), camphor (21.6%) and α-pinene (13.5%). More recently, Martos et al. (2010) reported that the major constituents of *R. officinalis* are 1,8-cineole (23.59%), camphor (20.7%) and α-pinene (18.21%), while Zaouali et al. (2010) determined that the main constituents of *R. officinalis* var. *typicus* and var. *troglodytorum* are 1,8-cineole (47.2 to 27.5%) and camphor (12.9 to 27.9%). These differences in chemical compositions of oils could be attributed to the geographical location of where the plant is grown, the climatic effects on the plants and the genotypical differences (Sagnard et al., 2002; Adams et al., 2006).

Antioxidant activity

The screening of the potential activity of essential oil may require a combination of different methods to describe the antioxidant properties of the sample in more detail. Therefore, the antioxidant activity of the tested essential oil of *R. officinalis* was determined by different *in vitro* methods, such as: the DPPH free radical scavenging assay, *β*-carotene bleaching test and reducing power assay. The results were compared with the synthetic antioxidant BHT, which is an efficient synthetic antioxidant agent in food. All the assays were carried out in triplicate and the average values were considered.

DPPH radical-scavenging activity

DPPH is a free-radical compound which has been widely used to test the free-radical scavenging ability of various samples. The model of scavenging the stable DPPH radical is a widely used method to evaluate the free radical scavenging ability of various samples (Ebrahimzadeh et al., 2008). The antioxidant effect of the essential oil on DPPH radical scavenging may be due to their hydrogen donating ability and it reduces the stable violet DPPH radical to the yellow DPPH-H. Substances which are able to perform this reaction can be considered as antioxidants and therefore radical scavengers (Dehpour et al., 2009). Figure 1 depicts the effective concentrations of the essential oil required to scavenge DPPH radical and the scavenging values as an inhibition percentage at various concentrations. It can be seen that *R. officinalis* exhibited a dose-dependent increase with a radical scavenging effect of 61.00 ± 2.30% at 300 µg/ml, which is slightly lower than the DPPH %inhibition of the positive control BHT (80.70 ± 2.40%) at the same concentration. DPPH scavenging activity is usually presented by IC_{50} value, defined as the concentration of the antioxidant needed to scavenge 50% of DPPH present in the test solution. Comparing the DPPH scavenging activity of *R. officinalis* essential oil (110.20 µg/ml) and those expressed by BHT (40.50 µg/ml), it was shown that the essential oil exhibited the weakest antioxidant effects than BHT. Therefore, the antioxidant effect of the oil was about 3 times lower than that of the synthetic antioxidant BHT.

As can be seen from the results summarized in Table 1, the DPPH scavenging ability of this oil can be attributed to the presence of some components that have antioxidant activity, for example: 1,8 cineole, α-pinene, β-pinene (Houghton, 2004), camphor, α-thujone and β-thujone (Bozin et al., 2007).

β-carotene bleaching method

The *β*-carotene bleaching method is based on the loss of the yellow color of *β*-carotene due to its reaction with radicals that are formed by linoleic acid oxidation in an emulsion. In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxide arising from linoleic acid oxidation. *β*-carotene undergoes rapid discoloration in the absence of an antioxidant; however, the presence of antioxidant will minimize its oxidation. This test measures the potential of the plant to inhibit conjugated diene hydroperoxide formation from linoleic acid oxidation. As can be seen from Figure 2, the antioxidant activity was dose-dependent with a value of 69.09 ± 0.67 and 77.50 ± 1.00 at a final concentration of 70 µg/ml for the essential oil of *R. officinalis* and BHT, respectively. The potential of *R. officinalis* to inhibit lipid peroxidation was evaluated using the *β*-carotene/linoleic acid bleaching test by measuring the IC_{50} with a value of 27.28 and 20.00 µg/ml, obtained

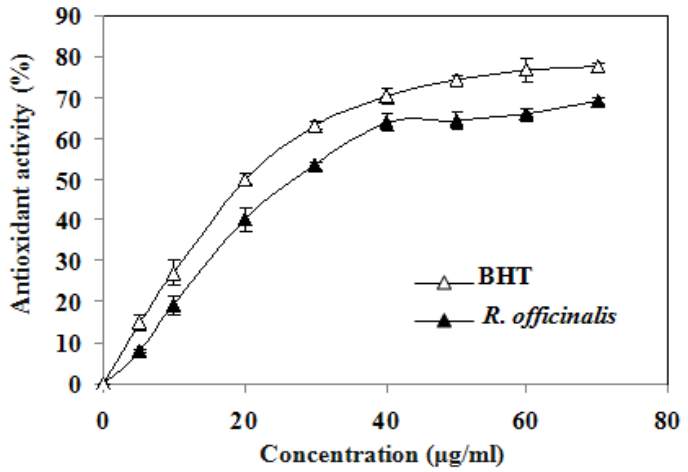

Figure 2. Antioxidant activity of *R. officinalis* essential oil and BHT determined by *β*-carotene bleaching test.

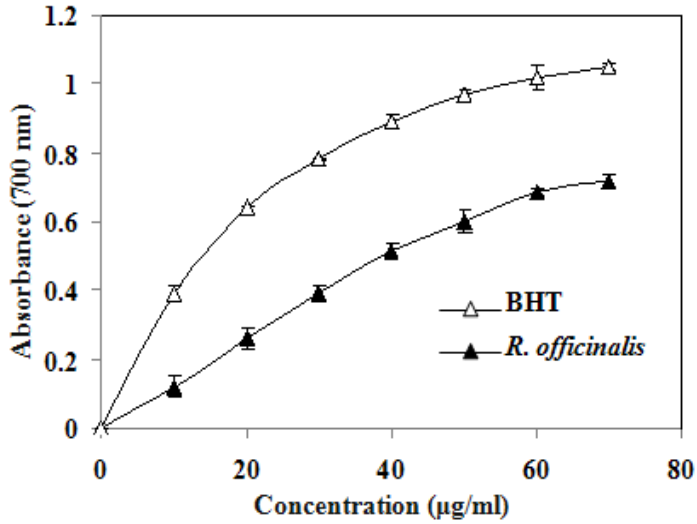

Figure 3. Reducing power of *R. officinalis* as compared to BHT.

for the oil and positive control BHT, respectively. This activity was moderately lower than that of BHT and was attributed to the presence of 1,8 cineole, α-pinene and β-pinene (Wang et al., 2008). Also, it was attributed to the presence of an appreciable amount of antioxidant compounds, such as: 1,8-cineole, transcaryophyllene, borneol, camphor and α-pinene, and to the absence of phenolic compounds (Ebrahimabadi et al., 2010).

Reducing power antioxidant

Antioxidant activity was also determined by ferric reducing power using a spectrophotometer at 700 nm. In this assay, the presence of reducers causes the transformation of Fe^{3+} into Fe^{2+} by donating an electron. Then, the amount of complex can be monitored by measuring the formation of Perl's Prussian blue ($Fe_4[Fe(CN)_6]_3$) at 700 nm. Reduction is often used as an indicator of electron donating activity, which is an important mechanism of phenolic antioxidant action. Increasing absorbance at 700 nm indicates an increase in reductive ability. Figure 3 illustrates dose-response curves for the reducing powers of the essential oil and synthetic antioxidant BHT, that is, there was an increase in the values by increasing the concentration, which indicated an increase in the ferric reducing power. The *R. officinalis* essential oil exhibited a lower reducing power with an absorbance value of 0.72 ± 0.02 vs. 1.05 ± 0.01 for the synthetic antioxidant BHT obtained at 70 µg/ml.

The EC_{50} (a concentration in which the absorbance is 0.5) value of *R. officinalis* essential oil was 38.68 µg/ml, about three times lower than BHT (13.80 µg/ml). The reducing power behavior may be mainly due to the low phenolic content of this oil related to the presence of a reductant agent, which can reduce the oxidized intermediates of lipid peroxidation processes and convert them to more stable products, thereby terminating radical chain reactions consequently.

By comparing the three assays, it is very difficult to attribute the antioxidant effect of the entire essential oil to one or few active compounds. This can be due to the high percentage of the main constituents, but may also be due to the presence of other constituents in small quantities or to synergy among them since both minor and major compounds make a significant contribution to the activity of the oil (Wang et al., 2008). However, the antioxidant activity of this oil seems to be directly related to the presence of monoterpenes.

Conclusion

This study is outlined to probe the chemical composition and *in vitro* antioxidant activity of the essential oil of *R. officinalis* collected from South-west Tunisia. The aerial parts of the plant was characterized by GC-MS and 15 volatile compounds were identified with new chemotypes, such as 1,8-cineole and trans-caryophyllene. A linear relationship between the results of the antioxidant effects of the investigated essential oil, obtained with different methods of assessment, point out that the essential oil could serve as an antioxidant agent not only in food and cosmetics production, but also as an important function in the prevention and treatment of various human diseases. Further isolation of *R. officinalis*' bioactive compounds and the determination of their biological activities (*in vitro* and *in vivo*) are needed to prevent the deterioration of foodstuffs, beverages and pharmaceuticals.

REFERENCES

Adams RP (2001). Identification of essential oil components by gas

chromatography /quadrupole mass spectroscopy. Allured Publishing Corporation, Carol Stream, Illinois.
Adams RP, Socorro M, Elizondo G, Elizondo MG, Slinkman E (2006). A new subalpine variety of *Juniperus blancoi Martinez* (Cupressaceae) from Durango, Mexico. Biochem. Syst. Ecol., 34: 205- 211.
Al-Sereiti MR, Abu-Amer KM, Sen P (1999). Pharmacology of rosemary (*Rosmarinus officinalis* Linn.) and its therapeutic potentials. Indian J. Exp. Biol., 37: 124-130.
Angioni A, Barra A, Cereti E, Barile D, Coisson JD, Arlorio M, Dessi S, Coroneo NDV, Cabras P (2004). Chemical composition, plant genetic differences, antimicrobial and antifungal activity - investigation of the essential of *Rosmarinus officinalis* L. J. Agric. Food Chem., 52: 3530-3535.
Bersuder P, Hole M, Smith G (1998). Antioxidants from a heated histidineglucose model system I: Investigation of the antioxidant role of histidine and isolation of antioxidants by high performance liquid chromatography. J. Am. Oil Chem. Soc., 75: 181-187.
Boussaada O, Ammar S, Saidana D, Chriaa J, Chraif I, Daami M, Helal AN, Mighri Z (2008). Chemical composition and antimicrobial activity of volatile components from capitula and aerial parts of Rhaponticumacaule DC growing wildin Tunisia. Microbiol. Res., 163: 87-95.
Bozin B, Mimica-Dukic N, Samojlik I, Jovin E (2007). Antimicrobial and antioxidant of rosemary and sage (*Rosmarinus officinalis* L. and *Slavia officinalis* L., Lamiaceae) Essential oils, J. Agric. Food Chem., 55: 7879-7885.
Buttner MP, Willekea K, Grinshpun SA (1996). Sampling and analysis of airborne microorganisms. In manual of Environmental Microbiology. Edited by: Hurst CJ, Knudsen GR, Mclnerney MJ, Stezenbach LD, Walter MV, ASM Press: Washington DC. pp. 629-640.
Dehpour AA, Ebrahimzadeh MA, Nabavi SF, Nabavi SM (2009). Antioxidant activity of methanol extract of *Ferula assafoetida* and its essential oil composition. Grasas Aceites, 60(4): 405-412.
Ebrahimabadi AH, Mazoochi A, Kashi, FJ, Djafari-Bidgoli, Z, Batooli H (2010). Essential oil composition and antioxidant and antimicrobial properties of the aerial parts of *Salvia eremophila* Boiss. from Iran. Food Chem. Toxicol., 48: 1371-1376.
Ebrahimzadeh MA, Pourmorad F, Bekhradnia AR (2008). Iron chelating activity screening, phenol and flavonoid content of some medicinal plants from Iran. Afr. J. Biotechnol., 7(18): 3188-3192.
Erenmemişoğlu A, Saraymen R, Ustün H (1997). Effect of Rosmarinus officinalis leaves extract on plasma glucose levels ın normoglycaemic and diabetic mice. Pharmazie., 52: 645-646.
Faid M, Bakhy K, Anshad M, Tantaoui-Elaraki A (1995). Alomond paste: Physiochemical and microbial characterizations and preservation with sorbic acid and cinnamon. J. Food Prod., 58: 547-550.
Fernandez LJ, Zhi N,Aleson CL, Perez AJA, Kur V (2005). Antioxidant and antibacterial activities of natural extracts, application in beef meat balls. Meat Sci., 69: 371-380.
Gachkar L, Yadegari D, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I (2007). Chemical and biological characteristics of *Cuminum cyminum* and *Rosmarinus officinalis* essential oils. Food Chem., 102: 898-904.
Gulcin I, Buyukokuroglu ME, Oktay M, Kufrevioglu OI (2002). On the *in vitro* antioxidative properties of melatonin. J. Pineal Res., 33(3): 167-171.
Houghton PJ (2004). Activity and constituents of Sage relevant to the potential treatment of symptoms of Alzheimer's disease. Herbal Gram., 61: 38-54.
Kültür Ş (2007). Medicinal plants used in Kırklareli province (Turkey). J. Endocrinol., 111: 341-364.
Koleva I, van Beek TA, Linssen JPH, de Groot A, Evstatieva LN (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. Phytochem. Anal., 13: 8-17.
Kovàts E (1958). Characterization of organic compounds by gas chromatography. Part 1 Retention indices of aliphatic halides, alcohols, aldehydes and ketones. Helv. Chim. Acta., 41: 1915-1932.
Martos MV, El Gendy AG S, Sendra E, Lopez JFN, Abdel Razik KA, Omer EA, Alvarez JAP (2010). Chemical Composition and Antioxidant and Anti-Listeria Activities of Essential Oils Obtained from Some Egyptian Plants. J. Agric. Food Chem., 58: 9063-9070.
Oluwatuyi M, Kaatza GW, Gibbons S (2004). Antibacterial and resistance modifying activity of *Rosmarinus officinalis*. Phytochem., 65: 3249-3254.
Perry RJ, Watson P, Hodges JR (2000). The nature and staging of attention dysfunction in early (minimal and mil) Alzheimer's disease: relationship to episodic and semantic memory impairment. Neuropsychol., 38: 252-271.
Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M Bruni R (2005). Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and anti-microbials in foods. Food Chem., 91: 621-632.
Sagnard F, Barberot C, Fady B (2002). Structure of genetic diversity in *Abies alba Mill*. from southwestern Alps: multivariate analysis of adaptive and nonadaptive traits for conservation in France. For. Ecol. Manag., 157: 175-189.
Shahidi F (2000). Antioxidants in food and food antioxidants. Nahrung, 44: 158-163.
Santoyo S, Cavero S, Jaime L, Ibanez E, Senorans FJ, Reglero G (2005). Chemical composition and antimicrobial activity of *Rosmarinus officinalis* L. essential oil obtained via supercritical fluid extraction, J. Food Protect., 68: 790-795.
Sibanda S, Chigwada G, Poole M, Gwebu ET, Noletto JA, Schmidt JM, Rea AI, Setzer WN (2004). Composition and bioactivity of the leaf essential oil of *Heteropyxis dehniae* from Zimbabwe. J. Ethnopharmacol., 92: 107-111.
Tomei PE, Cioni PL, Flamini G, Stefani A (1995). Evaluation of the chemical composition of the essential oils of some Lamiaceae from Serrania de Ronda (Andalucia, Spain). J. Essent. Oil Res., 7(3): 279-282.
Vagionas K, Ngassapa O, Runyoro D, Graikou K, Gortzi O, Chinou I (2007). Chemical analysis of edible aromatic plants growing in Tanzania. Food Chem., 105: 1711-1717.
Van de Braak SAAJ, Leijten GCJJ (1999). Essential Oils and Oleoresins: A survey in the Netherlands and other major Markets in the European Union. CBI, Centre for the Promotion of Imports from Developing Countries, Rotterdam, (pp. 116-119).
Wang SY, Chen CT, Sciarappa W, Wang CY, Camp MJ. (2008). Fruit quality, antioxidant capacity, and flavonoid content of organically and conventionally grown blueberries. J. Agric. Food Chem., 56: 5788-5794.
Yildirim A, Mavi A, Oktay M, Kara AA, Algur ÖF, Bilaloglu V (2000). Comparison of antioxidant and antimicrobial activities of tilia (Tilia argentea Desf Ex DC), sage (*Salvia triloba* L.) and black tea (*Camellia sinensis*) extracts. J. Agric. Food Chem., 48: 5030-5034.
Zaouali Y, Bouzaine T, Boussaid M (2010). Essential oils composition in two *Rosmarinus officinalis* L. varieties and incidence for antimicrobial and antioxidant activities. Food Chem. Toxicol., 48: 3144-3152.