Lipid Peroxidation Inhibition, Superoxide Anion and Nitric Oxide Radical Scavenging Properties of *Thymus daenensis* and *Anethum graveolens* Essential Oils

Dadashpour M (M.Sc. student)[1], Rasooli I (Ph.D.)[2]*, Sefidkon F (Ph.D.)[3], Rezaei MB (Ph.D.)[3], Darvish Alipour Astaneh S (Ph.D. student)[4]

1- Department of Biotechnology, College of Basic Sciences, Shahed University, Tehran, Iran
2- Medicinal Plants Research Center and Department of Biology, Shahed University, Tehran, Iran
3- Department of Medicinal Plants, Research Institute of Forests and Rangelands, Tehran, Iran
4- Department of Microbiology, College of Basic Sciences, Shahed University, Tehran-Iran
*Corresponding author: Department of Biology, College of Basic Sciences, Shahed University, Tehran-Iran
Email: rasooli@shahed.ac.ir

Receive: 4 Dec. 2010 **Acceptance:** 1 Feb. 2011

Abstract

Background: Safety and efficacy of the synthetic antioxidants used in the food industry are frequently questioned because such antioxidants are unstable and highly volatile, therefore, interest in finding naturally occurring antioxidants that have the potential to protect human beings from damage induced by oxidative stress has intensified.

Objective: Bioactivities of *Thymus daenensis* and *Anethum graveolens* essential oils with special reference to their antioxidative properties are studied.

Methods: Total phenolic content (TPC), lipid peroxidation inhibition (LPI), ferrous-ion *chelating* (FIC), superoxide anion and nitric oxide radical scavenging, and tyrosinase inhibition activities of the essential oils were determined.

Results: TPC of *T. daenensis* and *A. graveolens* oils were 644.07±6.79 and 174.91±2.05 mg GAE/100 g. *T. daenensis* and *A. graveolens* oils showed the highest LPI activity with FICs of 63.28±0.21 and 70.22±1.9 percent respectively. Superoxide anion and nitric oxide radical scavenging activities of the above oils had IC_{50} of 0.013, 0.001 and 0.005, 0.0014 mg, respectively. *A. graveolens* oil showed 6 fold higher anti - tyrosinase activity than *T. daenensis* oil.

Conclusion: There was not correlation between lipid peroxidation or ferrous ion chelating activities with total phenolics implying that the oils contain chelating ligands. The effects of antioxidant phytochemicals in the biological systems are ascribed on their ability to scavenge radicals, chelating metals, activate the antioxidant enzymes, and to inhibit the oxidases. *T. daenensis* and *A. graveolens* oils have good commercial potential in both food processing and cosmetic industries.

Keywords: Lipid peroxidation, Superoxide, Nitric oxide, Radical scavenging, *Thymus daenensis*, *Anethum graveolens,* Essential oils

[DOR: 20.1001.1.2717204.2011.10.37.12.3]

Introduction

Plant kingdom is a good source of natural preparations containing effective bioactive compounds which can be used for different applications, particularly as food additives and health promoting ingredients in the formulations of functional foods and nutraceuticals [1]. Thymus and Anethum species are well known as medicinal plants because of their biological and pharmacological properties. Genus Thymus comprises about 400 species of hardy, perennial, and aromatic evergreen or semi-evergreen herbaceous plants found mainly in the northern temperate region [2]. These species have been used as carminative, diuretic, urinary disinfectant and vermifuge [3]. *Anethum graveolens* (Dill) is an annual or biennial herb. In general, dill leaves (dill weeds) and seeds (small fragrant fruits) are used as seasoning. Dill seeds have been used to flavour cakes and pastries, soups, salads, potatoes, meats, sauerkraut and pickles. The seeds have antipasmodic, carminative, stomachic, emmenagogue and galactogogue properties. Food processors and consumers have expressed a desire to reduce the use of synthetic chemicals in food preservation. Common culinary herbs, spices and aromatic plants that exhibit biological activities could provide sources of acceptable, natural alternatives. Bioactive phytochemicals from these plants are often recovered as "essential oils" by hydrodistillation of whole tissues or seed. The essential oils and extracts of many Thymus species are widely used in pharmaceutical, cosmetic and perfume industry, and for flavouring and preservation of several food products. Antimicrobial activities of the oils from various varieties or species of thyme were reported by a number of researchers [4-8]. Chemical polymorphism is characteristic to the species of *Thymus*; numerous chemotypes have been defined, such as carvacrol and thymol, α-terpineol, thujone, geraniol, linalool and others [9]. The essential oils obtained from the aerial parts of *Thymus daenensis* subsp. *daenensis* were analyzed by using GC and GC/MS. Twenty six compounds representing 99.7% of *T. daenensis* oil were identified. The main ones were thymol (74.7%), *p*-cymene (6.5%), β-caryophyllene (3.8%) and methyl carvacrol (3.6%) [10]. Dill seed has an oil content of 2.3 – 3.5%, of which 40 – 60% is carvone, whilst dill weed has 0.4 – 0.8% oil composed of 40% carvone, 32% limonene and 20% phellandrene. (4*S*) - (+)-Carvone was found to be a good potato sprouting inhibitor [11]. The use of spices and herbs as antioxidants in processed foods is a promising alternative to the use of synthetic antioxidants. Many reports indicate that plant flowers have remarkable antioxidant activity [12-15]. Antioxidants are compounds that neutralize chemically active products of metabolism, such as free radicals which can damage the body. Plant phenols and polyphenols play a major role in the prevention of various pathological conditions such as cancer, cardiovascular and neurodegenerative diseases believed to be associated with oxidative stress. Plant phenols also exhibit significant antioxidant, antitumoral, antiviral, and antibiotic properties [16]. Safety and efficacy of the synthetic antioxidants used in the food industry are frequently questioned because such antioxidants are unstable and highly volatile [17], therefore, interest in finding naturally occurring antioxidants that have the potential to protect human beings from damage induced by oxidative stress has intensified [18]. Major reasons for increasing interest in natural antioxidants are: doubts on the safety of use of

[DOR: 20.1001.1.2717204.2011.10.37.12.3]

synthetic substances (butylated hydroxytoluene-BHT- and butylated hydroxyanisole-BHA), the antioxidative efficacy of a variety of phytochemicals, the consensus that foods rich in certain phytochemicals can affect the etiology, and pathology of chronic diseases and the ageing process and the public conception that natural compounds are innately safer than synthetic compounds and are thus more commercially acceptable [19]. Use of essential oils is not under regulatory control in many countries. In the present work, bioactivities of *Thymus daenensis* and *Anethum graveolens* essential oils with special reference to their antioxidative properties are reported.

Materials and Methods

Materials

The plant materials were collected from Dena region of Iran. They were identified and their essential oils were extracted at Department of Medicinal Plants, Research Institute of Forests and Rangelands, Tehran, Iran.

Total phenolic content (TPC)

Total phenolic content (TPC) of extracts was determined using the Folin – Ciocalteau assay [20]. Samples (300 µl) were introduced into test tubes followed by 1.5 ml of a Folin – Ciocalteau's reagent (10 x dilutions) and 1.2 ml of sodium carbonate (7.5% w/v). The tubes were allowed to stand for 30 min before measuring absorbance at 765 nm. TPC was expressed as gallic acid equivalent (GAE) in mg per 100 g material ($y = 0.0111x - 0.0148$; $r^2 = 0.9998$).

β-Carotene/linoleic acid bleaching assay

In this assay, antioxidant activity was determined by measuring the inhibition of volatile organic compounds and conjugated diene hydroperoxides arising from linoleic acid oxidation. The method described by Miraliakbari and Shahidi was used with slight modifications [21]. A stock solution of *β*-carotene and linoleic acid was prepared with 0.5 mg of *β*-carotene in 1 ml chloroform, 25 µl of linoleic, acid and 200 mg Tween 40. The chloroform was evaporated under vacuum and 100 ml of aerated distilled water was then added to the residue. The samples (2 g/l) were dissolved in DMSO and 350 µl of each sample solution was added to 2.5 ml of the above mixture in test tubes. The test tubes were incubated in a hot water bath at 50°C for 2 h, together with two blanks, one contained the antioxidant BHT as a positive control and the other contained the same volume of DMSO instead of the extracts. The test tube with BHT maintained its yellow color during the incubation period. The absorbencies were measured at 470 nm on an ultraviolet spectrometer (Cintra 6, GBC, Australia). Antioxidant activities (inhibition percentage, I%) of the samples was calculated using the following equation:

$$I\% = (A_{\beta\text{-carotene after 2h assay}} / A_{\text{initial } \beta\text{-carotene}}) \times 100$$

where $A_{\text{-}\beta\text{carotene after 2 h assay}}$ is the absorbance of *β*-carotene after 2 h assay remaining in the samples and $A_{\text{initial } \beta\text{-carotene}}$ is the absorbance of *β*-carotene at the beginning of the experiments. All tests were carried out in triplicate and inhibition percentages were reported as means ± SD of triplicates.

Ferrous-ion chelating (FIC) assa

$FeSO_4$ (2 mM) and ferrozine (5 mM) were prepared and diluted 20 times. Extracts (250, 500 and 1000 µl, diluted to 1 ml) were mixed with 1 ml diluted $FeSO_4$, followed by 1 ml of diluted ferrozine. The tubes were mixed well and allowed to stand for 10 min at room temperature. Absorbance of each extract was measured against blank at 562 nm [22]. The

[DOR: 20.1001.1.2717204.2011.10.37.12.3]

ability of the sample to chelate ferrous-ions was calculated and expressed as;

Chelating effect (%) = [1-($A_{sample}/A_{control}$)]×100%

Nitric oxide radical scavenging

The ability of the extract to scavenge nitric oxide free radicals was determined using a modification of the method described by Marcocci and co workers [23]. In brief, a 0.5 ml aliquot of extract (1 mg/ml) or positive control (1 mg/ml) dissolved in KH_2PO_4–KOH (50 mmol/l, pH 7.4) was mixed with 0.5 ml of (10 mmol/l) sodium nitroprusside solution. The mixture was incubated at 37°C for 2. 5 h under normal light condition. After incubation the sample was placed in dark for 20 min. Thereafter, 1 ml of Griess reagent (1 g/l *N*- (1-naphtyl) ethylenediamine and 10 g/l sulphanilamide dissolved in 20 ml/l aqueous H_3PO_4) was added and the absorbance was taken after 40 min at 546 nm. The percentage inhibition was calculated using the following equation:

Inhibition (%) = [($A_{control}$-A_{sample})/ $A_{control}$] ×100

Superoxide anion radical scavenging

The ability of the extract to scavenge superoxide anion radicals was determined by the method described by Lee et al. [24]. In brief, to a 100 µl aliquot of dissolved extract the following was added: 100 µl (30 mmol/l) Na_2EDTA, 100 µl (3 mmol/l) hypoxanthine in 50 mmol/l NaOH and 200 µl (1.42 mmol/l) nitroblue tetrazolium (NBT) in NaH_2PO_4–NaOH (50 mmol/l, pH 7.4). After a 3-min incubation period at room temperature, 100 µl (0.5 U/ml) xanthine oxidase in the NaH_2PO_4–NaOH buffer was added followed 2.4 ml NaH_2PO_4–NaOH buffer. The resulting solution was incubated at room temperature for 20 min and the absorbance at 560 nm was measured. The absorbance was also measured at 293 nm to detect if the extract inhibited uric acid generation. Once it was confirmed that uric acid formation is not inhibited, then the percentage inhibition at 560 nm was calculated using following equation and IC_{50} values were estimated using a non-linear regression.

Inhibition (%)=[($A_{control}$-A_{sample})/ $A_{control}$] ×100

Tyrosinase inhibition

Tyrosinase inhibitory activity was determined by a spectrophotometric method, as described by Chan et al. [25] using a modified dopachrome method with L-DOPA as the substrate. A 5 mg aliquot of the extract was weighed and dissolved in 2 ml of 50% DMSO. Then, 40 µl of sample was added to 80 µl of 0.1 M phosphate buffer (pH 6.8), 40 µl of 0.02 mg/ml tyrosinase and 40 µl of L-DOPA (2.5 mM) in a well of a 96-well microtiter plate. The samples were incubated for 30 min at 37°C. Each sample was accompanied by a blank that contains all components except L-DOPA. Absorbance was measured at 475 nm. Results were compared with the control containing 50% DMSO in place of the sample solution. Kojic acid and quercetin were used as the positive controls. The percentage of tyrosinase inhibition was calculated as:

Tyrosinase Inhibition (%)= [($A_{control}$-A_{sample})/ $A_{control}$] ×100

Anti-tyrosinase activity of the leaf extracts was also expressed as kojic acid equivalents (KAE) in mg/g of oil which were obtained from the following standard curve: (Kojic acid) $y = 0.292x + 0.423$ ($r^2 = 0.945$), where, y represents % inhibition and x represents concentration in µg/ml.

[DOR: 20.1001.1.2717204.2011.10.37.12.3]

Results

Total phenol contents of *T. daenensis* and *A. graveolens* oils were 644.07±6.79 and 174.91±2.05 mg GAE/100 g. The lipid peroxidation inhibition (LPI) activity of both plants oils as measured by the beta carotene bleaching assay is shown in Table 1. For all concentrations, *Anethum graveolens* showed higher LPI activity than *Thymus daenensis*. Both oils exhibited higher LPI than BHT or BHA, the synthetic antioxidants (Table 1). Iron chelating activity also was determined at different oil concentrations (Table 2) and EDTA (0 – 0.008 mg/ml), as standard compound. The maximum percentage of iron chelating activity of the oils is presented in Table 2. The standard of EDTA was observed to be the highest (74.6%) at concentration of 0.008 mg/ml and the lowest (30.85%) at 0.0001 mg/ml. *T. daenensis* and *A. graveolens* oils had ferrous ion chelating (FIC) activities of 63.28 ± 0.21 and 70.22 ± 1.9 percent at 1 and 0.002mg concentration levels respectively, while EDTA had 74.6 ± 1% chelating activity at 0.008 mg concentration. IC_{50} of *T. daenensis*, *A. graveolens* oils and EDTA were 0.73, 0.0013 and 0.00071 mg, respectively (Table 2). Percentage of superoxide radical scavenging activity and inhibition of uric acid formation were increased with increasing concentration of the oils. *Anethum graveolens oil exhibited stronger* superoxide radical scavenging activity than the *T. daenensis* oil (Table 3). Superoxide anion radical scavenging activities of *T. daenensis* and *A. graveolens oils* at 50% concentration (IC_{50}) were 0.013 and 0.001 mg, respectively (Table 3). Percentage of nitric oxide radical scavenging activity was also increased with increasing concentration of the oils. Lower concentrations of *A. graveolens oil exhibited stronger* radical scavenging activity than the higher concentrations of *T. daenensis* oil (Table 4). Nitric oxide radical scavenging activities of *T. daenensis* and *A. graveolens oils* at 50% concentration (IC_{50}) were 0.005 and 0.0014 mg, respectively (Table 4). Anti-tyrosinase activity of *T. daenensis* and *A. graveolens* oils, expressed in kojic acid equivalent values (KAE) showed 6 fold higher activity with A.graveolens oil as compared to *T. daenensis* oil (Table 5).

Table 1- β-Carotene bleaching activity of *Thymus daenensis and Anethum graveolens* essential oils

Essential oil	Mg	30 min	60 min	90 min
Thymus daenensis	0.3125	69.31 ± 1.7	88.22 ± 5.5	89.6 ± 2.3
Thymus daenensis	0.15625	61.68 ± 3.4	74.5 ± 1.5	81.4 ± 3.7
Thymus daenensis	0.07813	54.92 ± 1.8	72 ± 6	80.4 ± 0.5
Thymus daenensis	0.03906	53.8 ± 1	76.06 ± 1.4	80 ± 3.7
Thymus daenensis	0.01953	53.4 ± 0.6	73.36 ± 1.5	76.2 ± 2.5
Anethum graveolens	0.625	77.5 ± 6	80.23 ± 0.9	92.1 ± 3.6
Anethum graveolens	0.3125	72.3 ± 8	80.8 ± 7	92 ± 3
Anethum graveolens	0.15625	71.2 ± 6.5	77.3 ± 1.1	90.4 ± 5
Anethum graveolens	0.07813	68.9 ± 6	76.35 ± 1	86.5 ± 4
Anethum graveolens	0.03906	70.2 ± 6	72.31 ± 1.3	84.2 ± 7
BHT (1mM)	0.22 mg/ml	55 ± 2.5	78.6 ± 2.5	81.2 ± 2.3
BHA (1mM)	0.18 mg/ml	55.08 ± 2.6	78.56 ± 2.4	81.5 ± 2.5

[DOR: 20.1001.1.2717204.2011.10.37.12.3]

Table 2- Ferrous ion chelating (FIC) activities of essential oils from *T. daenensis* and *A. graveolens*

T. denansiensis oil concentrations	**1**	**0.5**	**0.25**	**0.125**	**0.0625**	**0.03125**	**IC_{50}**
Mean T. daenensis FIC (%)	63.28±0.2	41.06±1.8	23.7±0.6	15.1±0.5	13.1±0.7	5.6±0.4	0.73
Anethum graveolens oil concentrations	**0.0020**	**0.0010**	**0.0005**	**0.0003**			
Mean A. graveolens FIC (%)	70.2±2	42.4±5	27.5±2.5	22.3±1			0.0013
EDTA	**0.0080**	**0.004**	**0.002**	**0.001**	**0.0005**	**0.0003**	
Mean FIC by EDTA (%)	74.6±1	65.8±1.3	61.5±3	57.6±3	45.2±1.2	37.3±1.8	0.0007

Table 3- Percentage of superoxide anion free radical scavenging activity

T. denansiensis oil concentrations	**0.016**	**0.008**	**0.004**	**0.002**	**$IC_{50\ (mg)}$**
Mean superoxide anion free radical scavenging activity (%)	56.5±1.1	40.7±2.4	27.8±1.5	19.02±2	0.013
Anethum graveolens oil concentrations	**0.0016**	**0.0008**	**0.0004**	**0.0002**	
Mean superoxide anion free radical scavenging activity (%)	60.65±0.6	45.5±0.8	35.4±0.7	21.7±1	0.001

Table 4- Percentage of nitric oxide radical scavenging activity

T. denansiensis oil concentrations	**0.008**	**0.004**	**0.002**	**0.001**	**0.0005**	**$IC_{50\ (mg)}$**
Mean nitric oxide radical scavenging activity (%)	70.6±1.4	40.1±0.5	14.03±3.7	8.25±0.6	1.51±0.1	0.005
Anethum graveolens oil concentrations			**0.002**	**0.001**	**0.0005**	
Mean nitric oxide radical scavenging activity (%)	**ND**	**ND**	57.5±3.3	45.5±3.9	33.4±1.8	0.0014

Table 5- Anti-tyrosinase activity of T. daenensis and *A. graveolens oils*

T. denansiensis oil concentrations	**0.004**	**0.008**	**0.016**	**0.032**	**$IC_{50\ (mg)}$**
Mean Tyrosinase inhibition (%)	29.9±0.3	31±1.4	40.6±0.8	58.9±0.6	0.024
KAE (mg)	0.05	0.1	0.19	0.38	
Anethum graveolens oil concentrations	**0.0008**	**0.0016**	**0.0032**	**0.0064**	
Mean Tyrosinase inhibition (%)	35.6±1.5	37.2±0.9	46.7±0.5	56.8±2.5	0.004
KAE (mg)	0.05	0.9	0.17	0.34	

[DOR: 20.1001.1.2717204.2011.10.37.12.3]

Discussion

Reactive oxygen and nitrogen species (ROS/RNS), controlled by endogenous enzymes such as superoxide dismutase, glutathione peroxidase, catalase, are continuously produced in the human body. Valuable biomolecules (DNA, lipids, proteins) may be damaged upon over-production of these species and exposure to external oxidant substances [26]. Antioxidants have been reported to prevent oxidative damage by ROS/RNS and may prevent the occurrence of certain diseases such as cancer and the aging process. They can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals and also by acting as oxygen scavengers [26]. Essential oils containing high amount of thymol and carvacrol were reported to possess the highest antioxidant activity [27]. In the present study the total phenol content (TPC) of *T. daenensis* oil was higher than that of *A. graveolens* oil. Total phenolic content of the polar subfraction, as gallic acid equivalents, was reported to be 124.3 μg/mg [28]. The lipid peroxidation inhibition (LPI) activity of both plants (Table 1) showed that *Anethum graveolens* had the highest LPI activity than *Thymus daenensis*. The nonpolar subfraction of the methanol extract of aerial parts of *Thymus caramanicus* has shown the highest inhibition (84.4%), as assessed by the β-carotene/linoleic acid assay, which was only slightly lower than that shown by BHT (93. 3%) [28]. Antioxidant activities of ethanolic extract from dill flower and its various fractions were evaluated with 2, 2-diphenyl-1-picrylhydrazyl radical scavenging, Trolox equivalent antioxidant capacity, reducing power, chelating power, and β-carotene bleaching assays. In all assays, the flower extract showed higher antioxidant activity than the leaf and seed extracts [29]. Dill leaf, seed and their essential oil could provide good antioxidant activities [30]. In this study the LPI activity of both oils were higher than BHT or BHA (Table 1). In general, antioxidant and radical scavenging properties of plant extracts is associated with the presence of phenolic compounds possessing the ability to donate hydrogen to the radical. Numerous reports indicated good correlation between the RSA and the concentration of phenolic compounds measured by Folin–Ciocalteu method. A great number of simple phenolic compounds as well as flavonoids can act as antioxidants, however, their antioxidant power depends on some important structural prerequisites, particularly on the number and the arrangement of hydroxyl groups, the extent of structural conjugation and the presence of electron-donating and electron-accepting substituents on the ring structure [31]. However the present results show that there appears to be no correlation between LPI activity and TPC. This is consistent with findings of Lim and Quah [32] in which the TPC correlated with both AEAC and FRAP but not with LPI activity in methanolic extracts of six cultivars of Portulaca oleracea. LPI activity is mainly attributed to the hydrophobic character of the antioxidant molecules but TPC measures both types of antioxidants, hydrophobic and hydrophilic. Chelating activity of the oils was determined by the ferrozine assay. Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complex formation is disrupted with the result that the red color of the complex is decreased. Measurement of the rate of color reduction therefore allows estimation of the chelating activity of the coexisting chelator [33]. *T. daenensis* and *A. graveolens* essential oils inhibited the formation in a dose dependent

[DOR: 20.1001.1.2717204.2011.10.37.12.3]

variable with the IC_{50} values of 0.73 and 0.0013 mg/ml respectively. However, EDTA showed an excellent chelating ability with the IC_{50} values of 0.0007 mg/ml (Table 2). Due to its high reactivity, iron is the most important lipid oxidation pro-oxidant among the transition metals. Lipid oxidation is accelerated by breaking down of hydrogen peroxide and lipid peroxides by ferrous state of iron to reactive free radicals through the reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + {}^{\bullet}OH + {}^{-}OH$, known as the Fenton reaction. Radicals from peroxides are also produced by Fe^{3+} ion. However the rate is one tenth of Fe^{2+} ion [34]. These can lead to lipid peroxidation, modification of protein and damage to DNA. Metal ions could be inactivated by chelating agents and the metal-dependent processes could potentially be inhibited [35]. There is no correlation between ferrous ion chelating (FIC) and TPC.

Despite the high chelating activity of *Anethum graveolens* oil (Table 2), the relatively low TPC implies that this plant contain chelating ligands, which most probably are non-phenolic compounds that could act as secondary antioxidants, and they help in preventing the generation of hydroxyl radical via Fenton's reaction. This phenomenon has also been reported by Lim et al. [22] in chelating activity of M. tanarius and M. gigantean extracts. These results support the nutraceutical value of these spices and the potential of thymol and carvacrol in preventing the formation of toxic products by the action of reactive nitrogen species [36, 37]. Non-volatile antioxidants, such as flavonoids and vitamin E were also found in the extracts of *T. vulgaris* [38]. Therefore essential oils of thyme can be used as natural preservative ingredients in the food industry [39]. Superoxide radical is produced in human body by various oxidative enzymes in the form of one electron reduction of molecular oxygen. Xanthine oxidase is one of the major oxidative enzymes to produce superoxide radical as a result in tissue injury [40]. In vitro superoxide radical was generated by xanthine oxidase during the reaction; NBT undergoes oxidation and leads to water-soluble blue formazan [41]. The decrease in blue color formation after adding the solvent fractions in the reaction mixture was measured as superoxide radical scavenging. In this study the highest superoxide radical scavenging activity was observed in *A. graveolens* oil, which was at least 10-fold higher than *T. daenensis* oil (Table 3). Many flavonoids have been reported to be potent inhibitors of xanthine oxidase [42]. Iron also is playing a major role for the formation of lipid peroxidation in the body. Therefore, the effects of antioxidant phytochemicals in the biological systems are defense on their ability to scavenge radicals, chelating metals, activate the antioxidant enzymes, and to inhibit the oxidases [43]. Results of the present study disclosed the superoxide radical scavenging activity of the oils. These oils may serve as antioxidant defense mechanism by binding any catalytic iron generated during the course of cell destruction, minimizing hydroxyl radical-mediated tissue injury associated with neutrophil-oxidant production during inflammation and by binding lipopolysaccharide (LPS) with a consequent reduction in LPS bioactivity and ameliorating LPS-induced toxicity. Nitric oxide radical scavenging activities were concentration dependent with that of *A. graveolens oil stronger than T. daenensis* (Table 4). Increased levels of nitric oxide can be found in certain spasmodic conditions, for example, allergic rhinitis, adult respiratory distress syndrome and asthma immediate and late phase [44]. Plant extracts with antioxidant activity could also have immunomodulatory

[DOR: 20.1001.1.2717204.2011.10.37.12.3]

ability [45]. Table 5 summarizes the anti-tyrosinase activity of the oils, in which the oils were expressed in kojic acid equivalent values (KAE). *A. graveolens* oil showed the highest anti-tyrosinase activity with KAE of 0.05 mg/g. Tyrosinase inhibitors are chemical agents capable of reducing enzymatic reactions, such as food browning and melanisation of human skin. Therefore, these agents have good commercial potential in both food processing and cosmetic industries.

Conclusion

There appears to be no correlation between lipid peroxidation and ferrous ion chelating activities with the total phenol content implying that the oils contain chelating ligands. These results support the nutraceutical value of these spices and the potential of thymol and carvacrol in preventing the formation of toxic products by the action of reactive nitrogen species. Therefore, the effects of antioxidant phytochemicals in the biological systems are defense on their ability to scavenge radicals, chelating metals, activate the antioxidant enzymes, and to inhibit the oxidases. The oils under study may serve as antioxidant defense mechanism. Plant extracts with antioxidant activity could also have immunomodulatory ability. Therefore, *T. daenensis* and *A. graveolens* oils have good commercial potential in both food processing and cosmetic industries.

Acknowledgement

The authors wish to thank The Medicinal Plants Research Centre of Shahed University for the sanction of research grants to conduct the present study.

References

1. Loziene K, Venskutonis PR, Sipailiene A, Labokas J. Radical scavenging and antibacterial properties of the extracts from different Thymus pulegioides L. chemotypes. *Food Chem.* 2007; 103: 546 - 59.

2. Tepe B, Sokmen M, Akpulat HA, Daferera D, Polissiou M, Sokmen A. Antioxidative activity of the essential oils of Thymus sipyleus subsp. sipyleus var. sipyleus and Thymus sipyleus subsp. sipyleus var. rosulans. *J Food Eng.* 2005; 66: 447 - 54.

3. Mata AT, Proenta C, Ferreira AR, Serralheiro MLM, Nogueira JMF, Ara·jo MEM. Antioxidant and antiacetylcholinesterase activities of five plants used as Portuguese food spices. *Food Chem.* 2007; 103: 778 - 86.

4. Al-Bayati FA. Synergistic antibacterial activity between *Thymus vulgaris* and Pimpinella anisum essential oils and methanol extracts. *J. Ethnopharmacol.* 2008; 116: 403 - 6.

5. Bounatirou S, Smiti S, Miguel MG, Faleiro L, Rejeb MN, Neffati M, Costa MM, Figueiredo AC, Barroso JG, Pedro LG. Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian Thymus capitatus Hoff. et Link. *Food Chem.* 2007; 105: 146 - 55.

6. Giordani R, Hadef Y, Kaloustian J. Compositions and antifungal activities of essential oils of some Algerian aromatic plants. *Fitoterapia* 2008; 79: 199 - 203.

[DOR: 20.1001.1.2717204.2011.10.37.12.3]

7. Hazzit M, Baaliouamer A, Verissimo AR, Faleiro ML, Miguel MG. Chemical composition and biological activities of Algerian Thymus oils. *Food Chem.* 2009; 116: 714 - 21.

8. Nejad Ebrahimi S, Hadian J, Mirjalili MH, Sonboli A, Yousefzadi M. Essential oil composition and antibacterial activity of Thymus caramanicus at different phenological stages. *Food Chem.* 2008; 110: 927 - 31.

9. Thompson JD, Manicacci D, Tarayre M. Thirty-five years of thyme: A tale of two polymorphisms. Why so many females? Why so many chemotypes? *BioScience* 1998; 48: 805 - 15.

10. Nickavar B, Mojab F, Dolat-Abadi R. Analysis of the essential oils of two Thymus species from Iran. *Food Chem.* 2005; 90: 609 - 11.

11. Hartmans KJ, Diepenhorst P, Bakker W, Gorris LGM. The use of carvone in agriculture: sprout suppression of potatoes and antifungal activity against potato tuber and other plant diseases. *Ind. Crops Prod.* 1995; 4: 3 - 13.

12. Elzaawely AA, Xuan TD, Koyama H, Tawata S. Antioxidant activity and contents of essential oil and phenolic compounds in flowers and seeds of Alpinia zerumbet (Pers.) B.L. Burtt. & R.M. Sm. *Food Chem.* 2007; 104: 1648 - 53.

13. Ho SC, Hwang LS, Shen YJ, Lin CC. Suppressive effect of a proanthocyanidin-rich extract from longan (Dimocarpus longan Lour.) flowers on nitric oxide production in lps-stimulated macrophage cells. *J. Agric. Food Chem.* 2007; 55: 10664 - 70.

14. Kaur G, Alam MS, Jabbar Z, Javed K, Athar M: Evaluation of antioxidant activity of Cassia siamea flowers. *J. Ethnopharmacol.* 2006; 108: 340 - 8.

15. Susanti D, Sirat HM, Ahmad F, Ali RM, Aimi N, Kitajima M: Antioxidant and cytotoxic flavonoids from the flowers of *Melastoma malabathricum* L. *Food Chem.* 2007; 103: 710 - 6.

16. Apak R, Gulcu K, Demirata B, Ozyurek M, Celik SE, Bektasoglu B, Berker KI, Ozyurt D: Comparative evaluation of various total antioxidant capacity assays applied to phenolic compounds with the CUPRAC assay. *Molecules* 2007; 12: 1496 - 547.

17. Sokmen A, Gulluce M, Akpulat HA, Daferera D, Tepe B, Polissiou M, Sokmen M, Sahin F: The in vitro antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic Thymus spathulifolius. *Food Control* 2004; 15: 627 - 34.

18. Scalbert A, Manach C, Morand C, Remesy C, Jimenez L: Dietary polyphenols and the prevention of diseases. *Crit. Rev. Food Sci. Nutr.* 2005; 45: 287 - 306.

19. Dorman HJD, Hiltunen R: Fe (III) reductive and free radical-scavenging properties of summer savory (*Satureja hortensis* L.) extract and subfractions. *Food Chem.* 2004; 88: 193 - 9.

20. Kahkonen MP, Hopia AI, Vuorela HJ, Rauha JP, Pihlaja K, Kujala TS, Heinonen M: Antioxidant activity of plant extracts containing phenolic compounds. *J. Agric. Food Chem.* 1999; 47: 3954 - 62.

21. Miraliakbari H, Shahidi F. Antioxidant activity of minor components of tree nut oils. *Food Chem.* 2008; 111: 421 - 7.

[DOR: 20.1001.1.2717204.2011.10.37.12.3]

22. Lim TY, Lim YY, Yule CM. Evaluation of antioxidant, antibacterial and anti-tyrosinase activities of four Macaranga species. *Food Chem.* 2009; 114: 594 - 9.

23. Marcocci L, Maguire JJ, Droy-Lefaix MT, Packer L. The nitric oxide-scavenging properties of Ginkgo biloba extract EGb 761. *Biochem. Biophys. Res. Commun.* 1994; 201: 748 - 55.

24. Lee JC, Kim HR, Kim J, Jang YS. Antioxidant property of an ethanol extract of the stem of Opuntia ficus-indica var. saboten. *J. Agric. Food Chem.* 2002; 50: 6490 - 6.

25. Chan EWC, Lim YY, Wong LF, Lianto FS, Wong SK, Lim KK, Joe CE, Lim TY. Antioxidant and tyrosinase inhibition properties of leaves and rhizomes of ginger species. *Food Chem.* 2008; 109: 477 - 83.

26. Oke F, Aslim B, Ozturk S, Altundag S. Essential oil composition, antimicrobial and antioxidant activities of Satureja cuneifolia Ten. *Food Chem.* 2009; 112: 874 - 9.

27. Dapkevicius A, Venskutonis R, Van Beek TA, Linssen JPH. Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *J. Sci. Food Agric.* 1998; 77: 140 - 6.

28. Safaei-Ghomi J, Ebrahimabadi AH, Djafari-Bidgoli Z, Batooli H. GC/MS analysis and in vitro antioxidant activity of essential oil and methanol extracts of *Thymus caramanicus* Jalas and its main constituent carvacrol. *Food Chem.* 2009; 115: 1524-8.

29. Shyu YS, Lin JT, Chang YT, Chiang CJ, Yang DJ. Evaluation of antioxidant ability of ethanolic extract from dill (*Anethum graveolens* L.) flower. *Food Chem.* 2009; 115: 515 - 21.

30. Delaquis PJ, Stanich K, Girard B, Mazza G. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *Int. J. Food Microbiol.* 2002; 74: 101 - 9.

31. Miliauskas G, Van Beek TA, De Waard P, Venskutonis RP, Sudholter EJR. Identification of radical scavenging compounds in Rhaponticum carthamoides by means of LC-DAD-SPE-NMR. *J. Nat. Prod.* 2005; 68: 168 - 72.

32. Lim YY, Quah EPL. Antioxidant properties of different cultivars of Portulaca oleracea. *Food Chem.* 2007; 103: 734 - 40.

33. Yamaguchi F, Ariga T, Yoshimura Y, Nakazawa H. Antioxidative and anti-glycation activity of garcinol from Garcinia indica fruit rind. *J. Agric. Food Chem.* 2000; 48: 180 - 5.

34. Miller NJ, Sampson J, Candeias LP, Bramley PM, Rice-Evans CA: Antioxidant activities of carotenes and xanthophylls. *FEBS LETT.* 1996; 384: 240 - 2.

35. Finefrock AE, Bush AI, Doraiswamy PM. Current status of metals as therapeutic targets in Alzheimer's disease. *J. Am. Geriatr. Soc.* 2003; 51: 1143 - 8.

36. Cavar S, Maksimovic M, Solic ME, Jerkovic-Mujkic A, Besta R. Chemical composition and antioxidant and antimicrobial activity of two Satureja essential oils. *Food Chem.* 2008; 111: 648 - 53.

37. Prieto JM, Iacopini P, Cioni P, Chericoni S: In vitro activity of the essential oils of Origanum vulgare, Satureja montana and their main constituents in peroxynitrite-induced oxidative processes. *Food Chem.* 2007; 104: 889 - 95.

[DOR: 20.1001.1.2717204.2011.10.37.12.3]

38. Dapkevicius A, Van Beek TA, Lelyveld GP, Van Veldhuizen A, De Groot A, Linssen JPH, Venskutonis R: Isolation and structure elucidation of radical scavengers from Thymus vulgaris leaves. *J. Nat. Prod.* 2002; 65: 892 - 6.

39. Curtis OF, Shetty K, Cassagnol G, Peleg M. Comparison of the inhibitory and lethal effects of synthetic versions of plant metabolites (anethole, carvacrol, eugenol, and thymol) on a food spoilage yeast (Debaromyces hansenii). *Food Biotechnol.* 1996; 10: 55 - 73.

40. Haraguchi H, Ishikawa H, Mizutani K, Tamura Y, Kinoshita T: Antioxidative and superoxide scavenging activities of retrochalcones in Glycyrrhiza inflata. *Bioorganic & Medicinal Chem.* 1998; 6: 339 - 47.

41. Gulcin I, Uguz M, Oktay M. Evaluation of the antioxidant and antimicrobial activities of clary sage (*Salvia sclarea* L.). *Turk. J. Agric. For.* 2004; 28: 25 - 33.

42. Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, Park SH, Kim SK. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. *Plant Sci.* 2002; 163: 1161 - 8.

43. Kulkarni AP, Aradhya SM, Divakar S: Isolation and identification of a radical scavenging antioxidant - punicalagin from pith and carpellary membrane of pomegranate fruit. *Food Chem.* 2004; 87: 551 - 7.

44. Ashutosh K: Nitric oxide and asthma: A review. *Curr. Opin. Pulm. Med.* 2000; 6: 21 - 5.

45. Awah FM, Uzoegwu PN, Oyugi JO, Rutherford J, Ifeonu P, Yao XJ, Fowke KR, Eze MO. Free radical scavenging activity and immunomodulatory effect of Stachytarpheta angustifolia leaf extract. *Food Chem.* 2010; 119: 1409 - 16.

[DOR: 20.1001.1.2717204.2011.10.37.12.3]