Available online at www.sciencedirect.com
SCIENCE DIRECT
Food Chemistry 100 (2007) 584–589
Food Chemistry
www.elsevier.com/locate/foodchem

Investigation of the antioxidant properties of *Ferula orientalis* L. using a suitable extraction procedure

Nuket Kartal [a], Munevver Sokmen [a,*], Bektas Tepe [b], Dimitra Daferera [c], Moschos Polissiou [c], Atalay Sokmen [b]

[a] *Department of Chemistry, Faculty of Science and Literature, Cumhuriyet University, Sivas 58140, Turkey*
[b] *Department of Biology, Faculty of Science and Literature, Cumhuriyet University, Sivas 58140, Turkey*
[c] *Laboratory of General Chemistry, Agricultural University of Athens, Iera Odos 75, Athens 118 55, Greece*

Received 18 April 2005; received in revised form 30 September 2005; accepted 30 September 2005

Abstract

The present work examines the in vitro antioxidant properties of the essential oil and various extracts prepared from the herbal parts of *Ferula orientalis* A. (Apiaceae). The highest 2,2-diphenyl-l-picrylhydrazyl (DPPH) radical-scavenging activity was found in the polar extract, e.g. methanol–water (1:1), obtained from non-deodorised materials with IC_{50} values at 99.1 μg/ml. In the β-carotene/linoleic acid assay, the deodorised acetone extract exhibited stronger activity than the polar one. The relative antioxidant activities (RAA%) of the extracts ranged from 10.1% to 76.1%, respectively. Extraction with methanol–water (1:1) mixture was concluded to be the most appropriate method in terms of higher extract yield, as well as effectiveness, observed in both assays. Although the essential oil showed antioxidative potential, it was not as strong as that of positive control (BHT). GC/MS analysis of the essential oil resulted in the identification of 39 compounds, β-phellandrene (23.6%), (*E*)-β-ocimene (13.8%), α-pinene (12.5%), α-phellandrene (11.5%) and dehydro-sesquicineole (10.1%) being the major components.

Keywords: *Ferula orientalis* L. (Apiaceae); Essential oil; Antioxidant activity; DPPH; Linoleic acid oxidation

1. Introduction

Plant-derived natural chemicals, known as secondary metabolites, are effective in their roles of protection, adaptation and pollination. Secondary metabolites are mainly used in food, pharmaceutical, chemical, cosmetic industries and agriculture (Philipson, 1990; Sokmen & Gurel, 2001, chap. 7).

Plant-derived natural products are highly abundant; many exhibit numerous biological activities and some can be employed as food additives. Synthetic antioxidants have been used in the food industry since the 1940s, but trends in many health-related industries tend to shift preferences to natural sources. Therefore, investigation of natural antioxidants has been a major research interest for the past two decades as many research groups and institutions have been screening plant materials for possible antioxidant properties.

Extraction procedures to obtain active principles are mainly focussed on the use of methanol or ethanol as solvents. Since active compounds in plants exhibiting biological activity are in low concentrations, selective extraction methods should be used. Activity may be varied when different solvents are used for conventional extraction. Extraction with non-polar solvents, such as hexane, petrol ether, provided better antioxidant properties than did methanol or acetone (Chen, Shi, & Ho, 1992; Chevolleau,

* Corresponding author. Tel.: +90 346 219 10 10x2922; fax: +90 346 219 11 86.
E-mail addresses: msokmen@cumhuriyet.edu.t (M. Sokmen), asokmen@cumhuriyet.edu.tr (A. Sokmen).

0308-8146/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2005.09.084

Mallet, Ucciani, Gamisans, & Gruber, 1992; Kramer, 1985). Dapkevicius, Venskutonis, Van Beek, and Linssen (1998) investigated the extracts of some aromatic herbs grown in Lithuania. A sequential extraction procedure was applied by this group and the best extraction procedure for investigated plant species was recommended. As reported above, studies on extraction procedures are directly related to plant species and suitable procedures for each species should be investigated. However, yields of the extracts as much as their antioxidant activities, should be considered for their possible industrial uses.

Presence of antioxidant component in plant materials is determined by many methods since the antioxidants act by different mechanisms. They play an important role for the scavenging of free radicals and chain-breaking and these types of compounds have been called primary antioxidants and act as deactivators of metals, inhibitors of lipid hydroperoxide breakdown, regenerators of primary antioxidants and quenchers of singlet oxygen (Gordon, 1990; Koleva, van Beek, Linssen, de Groot, & Evstatieva, 2002).

Several methods have been recommended for the evaluation of antioxidant properties of plant materials and some methods in current use were compared (Gordon, 1990; Halliwell, Gutteridge, & Aruoma, 1987; Halliwell & Gutteridge, 1989; Halliwell, 1990; Koleva et al., 2002; Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002). 2,2′-Diphenyl-1-picrylhydrazyl radical (DPPH·) assay is a well known method for the evaluation of free radical-scavenging activity. The method is sample polarity-independent very rapid, simple and reproducible (Koleva et al., 2002). On the other hand, fatty acid decomposition is one of the main causes of food spoilage and inhibition of fatty acid oxidation is an important issue in the food industry. Food preservers or antioxidants are mainly used as inhibitors of the oxidation of fatty acids. Therefore, the inhibition of linoleic acid oxidation can be measured in the presence of β-carotene that is used as a marker (Dapkevicius et al., 1998). Linoleic acid oxidation produces conjugated dienes and other volatile products that attack β-carotene and bleach its characteristic colour (pale yellow in aqueous emulsion). In general, both free radical-scavenging and inhibition of linoleic acid oxidation are desired in the food industry.

Fresh peeling stalks of *Ferula orientalis,* known as ''at kasnisi'', is consumed as a flavouring additive in pickles by local people. As far as our literature survey could ascertain, some *Ferula* species have been used in pharmacy as stimulants, antispasmodics and expectorants (Duke, 2002). However, no report was available concerning its antioxidant features. This plant, 100–150 cm in height, grows on rocky steps at 1600–2900 m and has distinguishable yellow flowers, with a flowering stage in late May and June (Davis, 1965–1984).

The aim of this study was to investigate suitable extraction procedures for *F. orientalis*, employing the above test methods, as well as analysing the essential oil obtained from herbal parts.

2. Materials and methods

2.1. Collection of plant material

F. orientalis was collected from roadsides, Zara to Susehri (Geminbeli Valley), Sivas, Turkey (1000–1100 m), when flowering (June, 2001). The plant collected was identified by a senior botanist, Prof. Semsettin CIVELEK, Department of Biology, Fırat University, Elazig, Turkey and the voucher specimens have been deposited at the Herbarium of the Department of Biology, Cumhuriyet University, Sivas, Turkey (CUFH-Voucher No: ED 6223).

Collected plant materials were dried in the shade and ground in a grinder with a 2 mm diameter mesh.

2.2. Isolation of the essential oil (EO)

A portion of dried sample (100 g) from aerial parts of plant material was submitted, for 3 h, to water-distillation using a Clevenger-type apparatus (Ildam, Ankara, Turkey) (yield 0.51%, v/w). The obtained essential oil (EO) was dried over anhydrous sodium sulphate and, after filtration, stored at +4 °C until tested and analysed.

2.3. Preparation of the extracts

2.3.1. General

Five different extracts, employing different solvent/solvent mixtures and essential oil, were obtained. Dried aerial parts of the plant were used for extraction. All of the extraction procedures are given in Scheme 1.

2.3.2. Deodorized water extract (DWE)

After completion of hydrodistillation, the liquid retentate was collected, filtered and lyophilised (Edwards, Crawley, Sussex, England). This procedure gave deodorized water extract (DWE) (yield 21.21%, w/w).

2.3.3. Deodorized acetone oleoresins (DAO)

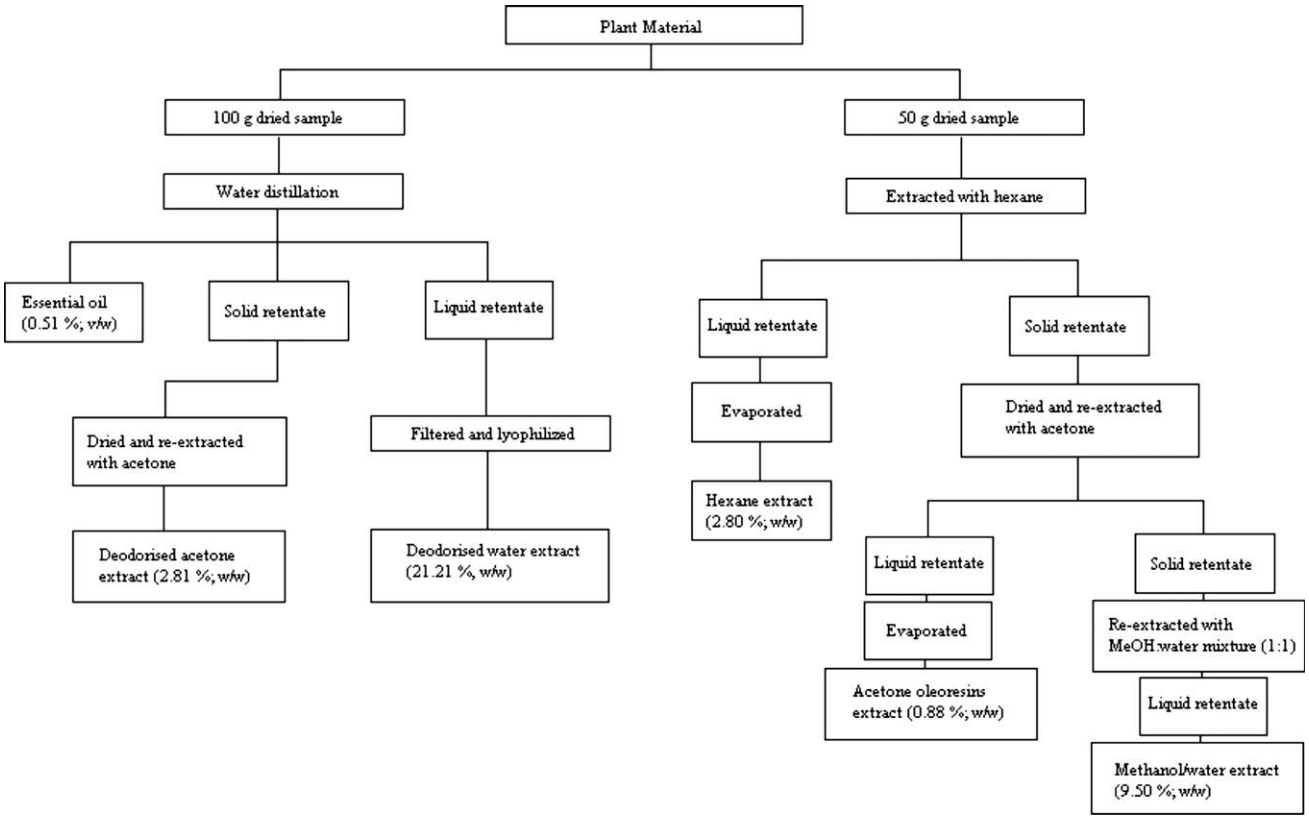
The solid retentate of the hyrodistillation was dried and re-extracted with acetone at 30 °C for 24 h. The resulting extract (DAO) was evaporated to dryness (yield 2.81%, w/w).

2.3.4. Hexane extract (HE)

Another portion (50 g) of dried plant material was extracted with hexane (HE) in a Soxhlet apparatus (Isolab, Wetheim, Germany) for 6 h. Hexane phase was separated and evaporated to dryness (yield 2.80%, w/w).

2.3.5. Acetone oleoresins extract (AO)

The solid retentate, after hexane extraction, was dried and re-extracted with acetone at 30 °C for 24 h. The resulting extract (AO) was evaporated to dryness (yield 0.88%, w/w).



Scheme 1. Extraction procedure.

2.3.6. Methanol–water extract (MWE)

The solid retentate, after acetone extraction, was dried and re-extracted with a methanol–water mixture (1:1, 250 ml) at 30 °C for 24 h. Aqueous extract was separated, filtered and evaporated under vacuum to 10–15 ml then dried at −50 °C in a lyophiliser (yield 9.5%, w/w).

All extracts obtained were lyophilised and kept in the dark at +4 °C until used.

2.4. Gas chromatography (GC) analysis

The essential oil was analyzed using a Hewlett–Packard 5890 II GC equipped with a FID detector and HP-5ms capillary column (30 m × 0.25 mm, film thickness 0.25 μm). Injector and detector temperatures were set at 220 and 290 °C, respectively. Column temperature was initially kept at 50 °C for 3 min, then gradually increased to 240 °C at a rate of 3 °C/min. Helium was the carrier gas, at a flow rate of 1 ml/min. Diluted samples (1/100 in acetone, v/v) of 1.0 μl were injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data without the use of correction factors.

2.5. Gas chromatography/mass spectrometry (GC/MS) analysis

Analysis of the oils was performed under the same conditions as for GC, using a Hewlett–Packard 5890 II GC, equipped with a HP 5972 mass selective detector and a HP-5 ms capillary column (30 m × 0.25 mm, film thickness 0.25 μm). For GC/MS detection, an electron ionization system with ionization energy of 70 eV was used. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. *n*-Alkanes were used as reference points in the calculation of the Kovats indices (K.I.). Tentative identification of the compounds was based on the comparison of their relative retention times and mass spectra with those of NBS75K and Wiley275 library data of the GC/MS system and literature data (Adams, 2001).

2.6. Antioxidant activity

2.6.1. DPPH assay

The hydrogen atom- or electron-donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of the purple-coloured methanol solution of DPPH. This spectrophotometric assay (Pharmacia, Uppsala, Sweden) LKB-Novaspec (II) uses stable radical diphenylpicrylhydrazyl (DPPH) as a reagent (Sigma–Aldrich) (Burits & Bucar, 2000; Cuendet, Hostettmann, & Potterat, 1997). Fifty μl of various concentrations of the extracts in methanol were added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517 nm. Inhibition of free radical DPPH in percent (*I*%) was calculated as follows:

$$I\% = (A_{blank} - A_{sample}/A_{blank}) \times 100,$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotting inhibition percentage against extract concentration. Tests were carried out in triplicate and butylated hydroxytoluene (BHT) and ascorbic acid (AA) were used as positive controls.

2.6.2. β-Carotene–linoleic acid assay

In this assay, antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Barriere et al., 2001).

A stock solution of β-carotene/linoleic acid (Sigma–Aldrich) was prepared as follows: first, 0.5 mg of β-carotene was dissolved in 1 ml of chloroform (HPLC grade), then 25 μl of linoleic acid and 200 mg of Tween 40 (Merck) were added. The chloroform was subsequently evaporated using a vacuum evaporator (Büchi, Flawil, Switzerland). Then, 100 ml of distilled water saturated with oxygen (30 min at 100 ml/min) were added with vigorous shaking. Aliquots (2.5 ml) of this reaction mixture were transferred to test tubes, and 350 μl portions of the extracts (2 g/l in ethanol) were added before incubating for 48 h at room temperature. The same procedure was repeated with BHT at the same concentration and a blank containing only 350 μl of ethanol. After the incubation period, the absorbances of the mixtures were measured at 490 nm. Antioxidant capacities of the samples were compared with those of BHT and the blank.

3. Results and discussion

3.1. Extract yields

Extract yields are given in Section 2. Although there is no linear correlation between the yield and the polarity, deodorised methanol/water (DWE) extract gave the highest extract yield when compared to that of acetone oleoresin. This might be attributed to the solvent strength.

3.2. Chemical composition of the essential oil

The chemical composition of the essential oil obtained by hydrodistillation from the aerial parts of *F. orientalis* was determined by GC and GC–MS (Table 1). Thirty nine compounds were identified, representing 90.3% of the oil. The oil was found to be rich in the monoterpenes, β-phellandrene (23.6%), (*E*)-β-ocimene (13.8%), α-phellandrene (11.5%), α-pinene (12.5%) and (*Z*)-β-ocimene (3.5%). The oil of *F. orientalis* is also characterized by the presence of dehydro-sesquicineole (10.1%), which is an oxygenated sesquiterpene compound. Generally, many sesquiterpenes, either hydrocarbons or oxygenated, are included in the oil composition (Table 1).

Table 1
Chemical composition of the aerial parts of *Ferula orientalis* essential oil (%)

	K.I.[a]	Compounds	% Composition
1	911	α-Thujene	0.1
2	917	α-Pinene	12.5
3	933	Camphene	1.3
4	961	Sabinene	tr[b]
5	964	β-Pinene	0.8
6	981	β-Myrcene	2.0
7	996	α-Phellandrene	11.5
8	1021	*p*-Cymene	tr
9	1026	β-Phellandrene	23.6
10	1039	(*Z*)-β-Ocimene	3.5
11	1050	(*E*)-β-Ocimene	13.8
12	1064	γ-Terpinene	0.1
13	1097	Terpinolene	0.9
14	1105	3-Methyl-phenol	tr
15	1139	(*cis*)-*p*-Menth-2-en-1-ol	0.1
16	1147	(allo)-Ocimene	0.5
17	1162	(neo-allo)-Ocimene	0.1
18	1196	2,5-Dimethyl-3-(1-hydroxyethyl)-furan	2.4
19	1206	Terpinen-4-ol	0.1
20	1216	Cryptone	0.3
21	1318	*p*-Menth-1-en-7-al	tr
22	1323	4-Hydroxy-3-methylacetophenone	0.4
23	1329	Bornyl acetate	0.9
24	1360	Carvacrol	0.2
25	1432	Daucene	0.1
26	1511	α-Caryophyllene	0.2
27	1527	Dehydro-sesquicineole	10.1
28	1539	γ-Curcumene	0.4
29	1542	αr-Curcumene	0.1
30	1555	α-Zingiberene	0.1
31	1558	Bicyclogermacrene	0.1
32	1567	β-Bisabolene	0.1
33	1583	β-Sesquiphellandrene	0.1
34	1590	(*E*)-γ-Bisabolene	0.2
35	1601	α-Bisabolene[c]	0.4
36	1684	β-Atlantol	1.1
37	1696	Gossonorol	0.1
38	1725	α-Eudesmol	0.1
39	1739	α-Bisabolol	2.2
Total			90.3

[a] Kovats index on non-polar HP-5ms column in reference to *n*-alkanes.
[b] tr: trace ⩽ 0.06%.
[c] Correct isomer not identified.

To the best of our knowledge, the essential oil of *F. orientalis* has not been the subject of previous studies. In the literature, there are data for the essential oils of other *Ferula* species, such as *Ferula assa-foetida*, *Ferula stenocarpa*, *Ferula elaeochytris*, and *Ferula flabelliloba* (Baser et al., 2000; Filippini, Tomi, & Casanova, 2000; Rustaiyan, Assadian, Monfared, Masoudi, & Yari, 2001; Rustaiyan, Monfared, & Masoudi, 2001; Sefidkon, Askari, & Mirza, 1998).

The essential oil profile of *F. orientalis* was quite different from the oils of the above-mentioned species, although some of the main components are common. (*Z*)-β-Ocimene and (*E*)-β-ocimene were found in the oil of *F. assa-foetida* at the percentages of 11.9% and 9.0%, respectively (Sefidkon et al., 1998). β-Phellandrene was one of the main components (16.6%) of *F. arrigoni* essential oil (Chen et al.,

1992), whereas α-pinene was detected to the oils of *F. stenocarpa*, *F. elaeochytris* and *F. flabelliloba* at percentages varying from 48.8% to 10.0% (Baser et al., 2000; Rustaiyan et al., 2001; Rustaiyan et al., 2001).

3.3. Free radical-scavenging activity

Free radical scavenging actions of the extracts are concentration-dependent. For example, the inhibition percentage of DAO and AO extracts of *F. orientalis* ranged from 0.02 to 0.4 mg/ml and 100% inhibition was observed over this concentration (Fig. 1). Extract concentrations providing 50% inhibition of *F. orientalis* are given in Fig. 2. As shown, the non-deodorised extract (MWE) of the plant was able to reduce the stable radical DPPH· to the yellow-coloured diphenylpicrylhydrazine with IC_{50} values at 99.1 ± 0.5 μg/ml. Also, acetone extracts (DAO and AO) showed activity with an IC_{50} of 118 ± 0.8 μg/ml and 310 ± 0.5 μg/ml, respectively. The essential oil obtained from herbal parts exhibited similar activity (423 ± 0.8 μg/ml). On the other hand, HE and DWE were not effective at low concentrations and scavenging action over 10.0 mg/ml concentration was concluded to be possessing no activity. The concentrations of the positive controls, BHT and AA, scavenging 50% of the free radical (IC_{50}) were found to be 19.8 ± 0.5 and 3.76 ± 0.3 μg/ml, respectively.

Essential oil of *F. orientalis* was applied on to a silica gel TLC plate and 11 main zones were determined. With DPPH reagent, only two yellow spots appeared after spraying the TLC plate. Among the oil components of *F. orientalis*, only β-ocimene (*Z*/*E*) isomers and terpinolene were reported as the compounds responsible for the activity (Ruberto & Baratta, 2000). Some pure components, such as α-pinene and β-phellandrene, were tested, employing the above-mentioned methods, and showed no or very weak activity. But other components of the oil were not tested since the oil has a lower activity among the extracts tested exhibiting higher IC_{50} values. Therefore, detailed studies with oil components were not carried out.

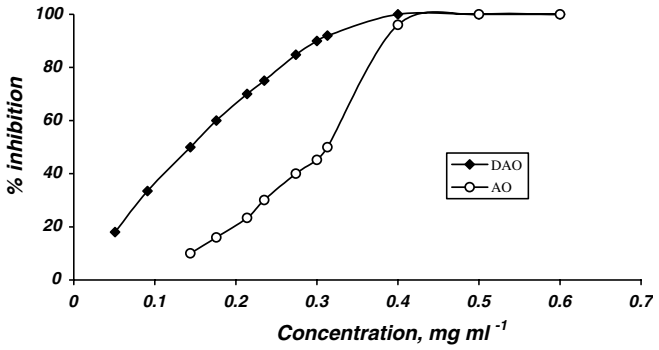


Fig. 1. Inhibition ratio (%) against increasing *F. orientalis* concentration in DPPH assay. (Standard deviation of each parameter is ca. 0.05. Therefore, standard deviation bars are not included in the figure.)

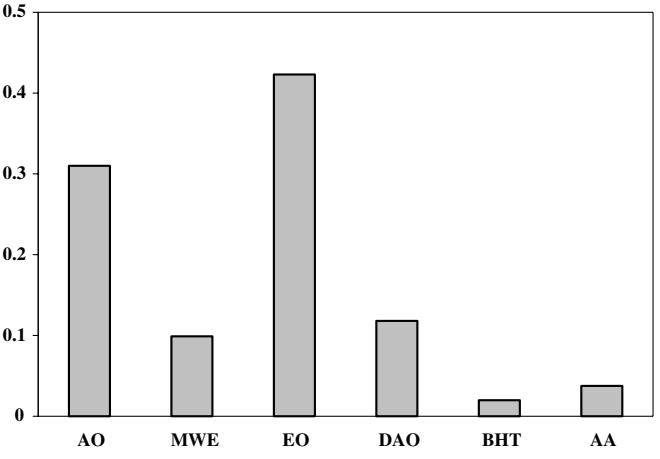


Fig. 2. IC_{50} (mg ml^{-1}) values of the extracts of *F. orientalis* in DPPH assay. (Extracts exhibiting weak or no activity were not included; AO, acetone oleoresins; MWE, methanol water extract; EO, essential oil; DAO, deodorized acetone oleoresins; BHT, butylated hydroxy toluene; AA, ascorbic acid.)

3.4. Inhibition of linoleic acid oxidation

In general, non polar DAO extracts seem to inhibit the oxidation of linoleic acid that is an important issue in food processing and preservation (Fig. 3).

Antioxidants minimize the oxidation of lipid components in cell membranes or inhibit the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation that are known to be carcinogenic. Relative antioxidant activities (RAA%) of the extracts were calculated from the equation given below:

$$RAA\% = A_{Sample}/A_{BHT} \times 100,$$

where A_{BHT} is the absorbance of the positive control BHT and $A_{Sample}$is the absorbance of the extract. Calculated RAA% of the extracts are given in Table 2.

The highest inhibition was provided by DAO (76.1%), followed by AO (73.8%) and MWE (66.3%). Oxidation of linoleic acid was inhibited at 10.1% in the presence of

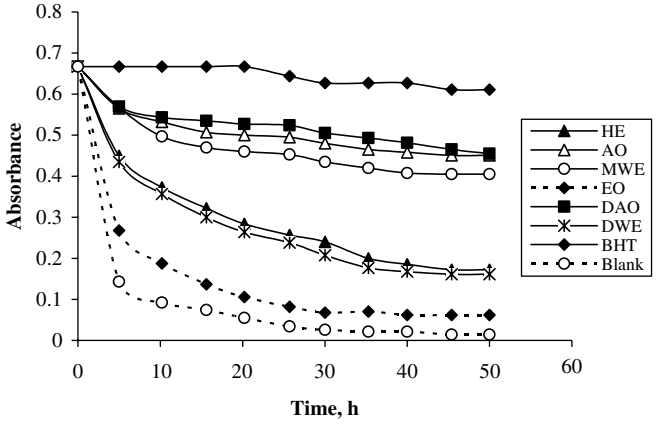


Fig. 3. Absorbance change of β-carotene at 490 nm in the presence of *F. orientalis* extracts, control and positive control BHT. (Standard deviation of each parameter is ca. 0.05. Therefore, standard deviation bars are not included in the figure.)

Table 2
Relative antioxidant activity of *F. orientalis* extracts and positive control (BHT) in β-carotene/linoleic acid assay

Extracts	RAA[a] %
HE	28.0
AO	73.8
MWE	66.3
EO	10.1
DAO	76.1
DWE	26.4
BHT	100

[a] ±0.1% Standard deviation.

2 mg/ml essential oil (EO) and that can be concluded to be weak activity. However, presence of high concentrations of non-polar phenolics in the extract might promote the inhibiting effect on linoleic acid oxidation. Polar extract MWE (66.3%) was also effective at this concentration.

The active component in MWE of *F. orientalis* was tentatively determined by TLC to be ferulic acid.

4. Conclusion

Both extract yields and economical aspects of the extraction methods should be considered since cheaper solvents (e.g., water) or solvent mixtures would cut the cost. Therefore, extraction with methanol–water (1:1) mixture could be used for *F. orientalis*. It gave higher extract yield and was highly effective for radical scavenging and inhibition of linoleic acid oxidation.

Acknowledgement

Contract/grant sponsor: Research Council of Cumhuriyet University, Sivas, Turkey. Contract/Grant No. F-124/2000.

References

Adams, R. P. (2001). *Identification of essential oils components by gas chromatography/quadrupole mass spectroscopy*. IL, USA: Allured Publishing Corporation.

Barriere, C., Centeno, D., Lebert, A., Leroy-Setrin, S., Berdague, J. L., & Talon, R. (2001). Roles of superoxide dismutase and catalase of *Staphylococcus xylosus* in the inhibition of linoleic acid oxidation. *FEMS Microbiology Letters, 201*, 181–185.

Baser, K. H. C., Ozek, T., Demirci, B., Kurkcuoglu, M., Aytac, Z., & Duman, H. (2000). Composition of the essential oils of *Zosima absinthifolia* (Vent.) link and *Ferula elaeochytris* Korovin from Turkey. *Flavour and Fragrance Journal, 15*, 371–372.

Burits, M., & Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytotherapy Research, 14*, 323–328.

Chen, Q., Shi, H., & Ho, C. T. (1992). Effects of rosemary extracts and major constituents on lipid oxidation and soybean lipoxygenase activity. *Journal of the American Oils Chemists Society, 69*, 999–1002.

Chevolleau, S., Mallet, J. F., Ucciani, E., Gamisans, J., & Gruber, M. (1992). Antioxidant activity in leaves of some mediterranean plants. *Journal of the American Oils Chemists Society, 69*, 1269–1271.

Cuendet, M., Hostettmann, K., & Potterat, O. (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helvetica Chimica Acta, 80*, 1144–1152.

Dapkevicius, A., Venskutonis, R., Van Beek, T. A., & Linssen, P. H. (1998). Antioxidant activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *Journal of the Science of Food and Agriculture, 77*, 140–146.

Davis, P. H. (1965–1984). *Flora of Turkey and the East Aegean Islands* (Vol. 7). Edinburgh: Edinburgh University Press.

Duke, J. A. (2002). Dr. Duke's phytochemical and ethnobotanical databases. *Agricultural Research Service Publication*.

Filippini, M. H., Tomi, F., & Casanova, J. (2000). Composition of the leaf oil of *Ferula arrigonii* Bocchieri. *Flavour and Fragrance Journal, 15*, 195–198.

Gordon, M. H. (1990). The mechanism of antioxidant action in vitro. In B. J. F. Hudson (Ed.), *Food Antioxidants* (pp. 1–18). London: Elsevier.

Halliwell, B., Gutteridge, J. M. C., & Aruoma, O. I. (1987). The deoxyribose method: a simple 'test tube' assay for determination of rate constants for reaction of hydroxyl radicals. *Analytical Biochemistry, 165*, 215–219.

Halliwell, B., & Gutteridge, J. M. C. (1989). *Free radicals in biology and medicine* (pp. 416–494) (2nd ed.). Oxford: Clarendon Press.

Halliwell, B. (1990). How to characterize a biological antioxidant. *Free Radical Research Community, 9*, 13–14.

Koleva, I. I., van Beek, T. A., Linssen, J. P. H., de Groot, A., & Evstatieva, L. N. (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochemical Analysis, 13*, 8–17.

Kramer, R. E. (1985). Antioxidants in clove. *Journal of the American Oils Chemists Society, 62*, 111–113.

Ou, B., Huang, D., Hampsch-Woodill, M., Flanagan, J. A., & Deemer, E. K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assay: a comparative study. *Journal of Agricultural and Food Chemistry, 50*, 3122–3128.

Philipson, J. D. (1990). Plants as sources of valuable products. In B. V. Charlwood & M. J. Rhodes (Eds.), *Secondary products from plant tissue culture* (pp. 1–22). Oxford: Clarendon Press.

Ruberto, G., & Baratta, M. T. (2000). Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chemistry, 69*, 167–174.

Rustaiyan, A., Assadian, F., Monfared, A., Masoudi, S., & Yari, M. (2001). Composition of the volatile oil of *Ferula stenocarpa* Boiss. and Hausskn. *Journal of Essential Oil Research, 13*, 181–182.

Rustaiyan, A., Monfared, A., & Masoudi, S. (2001). The essential oil of *Ferula flabelliloba* Rech. F. et Aell. *Journal of Essential Oil Research, 13*, 403–404.

Sefidkon, F., Askari, F., & Mirza, M. (1998). Essential oil composition of *Ferula assa-foetida* L. from Iran. *Journal of Essential Oil Research, 10*, 687–689.

Sokmen, A., & Gurel, E. (2001). Bitki Biyoteknolojisi "Plant biotechnology". In M. Babaoglu, E. Gurel, & S. Ozcan (Eds.), *Sekonder Metabolit Uretimi (Secondary metabolite production)* (pp. 211–261). Konya: Selcuk University Press.