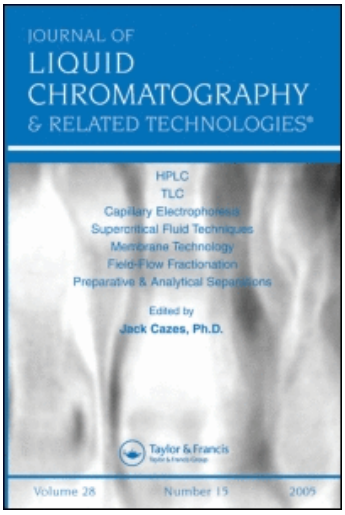

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Antioxidant Activity of S-Carvone Isolated from Spearmint (*Mentha Spicata L. Fam Lamiaceae*)

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To cite this Article Elmastaş, Mahfuz , Dermirtas, Ibrahim , Isildak, Omer and Aboul-Enein, Hassan Y.(2006) 'Antioxidant Activity of S-Carvone Isolated from Spearmint (*Mentha Spicata L. Fam Lamiaceae*)', Journal of Liquid Chromatography & Related Technologies, 29: 10, 1465 — 1475
To link to this Article: DOI: 10.1080/10826070600674893
URL: http://dx.doi.org/10.1080/10826070600674893

Journal of Liquid Chromatography & Related Technologies®, 29: 1465–1475, 2006

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070600674893

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Abstract: S-Carvone was isolated from *Mentha spicata* (*Fam. Lamiaceae*) and purified using chromatographic methods, and also characterized with GS-MS, FTIR, and NMR techniques. The antioxidant activity of S-carvone obtained from *Mentha spicata* was evaluated as *in vitro* using a total antioxidant activity test. Results were compared with a standard antioxidant, α-tocopherol. The results indicate that S-carvone possess high antioxidant activity compared to α-tocopherol.

Keywords: *Mentha spicata*, S-Carvone, Antioxidant activity

INTRODUCTION

The life supporting oxygen becomes toxic to most aerobic organisms when exposed to greater concentrations. Reasons for this toxicity are due to the formation of superoxide (O_2^-) hydrogen peroxide (H_2O_2) and the hydroxyl radical ($-OH^{\cdot}$) during the conversion of oxygen to water in the mitochondria. The free radicals generated from environmental contaminants and by

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exogenous factors such as drugs, toxins, and stress cause oxidative damage to biological macromolecular structure and function.[1]

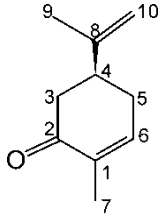
Many species of fruits, vegetables, herbs, cereals, sprouts, and seeds have been investigated for antioxidant activity during the last decade.[2,3] Natural antioxidants are extensively studied for their capacity to protect organisms and cells from damage which are induced by oxidative stress, the latter being considered a cause of ageing, degenerative diseases, and also cancer. Herbs and spices are excellent sources for obtaining natural antioxidant.

Obviously, there has been an increasing demand to evaluate the antioxidant properties of direct plant extracts or isolated products from plant origins, rather than looking for synthetic ones. It is an established fact that polyphenolic compounds, such as flavonoids, anthrequinones, anthocyanids, and xanthones, possess remarkable antioxidant activities, which are present quite commonly in the plants.[4] The high participation of fruits, vegetables, and herbs in the human diet, because of their ability to neutralize active oxygen species, is of utmost importance.

Carvone (*p*-mentha-6, 8-dien-2-one Figure 1), a main constituent of *Metha spicata*, has potential uses for inhibiting the growth of bacteria,[5] some fungi,[6] and as an insect repellent.[7] The most important technical application of carvone is its use as a reversible suppressant of sprouting in stored potatoes or flower bulbs.[8]

There are several published reports on the chemical composition of *Mentha spicata* (*MS*).[9–11]. However, there is only a preliminary study about *in vitro* antioxidant activity of *Mentha spicata.* The present paper describes the antioxidant activity and free radical scavenging activity. The main focus of this preliminary study is for the *in vitro* antioxidant activity of S-carvone isolated from *Mentha spicata*, which is compared with α-tocopherol and which is commonly used as food antioxidant.

In most cases, the interactions that invoke a biological response take place at receptor sites in the body that are chiral and are produced in only one enantiomeric form. Therefore, the two enantiomers of a biologically active material do not elicit the same response. Indeed, the differences are often dramatic. For example, both enantiomeric terpenes, (S)-carvone and



S-carvone

Figure 1. Structure of S-carvone.

(R)-carvone, have a distinctive odor, but the first one is a major contributor to the odor of MS, and the second to the odor of caraway seeds.

The purity of the isolated S-carvone was checked using GC, GC-MS, ^{1}H-NMR, and ^{13}C-NMR spectral analyses. The data obtained were compared with those obtained from authentic samples and literature data. The DEPT experimental data, three separate data sets, one each for 45, 90, and 135 were plotted to identify the number of protons attached to each carbon. This information then provided evidence for the most likely structure assigned to the compound of S-carvone.

EXPERIMENTAL

General

Infrared spectra were recorded on a Nicolet Magna 550 FTIR spectrometer using an attenuated total reflectance (ATR) ZnSe-plate for solid samples (unless otherwise noted). High resolution NMR spectra (^{1}H and ^{13}C) were obtained using Bruker Spectrospin Avance DMX 200 and DMX 300 spectrometers, where the reference compounds $SiMe_4$ or others are software controlled; *J*-values are given in Hz. Ultraviolet spectra were recorded using a Shimadzu UV-260 spectrophotometer, and mass spectra were obtained using a Micromass Instrument ProSpec Q. Dry solvents used in this study were stored under nitrogen over Molecular Sieve.

Chemicals and Plant Material

Ammonium thiocyanate was purchased from E. Merck (Darmstadt, Germany). Ferrous chloride and α-tocopherol were purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). *Mentha spicata* was harvested from the Black Sea region of Turkey. It was stored at the Gaziosmanpasa University Faculty of Agriculture, Medicinal Plant Herbarium Laboratory (No: LM-010). 1,1-diphenyl-2-picryl-hydrazyl (DPPH·) was purchased from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany). All other chemicals used were of analytical grade and were obtained from Sigma (Sigma-Aldrich GmbH, Sternheim, Germany).

Extraction and Isolation

The dried and ground samples (25 g) of Mentha Spicata were sequentially extracted with ethanol (500 mL). The residue was re re-extracted under the same condition until the extraction solvents became colourless. The obtained extract was filtered over Whatman No.1 paper and the filtrate was collected; then the ethanol was removed with a rotary evaporator at 40°C to

obtain a dry extract. The crude material (2.1 g) was applied to the column chromatography using the silica gel (50 g), using hexane:ethyl acetate (9:1 v/v) as eluant. The eluted fractions were collected in 15 mL sample tubes, monitored with UV, and the fractions (3–12) combined. The purified substance (245 mg of S-carvone) was obtained as a yellow oil. The compound was characterized using GC, GC-MC, and by FTIR (Figures 2–4, respectively) proton and carbon NMR.

(S)-Carvone; ^{1}H-NMR ($CDCl_3$): 1.75 (s, 6H, H-7,9), 2.28–2.82 (m, 5H, H-3,4,5), 4.77 (s, H-10), 6.75 (H-6). ^{13}C-NMR ($CDCl_3$): 14.85 (C-9), 19.66 (C-7), 30.42 (C-5), 41.69 (C-4), 42.31 (C-3), 109.81 (C10), 134.64 (C-1), 146.04 (C-8), 198.66 (C-2).

The rest of fractions are being examined for antioxidant activity as an ongoing experiment.

Determination of Total Antioxidant Activity

The thiocyanate method[12] for the measurement of antioxidant activity was applied to S-carvone in the present work. *MS* extract, S-carvone, or standard samples of 100 and 250 μg/mL in 2.5 mL of potassium phosphate buffer (0.04 M, pH 7.0), were added to linoleic acid, 2.5 mL of emulsion in potassium phosphate buffer (0.04 M, pH 7.0). The mixed solution was incubated at 37°C in the dark. The mixture was stirred for 3 min, and the peroxide value was determined by reading the absorbance at 500 nm in a

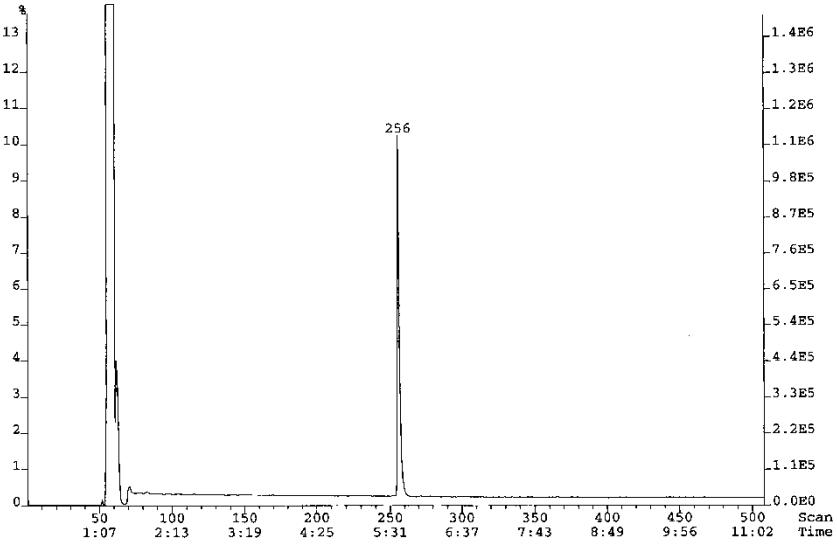


Figure 2. GC Chromatogram of carvone isolated from *Mentha spicata*.

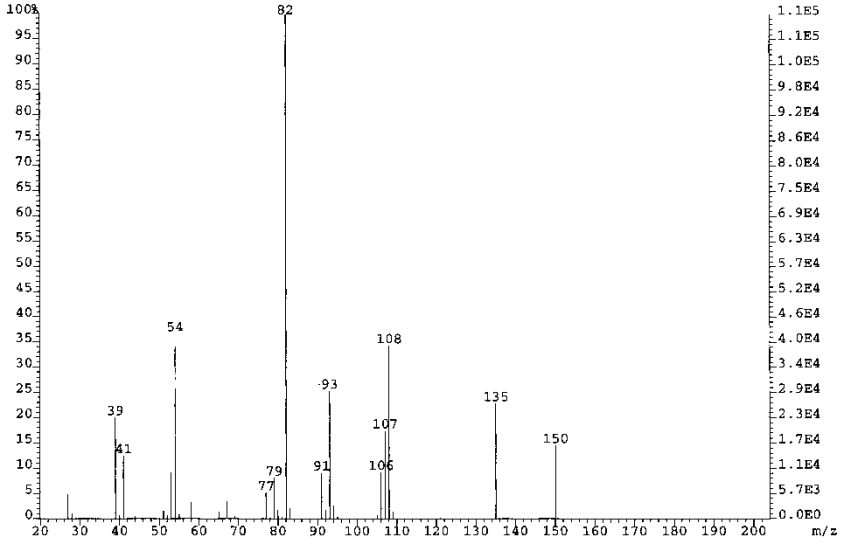


Figure 3. GC-MS result of the purified extract of *Mentha spicata.*

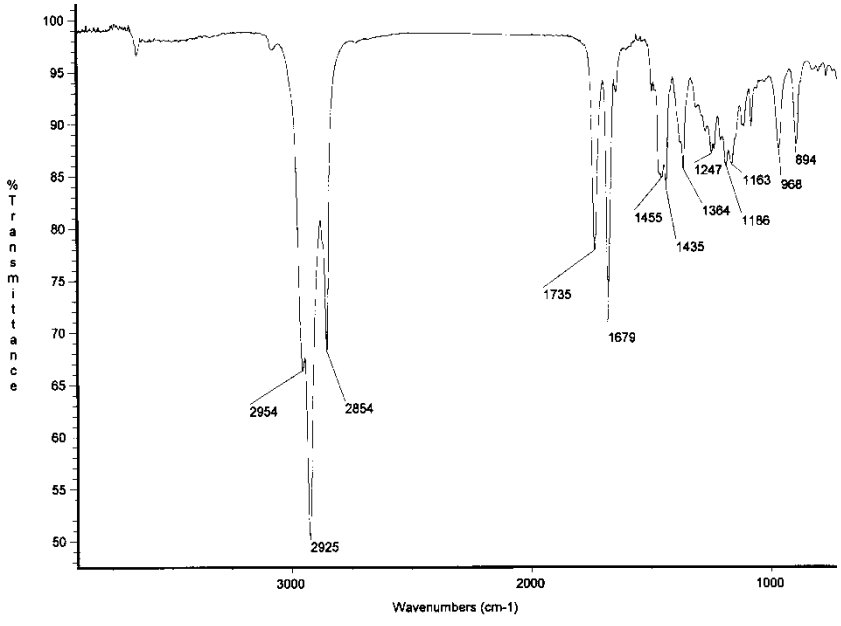


Figure 4. FTIR spectrum of carvone isolated from *Mentha spicata*.

spectrophotometer after reaction with $FeCl_2$ and thiocyanate, at intervals during incubation. During the linoleic acid oxidation, peroxides formed and these compounds oxidize Fe^{+2} to Fe^{+3}. The latter ions form a complex with SCN^- and this complex has maximum absorbance at 500 nm. Therefore, high absorbance indicates high linoleic acid oxidation. The solutions without extracts are used as blank samples. All data about total antioxidant activity are the average of triplicate analyses. The inhibition of lipid peroxidation percent was calculated by following equation:

$$\% \text{ Inhibition} = 100 - [(A_1/A_o) \times 100]$$

Where A_o was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample of *MS* extract, S-carvone or standards.

Free Radical Scavenging Activity

The free radical scavenging activity of S-carvone was measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH·) using the method of Blois.[13] Briefly, 0.1 mM solution of DPPH· in ethanol was prepared and 1 mL of this solution was added to 3 mL of S-carvone solution in ethanol at different concentrations (60, 120, 180 μg/mL). The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes. Then, the absorbance was measured at 517 nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The DPPH· concentration (mM) in the reaction medium was calculated from the following calibration curve, determined by linear regression (R^2: 0.997):

$$\text{Absorbance} = 0.0003 \times [\text{DPPH}\cdot] - 0.0174$$

The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH} \cdot \text{Scavenging Effect } (\%) = [(A_o - A_1/A_o) \times 100]$$

Where A_o was the absorbance of the control reaction and A_1 was the absorbance in the presence of S-carvone solution or standard.

Reducing Power

The reducing power of the S-carvone solution was determined according to the method of Oyaizu.[14] Different concentrations of S-carvone solution (50, 100, 250, 500 μg/mL) in 1 mL of ethanol was mixed with a phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. A portion

(2.5 mL) of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 1,000 × g. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer. Higher absorbance of the reaction mixture indicated greater reducing power.

RESULTS AND DISCUSSION

Total Antioxidant Activity Determination in Linoleic Acid Emulsion

Numerous antioxidant methods and modifications have been proposed to evaluate antioxidant activity and to explain the function of antioxidants. Among these methods, total antioxidant activity determination in linoleic acid emulsion, is the most commonly used for the evaluation of antioxidant activities of extracts.[12,15,16]

S-carvone exhibited effective and powerful antioxidant activity at two concentrations. The effects of these concentrations of S-carvone (100 and 250 μg/mL) on peroxidation of linoleic acid emulsion are shown in Figure 5. The antioxidant activity of S-carvone increased with increasing concentration. The percentages of peroxidation of 100 and 250 μg/mL concentrations of MS and S-carvone in linoleic acid system were 96, 98%, respectively, and greater than that of 250 μg/mL of α-tocopherol (77%).

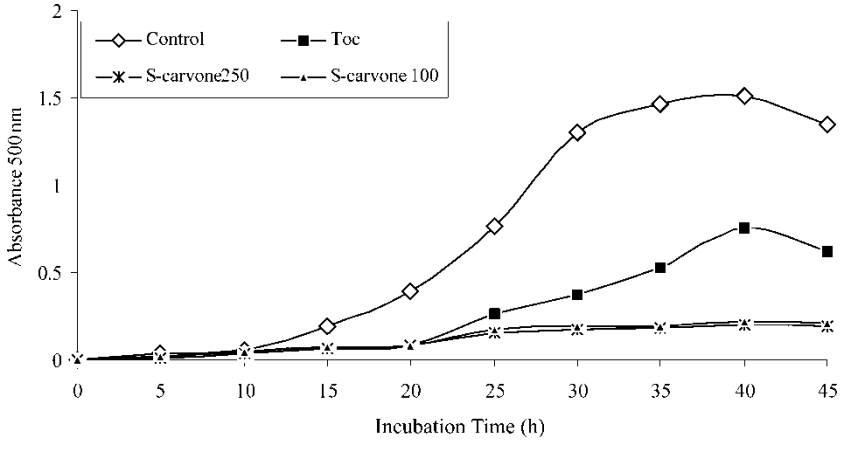


Figure 5. Total antioxidant activity of different concentration of S-carvone isolated from *Mentha spicata* and α-tocopherol in the linoleic acid emulsion was determined by the thiocyanate method (Toc: α-tocopherol, S-carvone100: 100 μg/mL S-carvone and S-carvone 250: 250 μg/mL S-carvone isolated *Mentha spicata*).

These results indicated that S-carvone possesses higher antioxidant activity compared to α-tocopherol.

The model of scavenging the stable DPPH radical is a widely used method to evaluate antioxidant activities in a relatively short time. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability.[17]

DPPH· is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.[18] The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm induced by antioxidants. The absorption maximum of a stable DPPH radical in ethanol was at 517 nm. The decrease in absorbance of a DPPH radical, caused by antioxidants, because of the reaction between antioxidant molecules and radical, occurs, which results in the scavenging of the radical by hydrogen donation. It is visually noticeable as a discoloration from purple to yellow. Hence, DPPH· is usually used as a substrate to evaluate antioxidative activity of antioxidants.[19,20] Figure 6 illustrates a significant ($P < 0.01$) decrease in the concentration of DPPH radical due to the scavenging ability of S-carvone and standards, namely BHA, BHT, and α-tocopheral. S-carvone showed strong DPPH scavenging activity. The scavenging effect of S-carvone and standards on the DPPH radical decreased in the order of BHA > S-carvone > α-tocopherol > BHT, and were 96, 95, 92, and 61 at the concentration of 180 μg/mL, respectively. These results indicated that S-carvone has a noticeable effect on the scavenging free radical. Free radical scavenging activity also increased with increasing concentration.

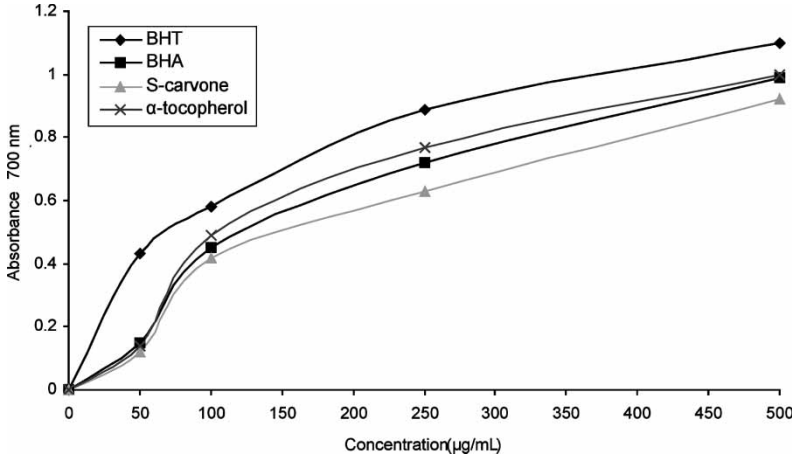


Figure 6. Reducing power of S-carvone, BHA, BHT, and α-tocopherol. (Spectrophotometric dedection of the Fe^{+3}-Fe^{+2} transformation, BHA: Butylated hydroxyanisole BHT: Butylated hydroxytoluene).

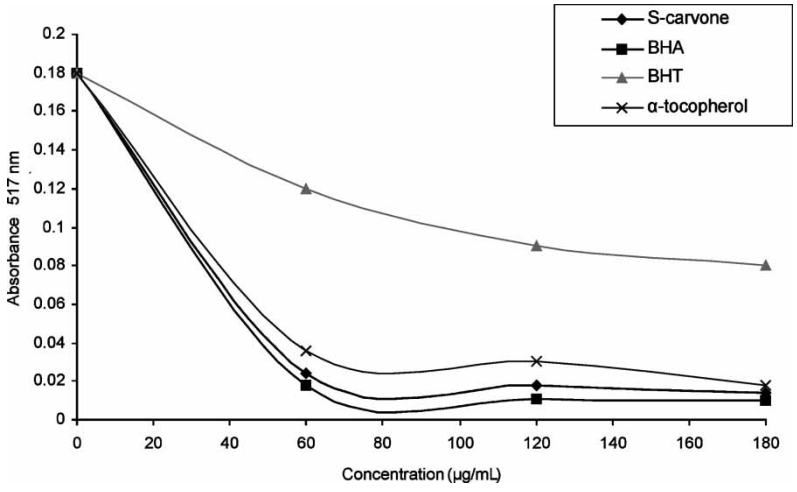


Figure 7. Free radical scavenging activity of S-carvone, BHA, BHT, and α-tocopherol by 1,1-Diphenyl-2-picrylhydrazyl radicals. (BHA: Butylated hydroxyanisole, BHT: Butylated hydroxytoluene).

Figure 7 shows the reductive capabilities of S-carvone compared to BHA, BHT, and α-tocopherol. For the measurements of the reductive ability, we investigated the Fe^{3+}-Fe^{2+} transformation in the presence of S-carvone samples using the method of Oyaizu.[14] The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity.[19] The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity, and radical scavenging.[20] Like the antioxidant activity, the reducing power of S-carvone increased with increasing concentration. All S-carvones showed higher activities than the controls, and these differences were statistically significant ($p < 0.01$). Reducing power of S-carvone and standard compounds was in the order: S-carvone $>$ BHA $>$ α-tocopherol $>$ BHT.

CONCLUSION

On the basis of the results of this study, it is clearly indicated that the ethanol extract of S-carvone isolated from *Mentha spicata* has significant antioxidant activity in the linoleic acid emulsion systems *in vitro*. Moreover, S-carvone can be used as an easily accessible source of natural antioxidants, and as a possible food supplement or in the pharmaceutical industry. It might be possible to use *Mentha spicata* for the same purpose.

ACKNOWLEDGMENTS

ME and OI are grateful to the University of Gaziosmanpasa for financial support (Grant Nr: 2003/33). ID is thankful to Department of Chemistry, Oslo University for providing the spectral facilities.

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Received December 24, 2005
Accepted January 15, 2006
Manuscript 6799