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ScienceDirect
Food Chemistry 108 (2008) 1019–1022
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidative activity of *Rosmarinus officinalis* L. essential oil compared to its main components

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Received 10 July 2007; received in revised form 22 October 2007; accepted 12 November 2007

Abstract

This study was designed to examine the in vitro antioxidant activities of *Rosmarinus officinalis* L. essential oil compared to three of its main components (1,8-cineole, α-pinene, β-pinene). GC–MS analysis of the essential oil resulted in the identification of 19 compounds, representing 97.97% of the oil, the major constituents of the oil were described as 1,8-cineole (27.23%), α-pinene (19.43%), camphor (14.26%), camphene (11.52%) and β-pinene (6.71%). The oil and the components were subjected to screening for their possible antioxidant activity by means of 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay and β-carotene bleaching test. In the DPPH test system, free radical-scavenging activity of *R. officinalis* L. essential oil, 1,8-cineole, α-pinene and β-pinene were determined to be 62.45% ± 3.42%, 42.7% ± 2.5%, 45.61% ± 4.23% and 46.21% ± 2.24% (v/v), respectively. In the β-carotene bleaching test system, we tested series concentration of samples to show the antioxidant activities of the oil and its main components, whereas the concentrations providing 50% inhibition (IC_{50}) values of *R. officinalis* L. essential oil, 1,8-cineole, α-pinene and β-pinene were 2.04% ± 0.42%, 4.05% ± 0.65%, 2.28% ± 0.23% and 2.56% ± 0.16% (v/v), respectively. In general, *R. officinalis* L. essential oil showed greater activity than its components in both systems, and the antioxidant activities of all the tested samples were mostly related to their concentrations. Antioxidant activities of the synthetic antioxidant, ascorbic acid and BHT, were also determined in parallel experiments as positive control.
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Keywords: *Rosmarinus officinalis* L.; 1,8-Cineole; α-Pinene; β-Pinene; Antioxidant activities; GC–MS

1. Introduction

Free radicals may play an important role in the origin of life and biological evolution, implication their beneficial effects on the organisms (McCord, 2000). For example, oxygen radicals exert critical actions such as signal transduction, gene transcription and regulation of soluble guanylate cyclase activity in cells (Lander, 1997). However, free radicals and other relative species could also cause the oxidation of biomolecules which leads to cell injury and death (Freidovich, 1999; Ignarro, Cirino, Casini, & Napoli, 1999; Lander, 1997; McCord, 2000).

Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods by free radicals. Since ancient times, spices in different types of food to improve flavors are well known for their antioxidant capacities (Madsen & Bertelsen, 1995). In recent decades, the essential oils and their components of plants have been of great interest as they have been the sources of natural products. In order to prolong the storage stability of foods, synthetic antioxidants are used for industrial processing. But the side effects of some synthetic antioxidants used in food processing such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have already been documented. For example, these substances can exhibit carcinogenic effects in living organisms (Ames, 1983; Baardseth, 1989). For this reason, governmental authorities and consumers are concerned about the safety of the food and also about the potential effects of synthetic additives on health. Recently, numerous reports have described antioxidants and compounds with

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doi:10.1016/j.foodchem.2007.11.046

radical-scavenging activity present in fruits, vegetables, herbs and cereals extracts (Gray, Clarke, Baux, Bunting, & Salter, 2002; Hou et al., 2005; Nuutila, Pimia, Aarni, & Caldenty, 2003).

Rosmarinus officinalis L. belongs to the Lamiaceae family of herbs, which in addition to being used as a food flavoring, is also known medicinally for its powerful antibacterial, antimutagenic properties, and as a chemopreventive agent (Oluwatuyi, Kaatz, & Gibbons, 2004). Owing to its antioxidant properties of leaves, *R. officinalis* L. has been widely accepted as one of the spices with the highest antioxidant activity (Peng, Yuan, Liu, & Ye, 2005). In this paper, we report the results of a study aimed to evaluate and compare the in vitro antioxidant properties of *R. officinalis* L. essential oil with three of its components – 1,8-cineole, α-pinene, β-pinene.

2. Materials and methods

2.1. Materials

Samples were obtained via steam distillation as pure essential oils from a number of commercial sources (Si Chuan Province, China); all the samples were stored in glass vials with Teflon sealed caps at -20 ± 0.5 °C in the absence of light.

2.2. Gas chromatography

The analysis of the essential oil was performed using a VG platform II GC–MS system equipped with a DB-5MS capillary column (30 × 0.25 mm i.d.; film thickness 0.25 μm). For GC–MS detection, an electron ionisation system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 ml/min. Injector and detector MS transfer line temperatures were set at 160 °C and 265 °C, respectively. Column temperature was initially kept at 50 °C, then gradually increased to 160 °C at 3 °C/min rate, held for 2 min and finally raised to 280 °C at 10 °C/min. 0.1 μl essential oil was injected manually. The components were identified by comparison of their relative retention times and mass spectra with those of standards (for the main components), NIST library data of the GC–MS system and literature data.

2.3. Biological activities

2.3.1. General

Antioxidant activity was assessed by 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and β-carotene bleaching test. All the data collected for each assay were the averages of three determinations of three independent experiments.

2.3.2. Free radical-scavenging activity: DPPH assay

The hydrogen atom or electron donation ability of the oil and the pure compounds was measured from the bleaching of purple-coloured ethanol solution of DPPH. This spectrophotometric assay uses the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a reagent (Amarowicz, Pegg, Moghaddam, Barl, & Weil, 2004). An aliquot of the sample (100 μl) was mixed with 1.4 ml of ethanol and then added to 1 ml of 0.004% DPPH (Sigma–Aldrich) in ethanol. The mixture was shaken vigorously and then immediately placed in a UV–Vis spectrophotometer (AWARENESS) to monitor the decrease in absorbance at 517 nm. Monitoring was continued for 70 min until the reaction reached a plateau. Ascorbic acid (Sigma–Aldrich), a stable antioxidant, was used as a synthetic reference. The radical-scavenging activities of samples, expressed as percentage inhibition of DPPH, were calculated according to the formula: Inhibition percentage $(I_p) = [(AB - AA)/AB] \times 100$ (Yen & Duh, 1994) where AB and AA are the absorbance values of the blank sample and of the tested samples checked after 70 min, respectively.

2.3.3. Antioxidant activity: β-carotene bleaching test

Antioxidant activity of the samples was determined using β-carotene bleaching test (Taga, Miller, & Pratt, 1984). Approximately 10 mg of β-carotene (type I synthetic, Sigma–Aldrich) was dissolved in 10 ml chloroform. The carotene–chloroform solution, 0.2 ml, was pipetted into a boiling flask containing 20 mg linoleic acid (Sigma–Aldrich) and 200 mg Tween 40 (Sigma–Aldrich). Chloroform was removed using a rotary evaporator (RE-52AA) at 40 °C for 5 min, and to the residue, 50 ml of distilled water was added, slowly with vigorous agitation, to form an emulsion. The emulsion (5 ml) was added to a tube containing 0.2 ml of the samples solution and the absorbance was immediately measured at 470 nm against a blank, consisting of an emulsion without β-carotene. The tubes were placed in a water bath at 50 °C and the oxidation of the emulsion was monitored spectrophotometrically by measuring absorbance at 470 nm over a 60 min period. Control samples contained 10 μl of water instead. Butylated hydroxytoluene (BHT, Sigma–Aldrich), a stable antioxidant, was used as a synthetic reference. The antioxidant activity was expressed as inhibition percentage with reference to the control after a 60 min incubation using the following equation: $AA = 100\ (DR_C - DR_S)/DR_C$, where AA = antioxidant activity; DR_C = degradation rate of the control = $[\ln(a/b)/60]$; DR_S = degradation rate in presence of the sample = $[\ln(a/b)/60]$; a = absorbance at time 0; b = absorbance at 60 min.

3. Results and discussion

3.1. Chemical composition

Nineteen components were identified in the *R. officinalis* L. essential oil (Table 1). The major components were 1,8-cineole (27.23%), α-pinene (19.43%), camphor (14.26%), camphene (11.52%) and β-pinene (6.71%), respectively.

Table 1
Composition percentage of *Rosmarinus officinalis* L. essential oil

No.	RT	Compounds	Molecular formula	MW	Relative percentage
1	5.75	α-Thujene	$C_{10}H_{16}$	136	0.43
2	5.99	α-Pinene	$C_{10}H_{16}$	136	19.43
3	6.39	Camphene	$C_{10}H_{16}$	136	11.52
4	7.24	β-Pinene	$C_{10}H_{16}$	136	6.71
5	7.81	β-Phellandrene	$C_{10}H_{16}$	136	1.23
6	8.03	α-Terpinene	$C_{10}H_{16}$	136	0.41
7	8.23	*p*-Cymene	$C_{10}H_{14}$	134	1.67
8	8.36	Limonene	$C_{10}H_{16}$	136	1.95
9	8.48	1,8-Cineole	$C_{10}H_{18}O$	154	27.23
10	9.14	Linalool	$C_{10}H_{18}O$	154	0.25
11	10.26	Isopulegol 2	$C_{10}H_{18}O$	154	1.19
12	11.80	Camphor	$C_{10}H_{16}O$	152	14.26
13	12.5	Borneol	$C_{10}H_{18}O$	154	3.17
14	12.72	Terpinene 4-ol	$C_{10}H_{18}O$	154	0.53
15	13.14	α-Terpineol	$C_{10}H_{18}O$	154	2.11
16	13.53	Verbenone	$C_{10}H_{14}O$	150	1.48
17	15.75	Bornyl acetate	$C_{12}H_{20}O_2$	196	1.13
18	19.65	β-Caryophyllene	$C_{15}H_{24}$	204	2.41
19	20.65	α-Caryophyllene	$C_{15}H_{24}$	204	0.86

Compounds, identified on the basis of comparison with MS database spectra, retention indices and pure reference chemicals, are listed in order of elution from a DB-5MS column. RT: retention time; MW: molecular weight.

3.2. Antioxidant activity

The antioxidant activities of the essential oil of *R. officinalis* L. and its main components studied here were determined by two complementary test systems, namely DPPH assay and β-carotene bleaching test.

Results of the antioxidant activity test systems can be seen in Figs. 1–3. In the DPPH test system, free radical-scavenging activity of *R. officinalis* L. oil was determined to be 62.45% ± 3.42%, whereas the values of 1,8-cineole, α-pinene, β-pinene were 42.74% ± 2.5%, 45.61% ± 4.23% and 46.21% ± 2.24%, respectively. When compared to 4.00% ascorbic acid (86.93% ± 3.21%), all the samples were less effective than this synthetic antioxidant agent.

We assessed the lipid peroxidation inhibitory activity of the essential oil and its components by the β-carotene bleaching test (Fig. 2). Results were consistent with data obtained from the DPPH test. Compared with butylated hydroxytoluene(BHT), *R. officinalis* L. essential oil, 1, 8-cineole, α-pinene and β-pinene bleached β-carotene by 96.94% ± 0.29%, 82.315% ± 1.19%, 93.34% ± 1.13% and 94.49% ± 0.61% at the concentration of 100%, whereas at the concentration of 50%–1.56% the bleached β-carotene abilities were in the range of 91.19% ± 2.20%–48.62%

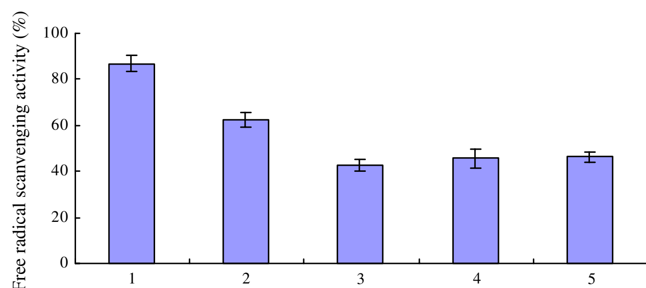

Fig. 1. Free radical-scavenging activity percentage of *Rosmarinus officinalis* L. essential oil (2), 1,8-cineole (3), α-pinene (4), β-pinene (5) evaluated by the 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay and by comparison with 4.00% (w/v) ascorbic acid (1).

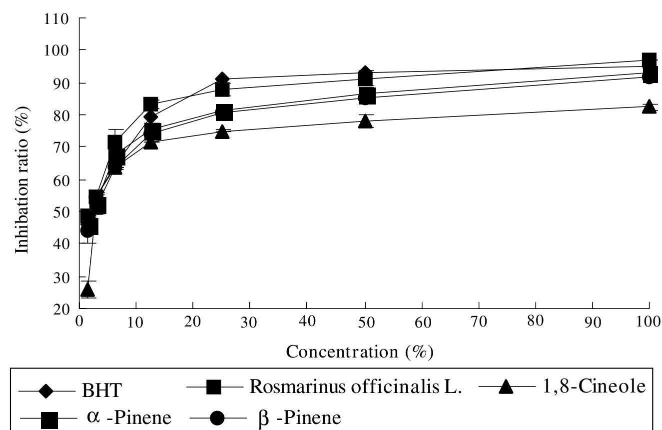

Fig. 2. Antioxidant activity percentage of *Rosmarinus officinalis* L. essential oil, 1,8-cineole, α-pinene, β-pinene determined by β-carotene bleaching test by comparison with the reference-butylated hydroxytoluene (BHT).

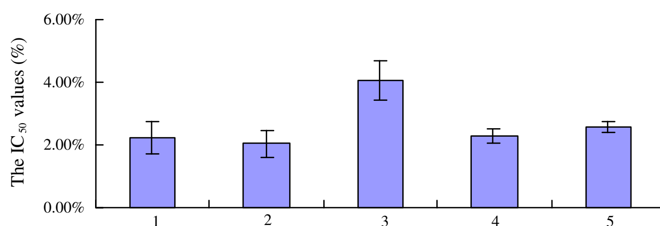

Fig. 3. The IC_{50} values of BHT (1), *Rosmarinus officinalis* L. essential oil (2), 1,8-cineole (3), α-pinene (4), β-pinene (5) evaluated by β-carotene bleaching test.

± 0.28%, 78.06% ± 1.72%–25.87% ± 2.59%, 86.79% ± 1.19–46.29% ± 2.71% and 85.41% ± 0.44%–44.20% ± 3.92%, respectively.

The concentrations providing 50% inhibition (IC_{50}) values of BHT, *R. officinalis* L. essential oil, 1,8-cineole, α-pinene and β-pinene were 2.25% ± 0.52%, 2.04% ± 0.42%, 4.05% ± 0.65%, 2.28% ± 0.23%, 2.56% ± 0.16%, respectively. It seemed the antioxidant activities of all the tested samples were mostly related to their concentrations. The IC_{50} of *R. officinalis* L. essential oil was lower than the synthetic antioxidant BHT.

In both test systems, all of the components and the oil exhibited remarkable antioxidant activities. In general, *R. officinalis* L. essential oil showed greater activity than its components in both systems.

It is very difficult to attribute the antioxidant effect of a total essential oil to one or a few active principles, because an essential oil always contains a mixture of different chemical compounds. In addition to the major compounds, also minor compounds may make a significant contribution to the oil's activity. From the results above we could infer that *R. officinalis* L. essential oil indicating the antioxidant activity is the cooperating results of their compositions.

4. Conclusion

Consumer demanding for healthy food products provide an opportunity to develop antioxidants as new functional foods. Following this idea, the antioxidant activities of *R. officinalis* L. essential oil, 1,8-cineole, α-pinene and β-pinene had been compared using the DPPH and β-carotene bleaching assay. In DPPH assay, *R. officinalis* L. essential oil showed a higher antioxidant activity than 1,8-cineole, α-pinene and β-pinene. And in β-carotene bleaching assay, various samples showed remarkable antioxidant activities. All the samples have strong antioxidant activity and may be rich sources of antioxidants. The result is very much needed by the food industry in order to find possible alternatives to synthetic preservatives. In this context, *R. officinalis* L. essential oil showed interesting results, being one of the best performing one in terms of ability to neutralize free radicals.

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