Antioxidant Property of Volatile Oils Determined by the Ferric Reducing Ability

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Some current oils and their main components were studied to determine their antioxidant values. This was done by using the modified method of ferric reducing ability of plasma. It has been established that volatile oils of medicinal plants have on average a reducing capacity of 3.5–220 mmol/kg oil. The reducing capacities of the main constituents of volatile oils are 0.165–65.5 mmol/kg in concentrated oils. The highest reducing capacity was showd for phellandrene (65.438 ± 0.166 mmol/kg) and anethole (50.087 ± 0.160 mmol/kg) while the lowest values were obtained for menthol (0.165 ± 0.023 mmol/kg) and menthone (0.168 ± 0.010 mmol/kg). It has been stated that the antioxidant values of the main constituents are lower than those of volatile oils. The reducing capacity of the main constituents of medicinal plant drugs at different concentrations was also determined.

Key words: Medicinal Plants, Volatile Oil, Antioxidant Activity

Introduction

Volatile oils have characteristic fragrances and tastes and are completely volatilized at room temperature. Volatile oils are mixtures of known and partially unknown compounds *e.g.* hydrocarbons, and contain terpene alcohols, aldehydes, ketones, phenols and esters. The antioxidant activity of drugs and their extracts has been studied and evaluated by various methods determining total antioxidant, free radical scavenging, superoxide anion radical scavenging (Malencic *et al.*, 2002; Parejo *et al.*, 2002), hydrogen peroxide scavenging and metal chelating activities, reducing power (Oktay *et al.*, 2003) and total phenolic content (Parejo *et al.*, 2002; Santos-Gomes *et al.*, 2002). The antioxidant capacity of the different extracts was measured in the following solvents: water (Oktay *et al.*, 2003; Dapkevicius *et al.*, 1998), ethyl acetate, hexane (Marinova and Yanishlieva, 1997), ethyl alcohol (Djarmati *et al.*, 1991), pentane (Guillen and Manzanos, 1996). The solvent extracts were obtained by different extraction methods, *e.g.* gamma-irradiated and microwave extraction (Farag and El-Khawas, 1998), supercritical fluid extraction with carbon dioxide (Yepez *et al.*, 2002). However, the antioxidant activity of volatile oils has not been studied so far.

The research work was to examine the antioxidant activity of some commercially available volatile oils, such as caraway, camomile, coriander, dill, eucalyptus, fennel, hyssop, juniper, lavender, parsley, peppermint, rosemary, sages, yarrow, and of some of the main components of these volatile oils by using the FRAP (ferric reducing ability of plasma) method. The FRAP assay depends upon the reduction of ferric tripyridyltriazine [Fe(III)-TPTZ] to ferrous tripyridyltriazine [Fe(II)-TPTZ] at low pH. The [Fe(II)-TPTZ] complex has an intensive blue colour and can be monitored at 593 nm (Benzie and Strain, 1996).

Materials and Methods

The volatile oils of caraway (*Carum carvi*), camomile (*Matricaria recutita*), coriander (*Coriandrum sativum*), dill (*Anethum graveolens*), eucalyptus (*Eucalyptus globulus*), fennel (*Foeniculum vulgare*), hyssop (*Hyssopus officinalis*), juniper (*Juniperus communis*), lavender (*Lavendulae officinalis*), muscat sage (*Salvia sclarea*), parsley (*Petroselium sativum*), peppermint (*Mentha piperita*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), and yarrow (*Achillea millefolium*) were purchased from the commercial network. The main constituents of volatile oils found in the me-

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dicinal plant drugs studied are summarized in Table II. The amount of the main components has been documented in area percentage in brackets.

All chemicals and reagents were analytical grade or of purest quality and were purchased from Merck (Darmstadt, Germany), Sigma-Aldrich (Seelze, Germany), Fluka (Buchs, Switzerland) or Reanal (Budapest, Hungary).

Characteristic parameters of the FRAP method (Benzie and Strain, 1996, 1999) are as follows:

Reagents:

1. 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate · 3 H_2O and 16 ml acetic acid in 1000 ml buffer solution).
2. 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 40 mM HCl
3. 20 mM $FeCl_3 \cdot 6\ H_2O$ in distilled water.

FRAP working solution: 25 ml of solution (1), 2.5 ml of solution (2) and 2.5 ml of solution (3). The working solution must be prepared always freshly. The aqueous solution of a known amount of Fe(II) was used for calibration.
Assay: Blank FRAP reagent.
Sample: 1.5 ml FRAP reagent and 50 μl sample solution.

The reaction was monitored up to 5 min at 594 nm, at 37 °C. The Fe(II) standard solution was tested in a parallel process. Calculations were made by a calibration curve.

The analysis of volatile components was carried out by gas chromatography and gas chromatography-mass spectrometry (GC-MS) (Lemberkovics, 1984; Blázovics *et al.*, 2003).

Gas chromatography measurements were carried out with a Bush 610 instrument (Darmstadt, Germany) equipped with a capillary silicon column (GC, column number 51130D, 25 m, 0.32 mm in diameter). The Temperature program was: 2 min at 60 °C, then heated at 8 °C/min up to 230 °C.

GC-MS parameters were as follows: HP 5890 instrument (Delaware, USA): column: 60 m; diameter: 0.25 mm; film thickness: 0.25 mm; stationary phase: RTX-5MS; column temperature: 40 °C, heated at 10 °C/min, 310 °C isotherm for 10 min; carrier gas: helium at a linear velocity of 32 cm/s: injected solution: 2 μl; Finningan MS detector: start: 9 min after injection, SCAN mode by electron impact ionization; mass range: 40–650; scanning rate: 1 analysis/s.

Evaluation of the results was made according to the software including a mass spectral library (Wiley), which was used for identifying organic compounds extracted from the oil.

Table I. Reducing capacity of volatile oils of medicinal plants determined by the FRAP method.

Medicinal plant		Reducing capacity
Drug	Volatile oil	[mmol/kg]
Asteraceae		
Chamomillae anthodium	Aetheroleum* chamomillae	145.107 ± 0.007
Achillae herba	Aetheroleum achillae	146.205 ± 0.017
Cupressaceae		
Juniperi fructus	Aetheroleum juniperi	215.901 ± 0.010
Labiatae		
Hyssopi herba	Aetheroleum hyssopi	30.573 ± 0.001
Lavandulae flos	Aetheroleum lavandulae	45.959 ± 0.004
Salviae sclarea	Aetheroleum salviae	43.479 ± 0.002
Menthae piperitae folium	Aetheroleum menthae piperitae	10.892 ± 0.001
Rosmarini folium	Aetheroleum rosmarini	31.473 ± 0.002
Salviae folium	Aetheroleum salviae	28.179 ± 0.002
Myrtaceae		
Eucalypti folium	Aetheroleum eucalypti	8.973 ± 0.002
Umbelliferae		
Carvi fructus	Aetheroleum carvi	27.422 ± 0.001
Coriandri fructus	Aetheroleum coriandri	3.582 ± 0.001
Anethi fructus	Aetheroleum anethi	42.642 ± 0.001
Foeniculi fructus	Aetheroleum foeniculi	47.308 ± 0.001
Petroselini herba	Aetheroleum petroselini	91.514 ± 0.002
Petroselini fructus	Aetheroleum petroselini	71.928 ± 0.003

* Aetheroleum is the volatile oil of the drug.

Monitoring of the data from the FRAP method was measured with a UV-VIS HP 8452 spectrophotometer.

Mean values and standard deviations (SD) were calculated from the results. For comparison of the mean values one-way analysis of variance (ANOVA) was used with the GraphPAD software version 1.14 (1990). Significance limit was $P < 0.0001$.

Results and Discussion

The antioxidant activities of volatile oils determined by the FRAP method (mmol/kg) are summarized in Table I. The unit mmol/kg means the quantity of Fe^{3+} in mmol that can be reduced to Fe^{2+} by 1 kg oil or component. The values are significantly different (ANOVA, $P < 0.0001$). The reducing ability of volatile oils of juniper (215.901 ± 0.010 mmol/kg), yarrow (146.205 ± 0.017 mmol/kg) and camomile (145.107 ± 0.007 mmol/kg) is very significant. The values of chamomile and yarrow are twice as high as the average values of the other plants. Most of the plants, such as lavender (45.959 ± 0.004 mmol/kg), rosemary (31.473 ± 0.002 mmol/kg), dill (42.642 ± 0.001 mmol/kg), caraway (27.422 ± 0.001 mmol/kg) have the same antioxidant values; while, surprisingly low values were obtained for coriander (3.582 ± 0.001 mmol/kg). There is some difference between the reducing capacities of volatile oils of parsley seed (71.928 ± 0.003 mmol/kg) and herba (91.514 ± 0.002 mmol/kg). For the measurement of the antioxidant activity of volatile oils the medicinal plants

Table II. The identified components of volatile oils of medicinal plant drugs determined by GC and GC-MS.

Family and plant drug	Volatile oil content [g/100 g]	Main components of volatile oils and concentration of other components*	
Asteraceae			
Chamomillae anthodium	0.3–0.56	α-bisabolol (45)	chamazulene, farnesol, farnesene
Achillae herba	0.1–0.5	tricyclene, pinene	champhene, sabinene, borneol acetate, cineole, limonene
Cupressaceae			
Juniperi fructus	1	terpineol-4, pinene (80)	champhene, cadinene, juniperol, thujone, sabinene
Labiatae			
Hyssopi herba	0.3–0.9	cineole	pinene, pinochamphene, isopinochamphene, pinocarvone
Lavandulae flos	1.50	linalyl acetate (35), linalool (20)	terpinenol, cineol, caryophyllene, geraniole
Salviae sclarea		linalool (65)	sclareol, manool, salvipinone
Menthae piperitae folium	1–2	menthol (67), menthone (13)	piperiton, methyl acetate, methyl valerate, menthofuran, jasmone, phellandrene, limonene, pulegon
Rosmarini folium	1–2	cineole (30)	borneol, camphor
Salviae folium	1–2	α-thujone (20), β-thujone (28)	cineole, camphor
Myrtaceae			
Eucalypti folium	0.3–3.5	1,8-cineole (85,6)	α-pinene, limonene, geraniole, camphene
Umbelliferae			
Carvi fructus	3–7	carvone (43), limonene (37)	*trans*-dehydrocarvone, *cis*-dehydrocarvone, *trans*-carveol, camphene, terpinene, linalool, pinene, fenchene
Coriandri fructus	0.2–1	linalool (17.5), linalool oxide (29)	phellandrene, camphor, pinene, cineole, limonene, camphene
Anethi fructus	2–4	carvone (45), limonene (28)	dehydrocarvone, phellandrene, eugenol, pinene
Foeniculi fructus	2–3	anethole (31)	limonene, fenchone, camphene, cineole, camphor, pinene
Petroselini herba		myristicin (18)	apiole, limonene, menthatriene, pinene
Petroselini fructus	3–6	myristicin(65)	apiole, limonene, pinene

* The concentrations are expressed in percentage in the brackets.

were selected from 5 families (Table II). Although a close correlation between antioxidant activity and the family of plant volatile oil has not been found, the highest activity can be observed in the Asteraceae and Cupressaceae families.

The reducing ability of the main constituents of volatile oils was studied to determine the correlation between antioxidant activity and the components responsible for it. The reducing capacities of the components are lower than the values obtained for volatile oils. The concentration range between 0.165 mmol/kg and 65.500 mmol/kg is considerably wide (Table III). Phellandrene has the highest reducing ability (65.500 mmol/kg), while menthol and menthone (0.165 and 0.168 mmol/kg) show the lowest. The best antioxidants are flavonoids (Vessal *et al.*, 2003; Molina *et al.*, 2003), therefore, the values are related to a well-known flavonoid antioxidant, quercetin, and to its glycoside, rutin. The antioxidant capacities of the compounds and of volatile oils show concentration dependence as seen in Fig. 1. Because of the solubility problems the reducing capacity of components could be measured in a lower concentration range as it can be seen in Fig. 1a. The reducing capacity of volatile oils of caraway, fennel, muscat sage related by the components could be determined at a higher concentration range (Fig. 1b). It can be explained by the presence of other components in oils that is supposed to facilitate the solubility of components. The main components of volatile oils are acyclic, monocyclic and bicyclic monoterpenes, phenyl propane derivatives as well as sesquiterpene lactones. No correlation was found between the type of the terpene component and the reducing capacity. In each case the structure of the molecule has been investigated and it has been found that the molecules with highest reducing capacity have delocalized electron structures and are capable of scavenging (Youdim *et al.*, 2002; Lee and Shibamoto, 2001; Teissedre and Waterhouse, 2000; Fadel *et al.*, 1999).

Table III. Reducing capacity of the main components of volatile oils determined by the FRAP method.

Volatile oil component	Reducing capacity [mmol/kg]
Menthol	0.165 ± 0.023
Menthone	0.168 ± 0.010
α-Bisabolol	2.676 ± 0.092
Carvone	15.707 ± 0.110
Linalyl acetate	20.853 ± 0.150
Linalool	25.732 ± 0.125
Camphor	28.381 ± 0.140
Borneol	28.724 ± 0.135
Limonene	37.440 ± 0.155
Anethole	50.087 ± 0.160
Phellandrene	65.438 ± 0.166
Quercetin	3832.727 ± 1.100
Rutin	5294.545 ± 1.250

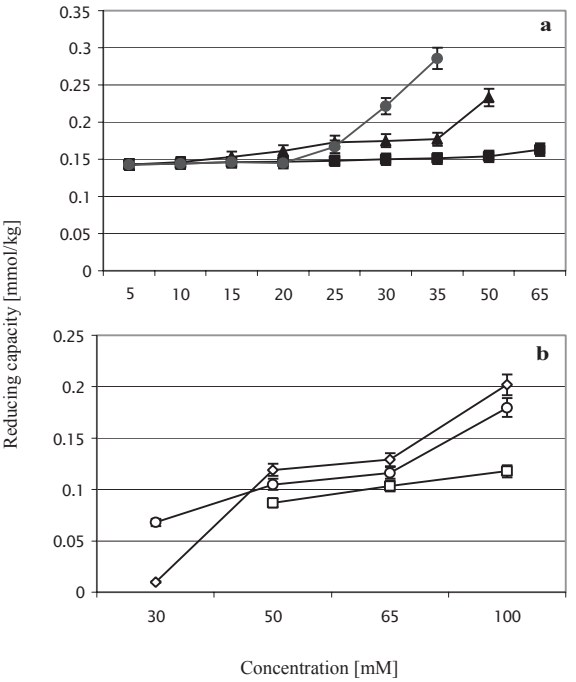

Fig. 1 Reducing capacity a) of volatile oils expressed in the main components' concentration: anethole (-●-), carvone (-■-), linalyl acetate (-▲-), and b) of the volatile oil component: anethole (-○-), carvone (-□-), linalool (-◇-) at different concentrations determined by the FRAP method.

Conclusion

It may be stated that the volatile oils examined have significant reducing capacity. The reducing abilities of the components of volatile oils are lower than those of volatile oils; therefore, the reducing capacities of volatile oils cannot be attributed solely to terpenes. In addition to the terpenes other biologically active compounds may also contribute to ferric reduction and in electron scavenging.

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