

Antioxidative properties of water extracts obtained from herbs of the species Lamiaceae

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Essential oils and extracts of aromatic herbs obtained by organic solvents have been extensively studied for their antioxidant activity in lipid substrates. Very little is known about the possible presence of antioxidants in polar extracts from herbs used in preparation of infusions and decoctions. In this work water extracts of six different herbs of the Lamiaceae family (dittany, lemon balm, mint, sage, sideritis and sweet marjoram) were prepared. The extracts were examined for their effect against lipid oxidation in comparison to a tea water extract. Sweet marjoram, sage and dittany extracts were found to have a remarkable capacity in retarding lipid oxidation. Examination by thin-layer chromatography of the freeze-dried extracts, before and after hydrolysis, showed that the extracts were rich in bound forms of phenolic compounds such as hydroxycinnamic acids and flavonoids. Rosmarinic and caffeic acids were detected in all extracts with the exception of those from mint and sideritis. These results indicate that certain plants used for the preparation of infusions could be further studied like tea as sources of antioxidants.

Introduction

Natural antioxidants are important for their physiological functions after absorption into the body (Harris & Haggerty, 1993; Offord *et al.*, 1997; Moon & Terao, 1998). Today, there are many research teams examining the chemical nature and levels of natural antioxidants in the plant material present in our diet. The great majority of active compounds isolated from higher plants are flavonoids or other phenolics. Some flavonoids present in tea infusions may have protective effects against coronary heart disease, cancer or allergy (Yen & Chen, 1994; Hertog *et al.*, 1993; Vinson *et al.*, 1995; Ho *et al.*, 1997; Wiseman *et al.*, 1997).

Aromatic plants have been extensively studied for the presence of natural antioxidants but emphasis has been given to essential oils or to hexane, acetone, ethanol (or methanol) and carbon dioxide extracts (Frag *et al.*, 1989; Lagouri *et al.*, 1993; Ramanathan & Das, 1993; Bertelsen *et al.*, 1995; Dorman *et al.*, 1995; Schwarz *et al.*, 1996; Nakatani, 1997; Tena *et al.*, 1997; Wang *et al.*, 1998; Dapkevicius *et al.*, 1998). Recently it has been shown that the aqueous extract of dittany is more effective than the methanol, ethanol and acetone extracts in scavenging hydroxyl radicals as generated by the Fenton reaction and in reducing oxygen

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consumption when initiated by metmyoglobin (Moller *et al.*, 1999).

The objective of this work was to evaluate the antioxidant activity of water extracts obtained from some aromatic plants of the Lamiaceae family grown wild (dittany, lemon balm, mint, sideritis and sage) or cultivated (sweet marjoram) in Greece. The dried parts of the plants selected for the study are used for the preparation of infusions, some of which are popular drinks in Greece. Antioxidant activity was assessed by the beta-carotene bleaching test according to Taga *et al.* (1984), with slight modification of the method, and by measurement of the Oil Stability Index using a Rancimat apparatus. The results were compared to the effect obtained by a tea infusion.

Materials and methods

Materials

Plant materials. The different kinds of wild and cultivated herbs were obtained from various places in Greece. Dittany (*Origanum dictamnus* L.) was from Crete, lemon balm (*Melissa officinalis* L.), mint (*Acinos suaveolens* Sm. Loudon) and sweet marjoram (*Origanum majorana* L.) were from Kavala (Northern Greece), sideritis (*Sideritis reaseri* Boiss. and Herdr., ssp. *raesari*) was from the mountain Pigeon (Northern Greece) and sage (*Salvia fruticosa* Miller) was from Thassos, an island of Northern Greece. Before use, they were characterised in the Laboratory of Systematic Botany and Phytogeography, Faculty of Biology, Aristotle University of Thessaloniki. China black tea, in packs of 2 g, was purchased from a local store in Thessaloniki.

Preparation of herb extracts. Each herb (10 g) was extracted with boiling water (250 g) for 10 min. The plant material remained for 15 min in the warm water and was then filtered; the filtrate was made to volume (200 ml) with the addition of water. Part of the water extract was used as such and the rest was freeze-dried. Samples were kept at -20°C until analysed. To prepare tea infusion, 4 g of China black tea were extracted with 200 ml boiling water for 1 min. After 5 min in the warm water the solution was filtered and the filtrate made to volume (200 ml).

Chemicals. Linoleic acid, 99% purity, was purchased from Sigma (St Louis, MO, USA); beta-carotene and Folin-Ciocalteu reagent from Merck (Darmstadt, Germany); methanol, chloroform, butanol, ethyl acetate, quercetin, rutin, silica gel 60 plates, chlorogenic acid and ferric chloride from Riedel-de-Haen (Seelze, Germany); caffeic acid from Fluka AG (Buchs, Switzerland); rosmarinic acid from Roth (Karlsruhe, Germany) and Tween 40 from Kock-Light Laboratories Ltd (Colnbrock, Bucks, England).

Methods

Screening for antioxidant activity. 15 mg beta-carotene were dissolved in 25 ml chloroform. In a conical flask 20 mg linoleic acid, 200 mg Tween 40 and 2 ml of the carotene solution were added. Excess chloroform was removed by evaporation at 40°C under a nitrogen stream. Then 50 ml water was slowly added to obtain emulsion A, which was further stabilised in a supersound bath. In the same way, emulsion B was prepared without the addition of beta-carotene. To measure the antioxidant activity of the extracts 200 μl of the water extract was mixed with 5 ml emulsion A and also with 5 ml emulsion B (blank). A mixture of 200 μl water and 5 ml emulsion A was used as control. For setting zero to the spectrophotometer a mixture of 200 μl water and 5 ml emulsion B was used. Readings were taken at 470 nm in a Hitachi model U-2000 UV-Vis spectrophotometer. The sweet marjoram water extract was used to establish from ten experiments the relative dispersion of the result (CV%, $\pm 3.9\%$).

Evaluation of the antioxidant activity by measurement of the Oil Stability Index. The induction period of 5 g refined sunflower oil samples containing 30 mg of each freeze-dried extract was measured twice with the Rancimat 617 (Metrohm AG, Herisau, Switzerland) at a temperature of 100°C and an airflow of 20 litres. h^{-1} . Six determinations on the refined sunflower oil used as substrate indicated good repeatability of the method (CV%, $\pm 3\%$).

Spectrophotometric determination of phenolic compounds. Total polyphenols in the water extracts, expressed as caffeic acid, were determined twice spectrophotometrically using

Folin-Ciocalteu reagent (Singleton *et al.*, 1999). Six determinations on the sweet marjoram water extract indicated good repeatability of the method (CV%, \pm 4.6%).

Acid hydrolysis. This was obtained by dissolving the freeze-dried herbal extract in water adding a small quantity of hydrochloric acid (0.5 ml per 2 ml of water solution containing 20 mg dry material) and heating at 105°C for 1 h. The hydrolysed product was extracted twice by 2 ml of diethyl ether, the solvent was evaporated and the residue was dissolved in methanol to be further examined by TLC.

TLC tests to identify the chemical composition of the extracts. The freeze-dried herbal extracts before and after acid hydrolysis, dissolved in methanol, were streaked on precoated silica gel plates (0.20 mm thick) to detect specific classes of phenols. The plates were developed with various solvents such as: chloroform-ethyl acetate (3:1 v/v) (Quinn & Tang, 1996), chloroform-ethanol-acetic acid (98:2:2 v/v/v) (Duve & White, 1991), chloroform-ethyl acetate-formic acid (5:4:1 v/v/v) (Onyeneho & Hettiarachchy, 1992), butanol-acetic acid-water (4:1:5 v/v/v) (Tian & White, 1994), ethyl acetate-formic acid-water (10:2:3 v/v/v) (Pratt & Miller, 1984). After development the plates were sprayed with reagents specific for compounds with characteristic structural features. These reagents were:

- A mixture of potassium ferricyanide 1% and ferric chloride 1%. Blue spots with all types of phenolics (Tian & White, 1994).
- Sodium carbonate 2% in water. Changes of fluorescence under UV light due to ionization of hydroxyl groups and increase of conjugation (Tian & White, 1994).
- Folin-Ciocalteu reagent. Blue spots with all types of phenolics (Onyeneho & Hettiarachchy, 1992; Quinn & Tang, 1996).
- Ferric chloride 2% in ethanol. Different colours depending on the number and position of the hydroxyl groups (Tian & White, 1994; Quinn & Tang, 1996).
- Aluminium trichloride 1% in ethanol. Characteristic yellow fluorescence of 3- or 5-hydroxylated flavonoids with a carbonyl at 4-position, such as flavones and flavonols (Markham, 1989).

Table 1. Solid residue content of water extracts

<i>Plant</i>	<i>Solid residue (% w/w)</i>
Dittany*	0.78
Lemon balm*	0.96
Sideritis*	0.68
Mint*	0.94
Sweet marjoram*	0.88
Sage*	0.72
Tea**	0.50

* 10 g in 200 ml water

** 4 g in 200 ml water

Plates were prepared in a dimly illuminated room. As standards, caffeic acid, chlorogenic acid, rosmarinic acid, quercetin and rutin dissolved in ethanol were used.

Results and discussion

All the extracts had a pleasant odour, which indicates that part of the essential oil was retained in the infusions. Solid residue contents of the water extracts obtained from the six aromatic plants and tea are given in Table 1. For the preparation of the water extracts, a four-times higher quantity of the herbs was used in relation to normal infusions for human consumption.

Antioxidant effect of the extracts and their content in phenolics are presented in Table 2. Antioxidant activity is expressed in antioxidant activity coefficients (AAC). The latter were calculated according to Chevolleau *et al.* (1992) from the equation:

$$AAC = 1000 \times (A_{180} - A_{C180}/A_{C0} - A_{C180})$$

Table 2. Total phenol content and antioxidant activity coefficients (AAC) of water extracts as measured by beta-carotene—linoleic acid coupled oxidation

<i>Plant</i>	<i>Phenol content (g caffeic acid/100 ml)</i>	<i>AAC</i>
Dittany	0.12	544
Lemon balm	0.13	373
Sideritis	0.08	335
Mint	0.07	251
Sweet marjoram	0.19	621
Sage	0.20	595
Tea	0.16	740

Table 3. Total phenol content of dried water extracts and oxidative stability of refined sunflower oil containing freeze-dried water extracts

<i>Plant extract</i>	<i>Phenol content (% w/w)</i>	<i>Oil Stability Index (h)</i>
Dittany	15.2	11.3
Lemon balm	13.7	10.5
Sideritis	12.5	10.4
Mint	7.7	10.3
Sweet marjoram	22.1	13.9
Sage	27.4	13.1
Tea	31.1	14.8

At time $t = 0$ min the absorbance of emulsion A at 470 nm is maximum and a coefficient 1000 is conventionally given. At time $t = 180$ min the minimum absorbance is observed for the control, and a coefficient 0 is given. For each extract an antioxidant activity coefficient ranging from 0 to 1000 is determined (A_{180} absorbance of the solution containing the additive at $t = 180$ min, A_{C0} and A_{C180} absorbance of the control at $t = 0$ and $t = 180$ min, respectively).

The results (mean values of four measurements) are calculated for the same level of water dilution for all the examined materials.

The order of antioxidant activity is tea > sweet marjoram > sage > dittany > lemon balm > sideritis > mint. This order was further confirmed by Oil Stability Index measurements using refined sunflower oil containing 0.6% w/w of the dry material of each extract obtained by freeze-drying as a substrate (Table 3). Refined sunflower oil was found to have an Oil Stability Index of 8.7 h.

It can be concluded that water extracts from sweet marjoram, sage and dittany are not as active as the tea water extract, but they have a remarkable capacity to retard lipid oxidation. This capacity is related to the content and the nature of phenolic compounds.

The main classes of phenolic compounds reported to be present in the plants of the Lamiaceae family are hydroxycinnamic acids and flavones, mainly in the form of derivatives such as esters and glycosides (Herrmann, 1980, 1995; Hegnauer, 1989). Sage and rosemary also contain phenolic diterpenes (Lindberg Madsen & Bertelsen, 1995). Most of the work carried

out is based on the use of solvents less polar than water such as hexane, diethyl ether, ethyl acetate, acetone, ethanol and methanol. It is therefore not known from the literature the extent to which water extracts of various herbs contain valuable bioactive ingredients. However, a dittany water extract was found recently to be richer in polar phenols and therefore more effective in retarding lipid oxidation and in scavenging of free radicals than acetone, ethanol and methanol extracts of the same plant material; it has been suggested that dittany is further explored as a source of water-extractable antioxidants (Moller *et al.*, 1999).

From the thin-layer chromatograms it can be concluded that:

- (1) All the extracts, except those of sideritis and mint, contained caffeic acid and other phenolic compounds in their free form. None of them had an Rf value equal to that of quercetin.
- (2) The extracts of sideritis and mint contained a bound form of caffeic acid, probably chlorogenic acid. All the other extracts contained rosmarinic acid, another caffeic acid derivative, which has been reported to be present at high levels in plants of the Lamiaceae family (Reschke, 1983). The latter seemed to be the most important phenolic compound of the extracts.
- (3) All the extracts contained bound forms of 3- or 5-hydroxylated flavonoids with a carbonyl at 4-position. None of them had an Rf value equal to that of rutin.
- (4) All the extracts also contained other phenolic derivatives, mainly glycosides.

The results of this work indicate that water extracts obtained from some aromatic herbs have a protective effect against lipid oxidation. This is due to a high content of phenols which may also be active in eradicating active oxygen species and suppressing their effects, which is believed to be the case with wine and tea phenols (Lin *et al.*, 1999). Further elucidation of the chemical nature of complex phenol mixtures in water extracts of such plants is now in progress in our laboratory.

Acknowledgement—This research was supported in part by the Greek General Secretariat for Research and Technology Program, EPET II.

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