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Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs

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Abstract

De-odourised aqueous extracts of four commonly consumed herbs belonging to the Lamiaceae family, i.e. oregano (*Origanum vulgaris* L.), rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.) and thyme (*Thymus vulgaris* L.), were investigated for their antioxidant properties. Various experimental models were used for the characterisation of the activity, including iron reduction capacity, DPPH•, $ABTS^{\bullet+}$ and •OH radical-scavenging activities and the capacity of the extracts to inhibit copper-induced oxidation of human low-density lipoproteins (LDL) ex vivo. The extracts showed varying degrees of reductive and radical scavenging capacity, and were capable of a marked prolongation of the lag-time in the LDL oxidation assay. The hierarchy of the observed antioxidant activity of the extracts was dependent on the type of assay used. The observed antioxidant characteristics were not fully related to the total phenolic contents of the extracts in any of the assays, but were presumably strongly dependent on rosmarinic acid, the major phenolic component present in this type of Lamiaceae extract.

Keywords: Lamiaceae family; Oregano; Rosemary; Sage; Thyme; Antioxidants; Free radicals; Peroxidation; Reduction; Low-density lipoprotein (LDL)

1. Introduction

Lipid peroxidation is a paradox of aerobic life, affecting both human health and the quality of modern life (Davies, 1995). Biological systems are lipid-rich matrices susceptible to autoxidation unless protected by either endogenous enzymatic or non-enzymatic mechanisms. Antioxidants can act by the following mechanisms in lipid peroxidation: (i) decreasing localized oxygen concentrations, (ii) preventing chain initiation by scavenging initiating radicals, (iii) binding catalysts, such as metal ions, to prevent initiating radical generation, (iv) decomposing peroxides so they can not be reconverted to initiating radicals, and (v) chain-breaking, to prevent continued hydrogen abstraction by active radicals. Plant phenolics are said to be multifunctional antioxidants (Shahidi & Wanasundara, 1992), and they might act at several levels in the oxidative sequence. Singlet oxygen quenching and thus decreasing of local oxygen concentrations has been suggested to take place by phenolic compounds (Beutner et al., 2001; Luiz, Biasutti, & Garcia, 2002). The transition metal chelating action by phenolics has been described (Brown, Khodr, Hider, & Rice-Evans, 1998; Chen & Ahn, 1998). The capability of different phenolic substances to scavenge various types of oxidation-initiating radicals has been reported in the polar phase (Bors, Heller, Michel, & Saran, 1990; Rice-Evans, Miller, & Paganga, 1996; Yen & Duh, 1994) and in the lipid phase (Dugas et al., 2000; Sawa, Nakao, Akaike, Ono, & Maeda, 1999). Phenolic compounds are also thought to be capable of regenerating endogenous α-tocopherol in the phospholipid bilayer of lipoprotein particles back to its active antioxidant form (De Whalley, Rankin, Hoult, Jessup, & Leake, 1990; Rice-Evans & Miller, 1996; Viana et al., 1996). They are also known to inhibit various types of oxidizing enzymes (Cos et al., 1998; Laughton, Evans, Moroney, Hoult, & Halliwell, 1991). These multiple potential mechanisms of antioxidant action make the diverse group of phenolic

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compounds an interesting target in the search of health-beneficial phytochemicals, and also offer a possibility to use phenolic compounds or extracts rich in them in lipid-rich foods in order to extend their shelf life (Hudson & Lewis, 1983).

Numerous techniques are available to evaluate the antioxidant activities of compounds and complex mixtures such as plant extracts (Anatolovich, Prenzler, Patsalides, McDonald, & Robards, 2002; Armstrong, 1998). Despite these various methods, just one procedure cannot identify all possible mechanisms characterising an antioxidant (Frankel & Meyer, 2000). In this study, four commonly consumed Lamiaceae herbs were extracted, using an aqueous extraction procedure, and the antioxidant properties of these aqueous extracts were characterised by the following techniques: iron (III) to iron (II) reduction (Oyaizu, 1986), DPPH• (Gyamfi, Yonamine, & Aniya, 1999), $ABTS^{\bullet+}$ (Re et al., 1998) and •OH (Halliwell, Gutteridge, & Aruoma, 1987) radical-scavenging. The capability of the extracts to inhibit copper-catalysed oxidation of human low-density lipoproteins (LDL) was assessed ex vivo (Esterbauer, Gebicki, Puhl, & Jürgens, 1992). The total phenolic content of each extract was estimated using the Folin-Ciocalteu method (Singleton, Orthofer, & Lamuela-Raventós, 1999). The purpose of the work was to (i) introduce a simple de-odourising organic solvent-free extraction procedure seldom used to extract antioxidants from Lamiaceae material, (ii) characterise the antioxidant activity of these extracts by various methods, and (iii) evaluate the relationship between the observed antioxidant properties and the phenolic content of the extracts. Based on the in vitro and ex vivo results, the potential exploitability of this type of antioxidative water-soluble herb extracts for different antioxidant usage, as well as the advisability of clinical studies required for health claims might be evaluated.

2. Materials and methods

2.1. Materials

2.1.1. Plant materials

Dried, cut leaves of *Origanum vulgare* L., *Rosmarinus officinalis* L., *Salvia officinalis* L. and *Thymus vulgaris* L. were obtained from Paulig Group Ltd., Finland.

2.1.2. Solvents and chemicals

Water used was of Millipore quality (Millipore, Bedford, MA, USA), and methanol was of HPLC-grade (Rathburn, Walkerburns, Scotland, UK). All reagents used were of the highest purity available and obtained from the usual suppliers.

2.2. Extraction

Fifty grammes of the herb material were placed in a 1000 ml round-bottom flask and 500 ml water were added. The flask was connected to a Ph. Eur. hydrodistillation apparatus and the water was left to boil for 2 h. The water from the flask was removed, 300 ml of fresh water were added, and the mixture was left to boil for 1 h. Water fractions were combined, filtered through qualitative No. 4 Whatman filter paper (Whatman International Ltd, Maidstone, England), freeze-dried and stored at 4 °C until used.

2.3. Total phenolics assay

Total phenolics were determined as gallic acid equivalents (GAE) (Singleton et al., 1999). The dry extract was diluted in water and 100 μl of this solution were transferred to a 10 ml volumetric flask, to which 0.5 ml undiluted Folin-Ciocalteu reagent was added. After 1 min, 1.5 ml 20% (w/v) Na_2CO_3 were added and the volume was made up to 10 ml with H_2O. After 1 h incubation at 25 °C, the absorbance was measured at 760 nm and compared to a pre-prepared gallic acid calibration curve. Values are presented as the mean of duplicate analyses.

2.4. Iron (III) to iron (II) reduction assay

The reductive capacities of the extracts were assessed using ferric to ferrous reductive activity as determined spectrophotometrically from the formation of Perl's Prussian blue coloured complex (Yildirim et al., 2000). One ml of each extract, in water, was mixed with 2.5 ml phosphate buffer (0.2 M, pH 7.0) and 2.5 ml of a 1% (w/v) potassium hexacyanoferrate $[K_3Fe(CN_6)]$ solution. After a 30 min incubation at 50 °C, 2.5 ml (10%, w/v) trichloroacetic acid was added and the mixture was centrifuged for 10 min (1800 rpm). Finally, 2.5 ml of the upper layer were mixed with 2.5 ml water and 0.5 ml (0.1%, w/v) $FeCl_3$ and the absorbance was recorded at 700 nm.

2.5. 1,1-Diphenyl-2-picrylhydrazyl scavenging assay

The ability of the extracts to scavenge the nitrogen centered DPPH• (1,1-diphenyl-2-picrylhydrazyl) radical was assessed spectrophotometrically (Gyamfi et al., 1999). A 50 μl aliqout of each extract, in Tris–HCl buffer (50 mM, pH 7.4), was mixed with 450 μl Tris–HCl buffer (50 mM, pH 7.4) and 1.0 ml 1,1-diphenyl-2-picrylhydrazyl (0.1 mM, in methanol). After a 30 min reaction period, the resultant absorbance was recorded at 517 nm. The percentage inhibition was calculated using Eq. (1) while the IC_{50}s (concentration of extract required for 50% inhibition of the radical absorbance at 517 nm) were estimated by a non-linear regression algorithm using SigmaPlot 2001 (version7.0, SPSS Inc., Chicago, IL, USA).

$$\text{Percentage Inhibition} = \left[\frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}}\right] \times 100 \quad (1)$$

2.6. $ABTS^{\bullet+}$ [2,2′-azinobis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt] scavenging assay

The determination of $ABTS^{\bullet+}$ radical scavenging was carried out as described by Re et al. (1998). The $ABTS^{\bullet+}$ radical was generated by reacting an (7 mM) ABTS aqueous solution with $K_2S_2O_8$ (2.45 mM, final concentration) in the dark for 12–16 h, at ambient temperature, and adjusting the $Abs_{734\ nm}$ to 0.700 (±0.020) at 30 °C. Extracts were diluted, such that, a 10 μl sample when added to 1 ml $ABTS^{\bullet+}$ resulted in a 20–80% inhibition of the blank absorbance. After 1 ml $ABTS^{\bullet+}$ was added to 10 μl extract/Trolox™ standards, the absorbance at 734 nm was recorded 1 min after initial mixing and subsequently (for 10 min). The percentage inhibition was plotted as a function of concentration and the Trolox equivalent antioxidant capacity (TEAC) was calculated against a Trolox™ calibration curve.

2.7. Fe^{3+}-EDTA/H_2O_2/ascorbate-catalysed deoxyribose oxidative degradation assay

The determination of •OH radical scavenging was carried out as described by Halliwell et al. (1987). A 1 ml reaction mixture containing 2.8 mM deoxyribose, 20 mM phosphate buffer (KH_2PO_4–KOH, pH 7.4), extract (variable concentrations), 100 μM $FeCl_3$, 104 μM EDTA, 1 mM H_2O_2 and 100 μM ascorbic acid was incubated at 37 °C for 60 min. Thereafter, 50 μl 2% (w/v) BHT, 1 ml 2.8% (w/v) trichloroacetic acid and 1 ml 1% (w/v) 2-thiobarbituric acid were added and the resulting mixture was incubated at 80 °C for 20 min. The reaction was stopped by a 5-min ice-water bath. To each tube, 2 ml of *n*-butanol were added, tubes were vortexed and centrifuged for 10 min and, finally, the degree of deoxyribose degradation was estimated from the absorbance of the organic layer (λ 532 nm). Percentage inhibition was calculated using Eq. (1).

2.8. Inhibition of LDL oxidation ex vivo

The protective action of the polar herb extracts against $CuSO_4$-induced oxidation of LDL ex vivo was evaluated by a method modified from that of Esterbauer et al. (1992). For possible further high-throughput screening purposes, a microwell-plate application of the technique was introduced. In addition to the multi-sample format, the microtitre adaptation also offers other advantages compared to conventional spectrophotometric applications as it requires minimal quantities of reagents and samples.

2.8.1. Isolation of the LDL fraction

Blood from healthy volunteers ($n=2$) was drawn into EDTA-containing (0.47 M) vacuum tubes. Plasma was separated by centrifugation (2500×*g*, 10 min, 4 °C). The lipoproteins were isolated by sequential ultracentrifugation (Havel, Eder, & Bragdon, 1955) at the following cut-off densities: VLDL ($d<1.006$ g ml^{-1}) and LDL ($1.006<d<1.063$ g ml^{-1}) using a Beckman Optima LE-80K ultracentrifuge and a Ti 50.4 rotor. The LDL fraction was collected, packed into cryo-tubes and stored at −80 °C until used (maximum 2 months).

2.8.2. Removal of EDTA

Prior to the oxidation experiment, the LDL fraction was gel-filtrated on a Sephadex G-25 column (dimensions 1×20 cm; Pharmacia Biotech, Uppsala, Sweden) to remove EDTA. The sample was applied in 2 ml of phosphate-buffered saline (PBS), which was also used as the elution buffer.

2.8.3. Determination of proteins

The protein level in the LDL fraction was determined after removal of EDTA by a method modified from Lowry, Rosebrough, Farr, and Randall (1951) using BioRad Protein Assay Reagents (BioRad Laboratories, Hercules, CA, USA) with a Perkin Elmer HTS7000+ BioAssay Reader (Buckinghamshire, UK) at 750 nm, after which the protein concentration was adjusted to 105 μg protein/ml with PBS.

2.8.4. LDL oxidation assay

Two hundred microlitres of the diluted LDL fraction were pipetted on UV microtitre plate wells. The dry herb extracts, diluted in water, were added to wells (except control) to final concentrations of 0.98 and 2.45 μg/ml. Oxidation was initiated by adding 1.67 μM (final concentration) $CuSO_4$ (aqueous solution) into the wells. The oxidation susceptibility of LDL was evaluated by measuring the formation of conjugated dienes at 235 nm with a Perkin Elmer HTS7000+ BioAssay Reader (Perkin-Elmer, Norwalk, USA) at 37 °C for 10 h, after which the lag-phases, before the propagation phase, were calculated from the kinetic oxidation curves. The effect of the extracts on the maximum rate of oxidation was evaluated from the slopes of the oxidation curves.

3. Results

3.1. The extraction yield and phenolic content of the extracts

The yields of the dry extracts (w/w) from the hydrodistillation procedure were 36% for oregano, 24% for rosemary, 25% for sage and 29% for thyme. The total phenolic contents of the extracts were determined to be:

oregano 149, rosemary 185, sage 166 and thyme 95.6 mg GAE/g. In these four aqueous extracts, rosmarinic acid was confirmed by HPLC analysis (data not shown) to be the main phenolic constituent, an example of which was shown for sage by Ollanketo, Peltoketo, Hartonen, Hiltunen, and Riekkola (2002).

3.2. Reductive capacity

In the ferric to ferrous reduction assay the electron donation capacity (reflecting the reductive power) of the samples was assessed, and compared to that of ascorbic acid, which is known to be a strong reducing agent. All the extracts showed some degree of electron donation capacity, but the capacities were, as expected, inferior to ascorbic acid (Table 1, Fig. 1). Sage and rosemary were the most active ones among the extracts, closely followed by oregano. Thyme extract clearly showed the weakest reductive power in the assay.

3.3. Radical scavenging activity

Table 2 shows that the extracts were capable of varying degrees of scavenging action against the three different radicals used. In the DPPH• assay, rosemary and sage clearly showed the highest activity, followed by oregano and thyme. Sage, rosemary and oregano were found to be rather similar in their scavenging action against $ABTS^{\bullet+}$ radicals, thyme again being the lowest in activity. Sage extract was shown to be by far the most effective against •OH radicals, followed by oregano. Thyme and rosemary did not practically differ from each other in terms of activity.

3.4. Inhibition of LDL oxidation ex vivo

In this ex vivo LDL oxidation assay, the aqueous herb extracts were capable of protecting isolated LDL particles from copper-induced oxidation. The lag-phase before the onset of oxidation was extended compared to the unprotected control at the concentration levels used (Fig. 2). The lag-times and standard deviations are presented in Fig. 3. Additions of rosemary and sage extracts yielded in most marked prolongations in the lag-times, followed by oregano. Again, in this assay, thyme extract was found to exhibit inferior antioxidant activity. The maximum oxidation rate (i.e. the slope of the oxidation curves, see Fig. 2) was not significantly affected by the antioxidant addition.

Table 1
Iron (III) to iron (II) reductive capacity and linear regression parameters

Sample	RRE[a]	Slope	Intercept ($\times 10^{-2}$)	r^2
Ascorbic acid	1.000	12.192±0.316	1.2±0.6	0.999
Origanum vulgare	0.160	1.987±0.171	2.2±3.0	0.996
Rosmarinus officinalis	0.188	2.339±0.059	2.9±1.2	0.996
Salvia officinalis	0.201	2.500±0.083	3.6±2.1	0.988
Thymus vulgaris	0.099	1.235±0.150	1.7±1.5	0.993

[a] Relative reductive efficiency (RRE) is the $\text{slope}_{\text{sample}}/\text{slope}_{\text{ascorbic acid}}$ ratio. Data are presented as $\bar{x}\pm 95\%$ confidence interval. Analysis of variance was performed by ANOVA procedures, with significant differences between means determined using Tukey's pairwise comparisons. Values for all the four samples are significantly ($P>0.05$) different.

4. Discussion

In most of the studies carried out to study the antioxidant activities or chemical composition of Lamiaceae herbs, the extraction procedures are based on the use of organic solvents, most often either methanol or acetone. This type of Lamiaceae extract contains some essential oil and thus, e.g., antioxidant monoterpene derivatives, such as thymol and carvacrol (Peltoketo et al., 2001). These compounds are mainly responsible for the typical strong aroma of Lamiaceae herbs. As reported by Zheng and Wang (2001), rosmarinic acid is the most abundant phenolic compound found in the acetone extract of sage, oregano and thyme, whereas in rosemary the main phenolic compounds are the diterpene derivatives carnosic acid and rosmanol, followed by rosmarinic acid. In our study, the extraction of the herbal material was carried out with water by a cheap, simple and organic solvent-free procedure—during which the volatile oil fraction containing monoterpene derivatives is removed. Furthermore, diterpenes are not extracted from Lamiaceae material by this extraction (Ollanketo et al, 2002). A large proportion of the components in the original plant material were extractable with water, and the obtained extracts were found to be rich in phenolic compounds, total phenolic content (GAE) varying from 9.6% (thyme) to 18.5% (rosemary). In all these four aqueous Lamiaceae extracts, rosmarinic acid was confirmed by HPLC analysis to be the main phenolic constituent, an example of which was shown for sage by Ollanketo et al (2002).

The reductive capacity assessed during this study is thought to be linked to important mechanisms of phenolic antioxidant action (Yildirim et al., 2000). The data from the iron reduction assay suggest that the extracts are able to donate electrons, and therefore should be able to donate electrons to reactive radicals, converting them into more stable and unreactive species. Sage and rosemary extracts, which were the most potent in the assay, contain the highest amounts of total phenolics, whereas thyme, containing the least phenolics, was weakest in activity. In the total phenolic assay and the reductive assay, functional chemical groups capable of reduction–oxidation reactions show high performance.

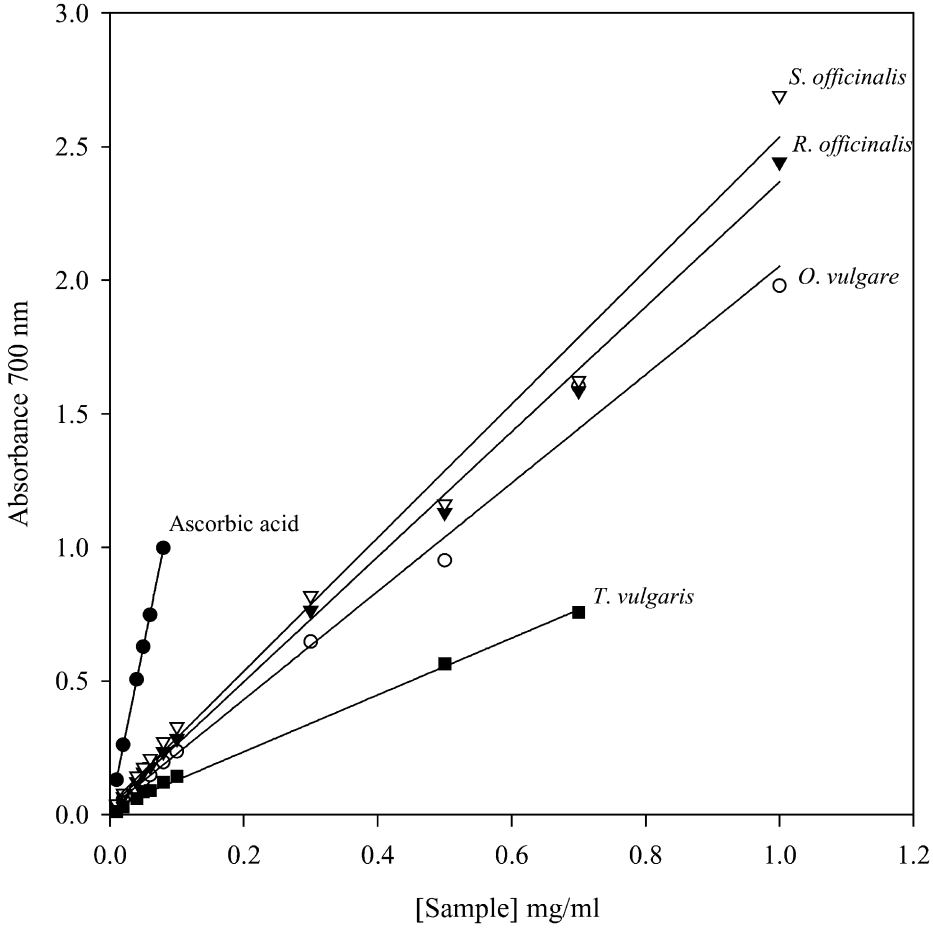

Fig. 1. The iron (III) to iron (II) reductive activities for the Lamiaceae extracts and ascorbic acid.

Table 2
Radical scavenging activities of the aqueous herb extracts against DPPH•, ABTS•$^+$ and •OH radicals

	Radical scavenging activity					
	DPPH•	ABTS•$^+$ (TEAC, mM Trolox)				•OH
Sample	IC_{50} [μg/ml]	1 min	4 min	6 min	10 min	IC_{50} [μg/ml]
Origanum vulgare	335.0±18.1a	9.4±1 .3a,A	12.0±1.4a,B	13.2±1.4a,C	14.9±1.5a,b,D	3375.1±242.6a
Rosmarinus officinalis	236.5±0.1b	10.3±3.2b,A	12.1±3.4a,B	12.9±3.7a,B,C	14.1±3.8b,C	3764.5±127.4b
Salvia officinalis	265.8±7.6b	11.5±0.9c,A	13.3±0.9b,B	14.2±1.0b,C	15.5±0.9a,D	2158.8±70.0c
Thymus vulgaris	382.4±28.3c	4.9±1.0d,A	5.8±1.4c,B	6.1±1.4c,B	6.8±1.7c,C	3747.3±320.7b

Data are presented as $\bar{x}\pm$95% confidence interval. Trolox™ equivalent antioxidant capacity (TEAC) is defined as the concentration of Trolox™ having the ABTS•$^+$ scavenging activity equal to a 1.0 mg/ml sample solution. Analysis of variance was performed by ANOVA procedures, with significant differences between means determined using Tukey's pairwise comparisons. Values with the same lowercase letter within each column are not significantly ($P>0.05$) different. Values with the same uppercase letter within each row are not significantly ($P>0.05$) different.

This may explain the correlation between the results from these two assays.

Scavenging of different types of reactive oxygen and nitrogen species, mostly free radicals, is thought to be one of the main mechanisms of the antioxidant action exhibited by phenolic phytochemicals. In the assessment of radical-scavenging properties, both synthetic and biologically-relevant free radicals are used. In this study, three different radical-scavenging models, based on three different radicals, DPPH•, ABTS•$^+$ and •OH radicals, were used. The synthetic nitrogen-centered DPPH• and ABTS•$^+$ radicals are not biologically relevant, but are often used as "indicator compounds" in testing hydrogen-donation capacity and thus antioxidant activity. •OH radicals are biologically relevant and extremely reactive oxygen species, which can rapidly react with and degrade susceptible food and biologically-relevant substrates, such as polyunsaturated fatty acids, proteins and

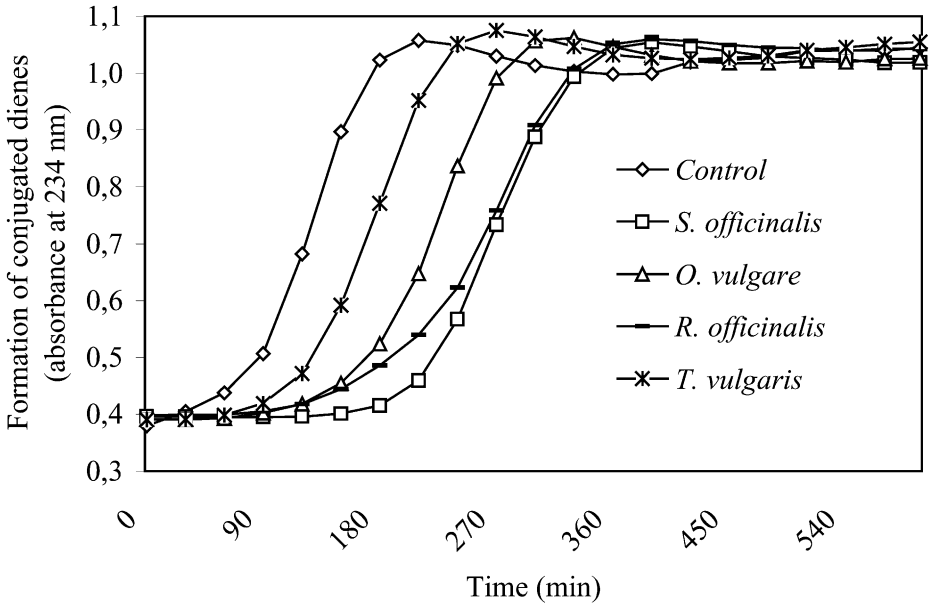

Fig. 2. Typical effects of the additions of aqueous Lamiaceae extracts (final concentration 2.45 μg/ml) on copper-mediated LDL oxidation kinetics compared to unprotected control.

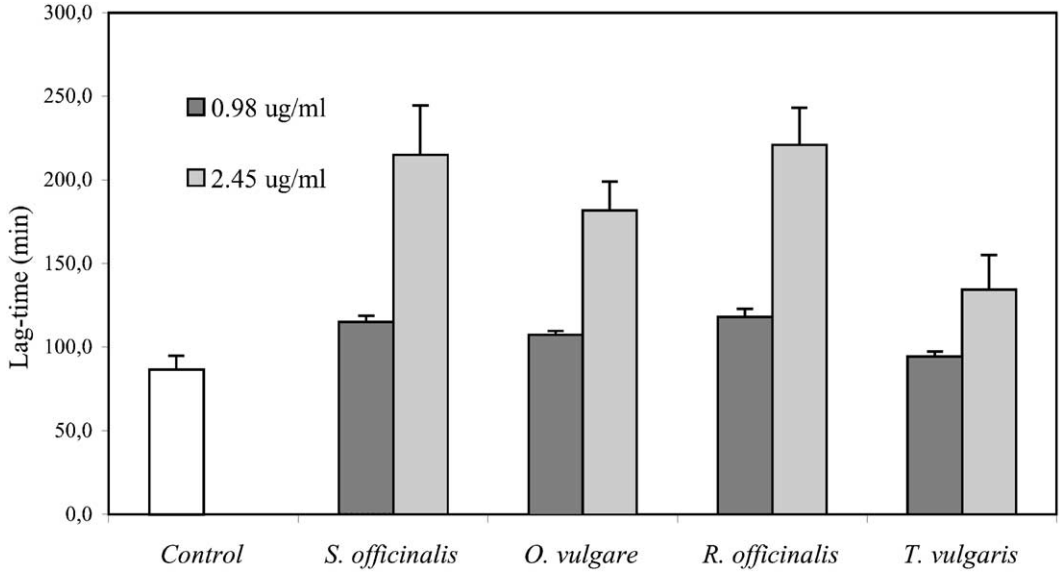

Fig. 3. The calculated lag-times for LDL, protected by additions of herb extracts, compared to unprotected control, consisting of diluted LDL-fraction and oxidation-inducer $CuSO_4$.

sugars (Halliwell, 1994). In the DPPH• and ABTS•$^+$ assays, polar-phase radical-scavenging is measured whereas, in the •OH radical scavenging assay, the scavenging action is determined by measuring •OH radical-mediated oxidation of the deoxyribose target molecules in a more complex matrix. The results from radical-scavenging assays show that the extracts, by hydrogen and/or electron donating action, might prevent reactive radical species from reaching biomolecules, such as lipoproteins, polyunsaturated fatty acids, DNA, amino acids, proteins and sugars, in susceptible biological and food systems (Halliwell, Aeschbach, Löliger, & Aruoma, 1995). As in the reductive capacity assay, rosemary and sage were the two most active extracts against nitrogen-centered radicals, followed by oregano. Thyme with the lowest total phenolic content was again inferior in activity. The results of the •OH radical-scavenging assay differed from the two other assays, based on synthetic nitrogen-centered radicals, possibly due to the reactivity of the •OH radical species and the nature of the assay matrix. The order of activity for the extracts did not seem to depend on the total phenolic content, as rosemary and thyme showed similar activities.

The findings from the use of three different radicals demonstrate the problem of using one-dimensional methods in evaluating multifunctional foods and biological antioxidants (Frankel & Meyer, 2000), and the use of just one radical species does not allow conclusions about the activity hierarchy in any other radical context.

The oxidation of low-density lipoprotein LDL is one of the most studied free radical-mediated processes occurring in the body, as it is believed to play a crucial role in the formation and progression of early atherosclerotic lesions linked to cardiovascular diseases (Diaz, Frei, Vita, & Keaney, 1997; Esterbauer et al., 1992; Steinberg, Parthasarathy, Carew, Khoo, & Witztum, 1989). Diets rich in vegetables, fruits, berries and wholegrain products, as well as some beverages, have been linked to decreased risk of cardiovascular diseases, and this finding has been thought to be explained by the presence of the diverse range of antioxidative phenolic compounds in these types of diets (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993; Hollman, Hertog, & Katan, 1996). In this study, the antioxidant protection of LDL was evaluated as the delay in the onset of oxidation (effect on the lag-phase) as well as the capacity to participate in the on-going peroxidation in the LDL particles (effect on the slope of the propagation phase). The extracts were found to prolong the lag-phase before the onset of copper-induced oxidation, but were not capable of delaying or inhibiting the propagation phase in the lipoprotein particles after the onset of oxidation. The highest prolongation of the lag-phase was observed with the extracts highest in the total phenolic content. The mechanism by which the extracts delayed the onset of LDL oxidation is not clear. Nardini et al. (1995) have suggested that the protection of LDL by phenolic acids in a copper-induced oxidation system could be due to both metal-chelating and radical scavenging action. Laranjinha, Almeida, and Madeira (1994) have summarised some possible explanations for the protecting effect on LDL by phenolic compounds: (i) scavenging of various radical species in the aqueous phase, (ii) interaction with peroxyl radicals at the LDL surface, (iii) partitioning into the LDL particle and terminating chain-reactions of lipid peroxidation by scavenging lipid radicals and (iv) regenerating endogenous α-tocopherol back to its active antioxidative form. The fact that the maximum oxidation rate (i.e. the slope of the oxidation curves, see Fig. 2) was not significantly affected by the antioxidant addition might suggest, that the phenolic compounds present in the extract may not be capable of chain-breaking antioxidant action during the propagation phase of oxidation in the lipoprotein particle surface. To be able to scavenge lipid radicals formed within the lipoprotein particles, the active components of the extracts should be able to be incorporated into the LDL particles, at least to some extent. Rosmarinic acid, the main component of the extracts, is rather polar, and its capability of incorporation into lipoproteins remains unclear.

To conclude, the aqueous de-odourised Lamiaceae extracts showed varying degrees of activity in all the assays used. The use of several assays showed, that the order of antioxidant activity found for a set of samples depends on the type of assay in question. In most of the assays, extracts with higher total phenolic contents were superior in activity. However, the activity hierarchy in the $^{\bullet}OH$ radical scavenging assay shows, that all the results were not explicable by the total phenolic content of the extracts. The results also depend on the chemical nature and structure of the phenolic compounds present in the extracts. The major contribution to the observed antioxidant activity could be explained by the presence of rosmarinic acid in the extracts.

Although the extracts were found to be effective natural antioxidant candidates in these systems, their potential exploitable beneficial effects and safety in humans need to be proven in clinical trials. To be able to act as antioxidants in vivo these substances should be found in adequate amounts in the body, meaning that they should be absorbed from the GI-tract, circulate in the blood and not be immediately metabolically deactivated. Consideration of these open questions will guide our future research. If shown clinically to be safe and having health beneficial effects in vivo, the herbs in question could be utilised in novel applications of the de-odourised antioxidant-rich extracts or as nutritional supplements, functional food components or food antioxidants.

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