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Abstract
In this study, the antioxidative capacity effect of essential oils and aqueous tea infusions obtained from oregano, thyme and wild thyme on the oxidation susceptibility of low-density lipoproteins (LDL) has been studied. The results indicate a dose-dependent protective effect of the tested essential oils and aqueous tea infusions on the copper-induced LDL oxidation. The protective effect of essential oils is assigned to the presence of phenolic monoterpenes, thymol and carvacrol, which are identified as the dominant compounds in these essential oils. The strong protective effect of aqueous tea infusions is proposed to be the consequence of large amounts of polyphenols, namely rosmarinic acid and flavonoids (quercetin, eriocitrin, luteolin-7-*O*-glucoside, apigenin-7-*O*-glucoside, luteolin, apigenin), with the most pronounced effect in the case of oregano. These findings may have implications for the effect of these compounds on LDL *in vivo*.

Keywords: *Low-density lipoprotein, oregano, thyme, wild thyme, essential oils, aqueous tea infusions*

Introduction

Low-density lipoproteins (LDL) oxidation is a free radical chain reaction during which peroxidation of the polyunsaturated fatty acids occurs, accompanied by the formation of very reactive aldehydes. Therefore, recognition of different antioxidants is important in protection of LDL against peroxidation and may be crucial in fighting against atheroclerosis.

Many studies have documented that the consumption of phenolic antioxidants in food products can act as the crucial factor responsible for reduced coronary diseases (Frankel et al. 1993; Bell et al. 2000; Teissedre and Waterhouse 2000). Plants of the *Lamiaceae* family have been often used in the process of extraction of active

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components (Lagouri et al. 1993). The antioxidant capacity of this diverse group of compounds depends on the individual structure and number of hydroxyl groups (Richelle et al. 2001).

This study was designed to investigate the influence of oregano, thyme and wild thyme essential oils, and aqueous tea infusions prepared from these herbs, on copper-induced LDL oxidation. The oxidative status of LDL was monitored spectrophotometrically by following the formation of conjugated dienes. It should be noted that the plants were collected in southern Croatia (Dalmatia) and the essential oils and aqueous tea infusions are therefore characteristic in their content.

Materials and methods

Materials

Dried plant material (100 g) from all individual samples was subjected to a 3 h hydrodistillation using a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous sodium sulphate and stored under nitrogen in a sealed vial at -20°C.

For preparing the tea infusions from oregano, thyme and wild thyme, 15 g each herb was infused into 150 ml boiling distilled water for 30 min, filtered through Whatman No. 1 paper and concentrated under vacuum until dryness. The obtained residue was re-dissolved in water to the final concentration of 60 g/l.

Gas chromatography–mass spectrometry

The analyses of the volatile compounds were run on a Hewlett-Packard GC-MS system (GC 5890 series II; MSD 5971A, Hewlett Packard, Wienna, Austria) as described in Kulisic et al. (2004).

High-performance liquid chromatography analysis of aqueous tea infusions from oregano, thyme and wild thyme

High-performance liquid chromatography analysis of aqueous tea infusions from oregano, thyme and wild thyme were performed under conditions described in Kulisic et al. (2006).

LDL isolation and oxidation

The procedure for the isolation and oxidation of LDL was described in Krisko et al. (2005). Samples prepared were 0.002 g/l, 0.004 g/l and 0.02 g/l essential oils and 0.0002 g/l, 0.02 g/l and 0.24 g/l aqueous tea infusions.

Results

Chemical composition of oregano, thyme and wild thyme essential oils and aqueous tea infusions

The phenolic monoterpenes thymol (oregano, 35.0%; thyme, 80.4%; and wild thyme, 30.0%) and carvacrol (oregano, 32.0%; thyme, 2.1%; and wild thyme, 49.4%) are identified as major compounds in oregano, thyme and wild thyme essential oils

(Kulisic et al. 2004). Other important constituents of essential oils were γ-terpinene (oregano, 10.5%; thyme, 5.5%; and wild thyme, 5.3%) and *p*-cymene (oregano, 9.11%; thyme, 11.6%; and wild thyme, 5.2%).

The results of high-performance liquid chromatography-photo diode array (PDA) analysis are summarized in Table I. Rosmarinic acid is a dominant component detected in oregano (123.22 mg/g), thyme (17.45 mg/g) and wild thyme (93.13 mg/g) aqueous tea infusions. Other identified components of the studied aqueous tea infusions were the flavonoids eriocitrin, luteolin, apigenin and quercetin.

It should be noted that the presence of eriocitrin was significant for aqueous tea infusions from oregano (17.20 mg/g) and wild thyme (10.26 mg/g). The amount of luteolin-7-*O*-glucoside was the highest in wild thyme infusion (10.37 mg/g), while the aqueous tea infusion from oregano showed a high content of apigenin-7-*O*-glucoside (5.97 mg/g).

In addition, the presence of phenolic acids was detected only in traces (0.05 mg/g *p*-hydroxybenzoic acid in thyme infusion, 0.02 mg/g caffeic acid in thyme and oregano tea infusions, and 0.03 mg/g caffeic acid in wild thyme tea infusion).

Effect of oregano, thyme and wild thyme essential oils and aqueous tea infusions on LDL oxidation

The kinetics of copper-induced LDL oxidation in the presence of different concentrations of essential oils of oregano, thyme and wild thyme are presented in Figure 1. The process, monitored over conjugated diene production, exhibits a lag phase, a propagation phase and a decomposition phase. The results indicate that LDL samples enriched with 0.004 g/l essential oils (Figure 1a) exhibit a prolonged lag phase (40 min for oregano, thyme and wild thyme essential oils) as compared with the native LDL sample (10 min). LDL samples enriched with 0.02 g/l essential oils (Figure 1b) also exhibit a prolonged lag phase (110 min for thyme) in comparison with native LDL (30 min). Furthermore, LDL samples exposed to 0.02 g/l essential oils of oregano and wild thyme never entered a propagation phase of oxidation. On the other hand, LDL samples enriched with 0.002 g/l applied essential oils did not have significant effect to the kinetics of LDL oxidation (data not shown).

The kinetics of copper-induced LDL oxidation in the presence of 0.02 g/l aqueous tea infusions of oregano, thyme and wild thyme are presented in Figure 2. It can be

Table I. Qualitative and quantitative data for aqueous tea infusions from oregano, thyme and wild thyme.

	Sample		
Identified compounds	Oregano	Thyme	Wild thyme
p-Hydroxybenzoic acid	Not detected	0.05 ± 0.01	Not detected
Caffeic acid	0.022 ± 0.01	0.02 ± 0.00	0.03 ± 0.00
Eriocitrin	17.20 ± 0.18	1.96 ± 0.09	10.26 ± 0.13
Rosmarinic acid	123.22 ± 10.57	17.45 ± 0.21	93.13 ± 9.85
Luteolin-7-*O*-glucoside	3.89 ± 0.03	1.36 ± 0.02	10.37 ± 0.11
Apigenin-7-*O*-glucoside	5.97 ± 0.12	2.37 ± 0.01	0.62 ± 0.01
Quercetin	0.70 ± 0.01	0.16 ± 0.00	0.31 ± 0.00
Luteolin	0.61 ± 0.01	0.41 ± 0.01	0.25 ± 0.00
Apigenin	0.03 ± 0.00	0.05 ± 0.00	0.44 ± 0.01

Data (mg/g sample) represent the average of triplicates ± standard deviation.

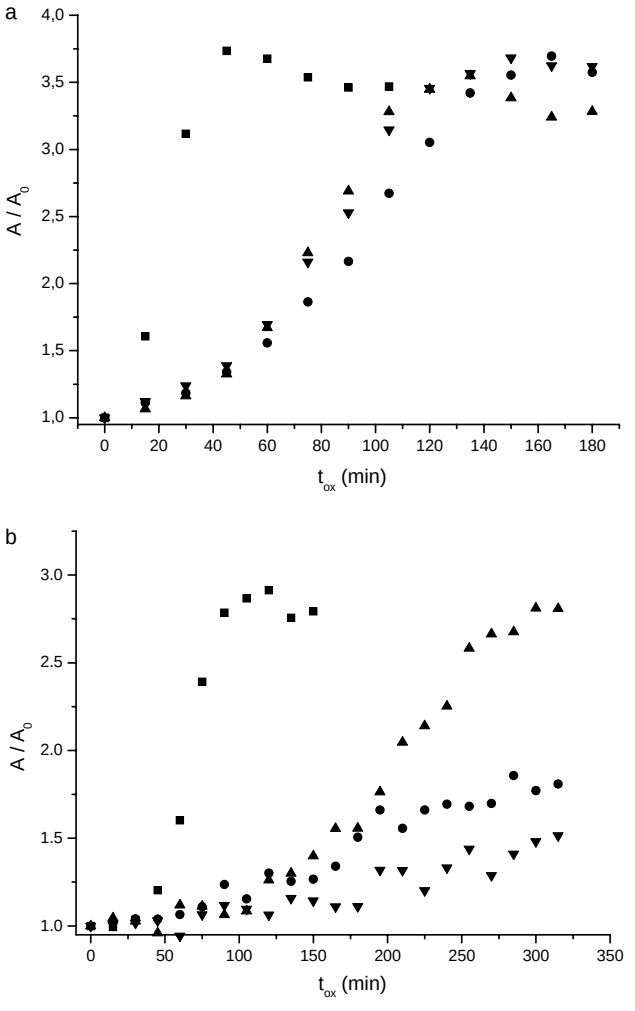

Figure 1. Oxidation of LDL (0.1 μM) induced by 2.5 μM copper. Measurements of conjugated diene formation at 234 nm were performed for native LDL (■) and for LDL in the presence of oregano (●), thyme (▲) and wild thyme (▼) essential oils at concentrations of (a) 0.004 g/l and (b) 0.02 g/l. The duration of the oxidation process is denoted t_{ox}. Absorbances at each time point of the oxidation process (A) are normalized with respect to the absorbance at the beginning of oxidation (A_0).

clearly seen that the aqueous tea infusion obtained from oregano inhibits the process of LDL oxidation, which never enters the propagation phase. Furthermore, the presence of thyme and wild thyme aqueous tea infusions leads to the prolongation of the lag phase of LDL oxidation from 30 min for native LDL to 250 min and 1200 min for LDL exposed to thyme and wild thyme, respectively. In addition, the amount of conjugated dienes detected in the decomposition phase is decreased in the presence of thyme and wild thyme aqueous tea infusions.

LDL samples enriched with 0.24 g/l all aqueous tea infusions inhibited the process of the copper-induced LDL oxidation. On the other hand, LDL samples enriched

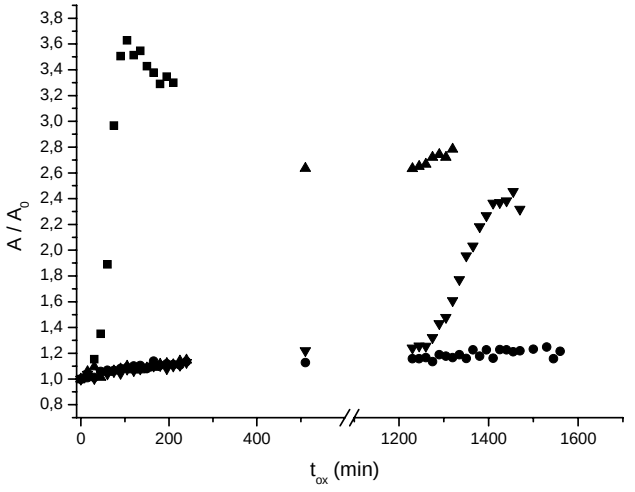

Figure 2. Oxidation of LDL (0.1 μM) induced by 2.5 μM copper. Measurements of conjugated diene formation at 234 nm were performed for native LDL (■) and for LDL in the presence of oregano (●), thyme (▲) and wild thyme (▼) aqueous tea infusions (0.02 g/l). The duration of the oxidation process is denoted t_{ox}. Absorbances at each time point of the oxidation process (A) are normalized with respect to the absorbance at the beginning of oxidation (A_0).

with 0.0002 g/l aqueous tea infusions did not have a significant effect on the kinetics of LDL oxidation (data not shown).

Discussion and conclusion

Analysis of the chemical composition of oregano, thyme and wild thyme essential oils proved the chemical similarity of all tested essential oils, and identified the phenolic monoterpenes thymol and carvacrol as their major components. In addition, we performed copper-induced LDL oxidation and provided experimental evidence that essential oils obtained from oregano, thyme and wild thyme have a protective role on LDL during the oxidation process. This conclusion is supported by the observation that the duration of the lag phase of LDL oxidation is increased in the presence of all essential oils in a dose-dependent manner. It is possible to suggest that the components of essential oils, predominantly thymol and carvacrol, act as antioxidants in these early stages of LDL oxidation. It cannot be excluded that these compounds also associate with LDL particles and provide a sterical barrier between the oxidation initiators and LDL. In addition, Teissedre and Waterhouse (2000) evaluated the antioxidant activities of 23 essential oils in inhibiting human LDL oxidation *in vitro* and also confirmed a protective role of essential oils from various plants on LDL during oxidation.

High-performance liquid chromatography-PDA analysis detected a dominant presence of rosmarinic acid in the investigated aqueous tea infusions. This is in accord with numerous studies that confirmed plants from *Lamiaceae* as a good source of rosmarinic acid (Lamasion et al. 1991; Exarchou et al. 2002; Bendini et al. 2002). Furthermore, recent studies on rosmarinic acid activity proved its high specific antioxidant activity as well as its ability to prevent LDL oxidation (Cartron et al. 2001). Flavonoid compounds and their glucosides (eriocitrin, luteolin-7-*O*-glucoside,

apigenin-7-*O*-glucoside, quercetin) were also detected in the composition of the aqueous tea infusions from oregano, thyme and wild thyme. It has been established previously that alcohol-free red wine extract and quercetin, one of its components, can inhibit LDL oxidation after *in vivo* supplementation (Chopra et al. 2000) as well as other flavonoids (Brown et al. 1998).

Aqueous tea infusions from oregano completely inhibit LDL oxidation even at the small dose of 0.02 g/l, and the process never enters the propagation phase. This is in accord with the results of Cervato et al. (2000) that also demonstrated very high polyphenol content and consequently strong antioxidant properties of aqueous and methanolic oregano extracts in the inhibition of all phases of the peroxidative process. In the presence of high concentrations of the tested aqueous tea infusions (0.24 g/l), the inhibition of LDL oxidation was complete.

The aqueous tea infusions prepared from oregano, thyme and wild thyme have a stronger protective effect on LDL during oxidation than the tested essential oils isolated from the same plants. Structural, physical and chemical properties of each phenolic compound are probably extremely important to explain these antioxidant activities. Oregano, thyme and wild thyme are a good source of compounds important to prevent oxidation of LDL. Their putative protective role *in vivo* could be influenced by multiple factors (bioavailability, transformation in the body, pharmacokinetics) and needs further investigation.

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