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Antioxidant Activity of Aqueous Tea Infusions Prepared from Oregano, Thyme and Wild Thyme

Tea Kulišić[1]*, *Verica Dragović-Uzelac*[2] *and Mladen Miloš*[1]

[1]Faculty of Chemical Technology, University of Split, Teslina 10/V, HR-21 000 Split, Croatia

[2]Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, HR-10 000 Zagreb, Croatia

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Summary

Using a multiple-method approach, antioxidant activity of aqueous tea infusions prepared from oregano (*Origanum vulgare* L. ssp. *hirtum*), thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) were tested in relation to their chemical composition. Total phenolic, flavonoid, catechin and anthocyanin content was determined by spectrophotometric methods. Oregano aqueous tea infusion had the highest amount of total phenols (12 500 mg/L gallic acid equivalent, GAE) and flavonoids (9000 mg/L GAE). Identification of polyphenolic compounds in aqueous tea infusions by HPLC-PDA analysis showed a dominant presence of rosmarinic acid (in mg/g): 123.11 in oregano, 17.45 in thyme and 93.13 in wild thyme. Antioxidant activity of aqueous tea infusions was evaluated using four antioxidative methods (the β-carotene bleaching method (BCB), the 2,2′-diphenyl-1--picrylhydrazyl (DPPH) radical scavenging method, the thiobarbituric acid reactive species (TBARS assay) and the induction period of lard oxidation (Rancimat assay)). The results were compared with natural (ascorbic acid and α-tocopherol) and synthetic antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). Oregano aqueous tea infusion showed the strongest antioxidant activity using three methods (the β-carotene bleaching method, DPPH radical scavening method and Rancimat assay), while thyme and wild thyme showed weaker and relatively similar antioxidant activity.

Key words: antioxidant activity, aqueous tea infusion, *Lamiaceae*, oregano, thyme, wild thyme

Introduction

Free radical formation is controlled naturally by various beneficial compounds known as antioxidants (*1*). The terminology describing the actions of antioxidants is unfortunately not completely clear because there are various types of antioxidants. It is known that plant phenolic compounds possess antioxidative properties acting as radical scavengers and chain-breaking antioxidants under certain conditions. Polyphenols such as flavonoids and anthocyanidins have metal chelating properties. They bind and stabilise redox-active metals, thus inhibiting their participation in deleterious reactions (*2*). Because of these reasons, plant waste rich in polyphenols represents a great challenge for food industry. Some flavonoids present in tea infusions may have protective effects against coronary heart disease, cancer or allergy (*3*).

Among the plants studied, those belonging to the *Lamiaceae* family have often been used to extract active components (*4*). The main classes of phenolic compounds reported to be present in those plants are hydroxycinnamic acids and flavones, mainly in the form of derivatives such as esters and glycosides (*5,6*). Reschke (*7*) found the presence of high levels of rosmarinic acid in plants of the *Lamiaceae* family.

*Corresponding author; Fax: ++385 21 384 964; E-mail: tea@ktf-split.hr

Among *Lamiaceae* species, oregano (*Origanum vulgare* L.), thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) have been studied widely for their antioxidant activity, due to the high content of phenolic compounds (*8–10*). Lagouri and Boskou (*11*) reported the presence of five major phenolic compounds with antioxidant activity in the extracts of oregano. These identified compounds were protocatechuic acid and its phenyl glucoside, caffeic acid, rosmarinic acid and a phenolic derivative of rosmarinic acid. Kikuzaki and Nakatani (*12*) identified tocopherol homologues in the dichloroethane extracts of oregano. Cervato *et al*. (*13*) confirmed the antioxidant activity of aqueous and methanolic extracts of oregano. In our previous study (*14*) significant antioxidant activity of compounds present in the essential oil from oregano, especially its phenolic monoterpenes – thymol and carvacrol, was proved. Fecka and Cisowski (*15*) identified rosmarinic acid, salvianolic acid, luteolin-7-*O*-β-glucuronide and luteolin glucoside as the main polyphenolic compounds in thyme and wild thyme.

In the present paper, different methods for evaluation of antioxidant activity of aqueous tea infusions prepared from Greek oregano (*Origanum vulgare* L. *hirtum*), thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) were applied. The antioxidant activity of aqueous tea infusions was related to their chemical composition and compared with the antioxidant activity of natural (ascorbic acid and α-tocopherol) and synthetic antioxidants (butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA)). The content of total phenolics, flavonoids, non-flavonoids, catechins and anthocyanins of aqueous tea infusions was determined using spectrophotometric methods. Identification of polyphenolic compounds from aqueous tea infusions was made by HPLC-PDA analysis.

Materials and Methods

Materials

Oregano (*Origanum vulgare* L. ssp. *hirtum*), thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) were collected in central Dalmatia (Croatia). The voucher specimens are deposited in the Laboratory of Biochemistry and Food Chemistry, Faculty of Chemical Technology, Split, Croatia.

For preparing tea infusions from oregano, thyme and wild thyme, 15 g of the air-dried herb (flowered tops and stalks) were infused into 150 mL of boiling distilled water for 30 min, filtered through Whatman No. 4 paper and then concentrated under vacuum until dry. The residue obtained was redissolved in water to the final concentration of 60 g/L.

Determination of total phenol, flavonoid, catechin and anthocyanin content in aqueous tea infusions from oregano, thyme and wild thyme

Determination of total phenolic content in aqueous tea infusions from oregano, thyme and wild thyme was carried out according to Amerine and Ough (*16*) and Singleton and Rossi (*17*) using a Folin-Ciocalteau colorimetric method, calibrating against gallic acid as the reference standard and expressing the results as gallic acid equivalents (GAE). The formaldehyde precipitation was used to determine flavonoid phenolic compounds in aqueous tea infusions (*18*). Formaldehyde reacts with 6′ or 8′ position on the 5,7-dihydroxy flavonoids forming a methylol derivative that will attach to another 6′ or 8′ position on another flavonoid and so on. These condensed molecules can be removed by filtration. The residual non-flavonoid phenolic tannin was tested by the Folin-Ciocalteau method. The content of flavonoids was calculated as the difference between total phenolic content and non-flavonoid content in the investigated aqueous tea infusions.

The amount of catechins was determined using the vanillin assay (*16*). In this assay, which is specific one for flavan-3-ols, proanthocyanins and dihydrochalcones, catechin reacted with vanillin and the resulting coloured compound could be quantitatively determined. (+)-Catechin, a monomeric flavan-3-ol, was used as a standard.

The anthocyanin content in aqueous tea infusions from oregano, thyme and wild thyme was determined by bisulphite bleaching method (*19*). Sulphur dioxide additions cause changes in absorbance in the unpolymerized pigments but not in the condensed or polymerized pigments. The molar absorbance value for cyanidin 3,5--diglucoside was used as a standard value (*20*).

HPLC analysis of aqueous tea infusions from oregano, thyme and wild thyme

The analytical HPLC system employed consisted of a Varian Pro Star System (Palo Alto, CA, USA) equipped with a Pro Star Solvent Delivery Module 230, Injector Rheodyne 7125 and Pro Star 330 UV-VIS-Photodiode Array Detector. Chromatographic separations were performed on a Pinnacle II C-18 column (250 × 4.6 mm i.d., 5 μm) including Pinnacle C-18 guard column (10 × 4 mm i.d., 5 μm) (Restek, Bellefonte, USA). Gradient elution was effected using a ternary nonlinear gradient of the solvent mixture methanol/water/acetic acid (10:88:2, by volume) (solvent A), methanol/water/acetic acid (90:8:2, by volume) (solvent B) and methanol (solvent C). The composition of solvent B was increased from 15 to 30 % in 15 min, then increased to 40 % in 3 min, held for 12 min and increased to 100 % in 5 min. The composition of solvent C was increased to 15 % in 2 min, then increased to 30 % in 11 min and returned to initial conditions in the next 2 min. Operating conditions were as follows: flow rate 0.7 mL/min, column temperature 20 °C, injection volumes 20 μL of the standards and sample extracts. The measurements were performed on the UV/VIS-photo diode array detector with detection at 278 nm.

Detection was performed with UV/VIS-photo diode array detector by scanning from 210 to 360 nm. Identification of separated compounds was carried out by comparing retention times and spectral data with those of authentic standards. Identified phenolic compounds were quantified using the external standard method and quantification was based on the peak area. Calibration curves of the standards were made by diluting stock solutions of standards in 80 % aqueous methanol to yield 5–50 mg/L of *p*-hydroxybenzoic acid, caffeic acid, luteolin and apigenin; 10–150 mg/L of quercetin, eriocitrin and luteolin-7-*O*-glucoside; and 10–100 mg/L of rosmarinic acid.

Apigenin-7-*O*-glucoside was quantified as apigenin. The samples were prepared and analysed in triplicate. Data are presented as the mean value ± standard deviation.

Determination of antioxidant activity using the β-carotene bleaching (BCB) method

Antioxidant activity of aqueous tea infusions from oregano, thyme and wild thyme was determined according to a slightly modified version of the β-carotene bleaching method (*21*). β-carotene (0.1 mg) was added to a boiling flask together with linoleic acid (20 mg) and Tween 40 (100 mg), all dissolved in chloroform. After evaporation to dryness, under vacuum at 50 °C using rotary evaporator, oxygenated distilled water (50 mL) was added and the mixture was emulsified for 1 min in the ultrasonic bath to form emulsion A. Two different concentrations (4 and 40 g/L) of ethanolic stock solution of the samples were prepared, of which 200 μL were mixed with 5 mL of emulsion A in open-capped cuvettes. A control, without antioxidant, consisted of 200 μL of ethanol and 5 mL of emulsion A. Emulsion B consisted of 20 mg of linoleic acid, 100 mg of Tween 40 and 50 mL of oxygenated water. The mixture of 200 μL of ethanol and 5 mL of emulsion B served as a blank. Readings of all samples were taken immediately (t=0) and at 15-minute intervals for 120 min on a Perkin-Elmer Lambda EZ 201 spectrophotometer at 470 nm. The cuvettes were incubated at 50 °C between measurements. All determinations were performed in duplicate. The antioxidant activity coefficient (AAC) was calculated from the data with the formula (*22*):

$$AAC=[(A_{A(120)}-A_{C(120)})/(A_{C(0)}-A_{C(120)}]\cdot 1000$$

where $A_{A(120)}$ is the absorbance of the antioxidant at t=120 min, $A_{C(120)}$ is the absorbance of the control at t=120 min, and $A_{C(0)}$ is the absorbance of the control at t=0 min.

Determination of antioxidant acivity using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method

The antioxidant activity of the aqueous tea infusions from oregano, thyme and wild thyme was measured in terms of hydrogen donating or radical scavenging ability, using the stable radical, DPPH (*23*). A volume of 50 μL of aqueous tea infusions of two different concentrations (1.05 and 10.5 g/L) was placed in a cuvette with 1 mL of $6\cdot10^{-5}$ M ethanolic solution of DPPH. Absorbance measurements commenced immediately. The decrease in absorbance at 517 nm was determined by Perkin-Elmer spectrophotometer after 1 h for all tested samples. Ethanol served as a blank. The absorbance of the DPPH radical without an antioxidant, *i.e.* control, was measured daily and special care was taken to minimize the loss of free radical activity of the DPPH stock solution (*24*). All determinations were performed in triplicate. The amount of sample necessary to decrease the absorbance of DPPH (IC_{50}) by 50 % was calculated graphically. Inhibition (in %) was plotted against the sample concentration in the reaction system. The inhibition percentage of the DPPH radical was calculated according to the formula of Yen and Duh (*25*):

$$\text{Inhibition}/\%=(A_{C(0)}-A_{A(t)}/(A_{C(0)})\cdot 100$$

were $A_{C(0)}$ is the absorbance of the control at t=0 min and $A_{A(t)}$ is the absorbance of the antioxidant at t=1 h.

Determination of antioxidant activity using thiobarbituric acid reactive species (TBARS) assay

A modified TBARS assay (*26*) was used to measure the potential antioxidant capacity using egg yolk homogenates as lipid rich media. Yolk homogenate (0.5 mL of 10 %, by mass per volume) and 0.1 mL of different concentrations of sample solutions (4.0, 20.0 and 40.0 g/L), prepared immediately before use, were added to a test tube and made up to 1.0 mL with distilled water. A volume of 0.05 mL of 2,2'-azobis(2-amidinopropane) dihydrochloride solution (0.07 M) in water was added to induce lipid peroxidation. Then 1.5 mL of 20 % acetic acid in 1.1 % (by mass per volume) sodium dodecyl sulphate solution was added and the resulting mixture was vortexed, and then heated at 95 °C for 60 min. After cooling, 5.0 mL of butan-1-ol were added to each tube, then vortexed and centrifuged at 1200 × g for 10 min. The absorbance of the organic upper layer was measured using a spectrophotometer (Perkin-Elmer Lambda EZ 201, Rome, Italy), set at 532 nm. All the values were based on the percentage of antioxidant index (AI/%):

$$\text{AI}/\%=(1-A_T/A_C)\cdot 100$$

where A_C is the absorbance value of the fully oxidized control, and A_T is the absorbance of the test sample.

Induction period of lard oxidation (Rancimat assay)

The induction period of lard with and without the addition of antioxidants was determined with the Rancimat model 743 (Metrohm, Switzerland) at 100 °C and the airflow of 20 L/h. A solution of the antioxidant (100 μL) was added to the lard (2.5 g), giving a final concentration of 0.16 % (by mass) of antioxidant. The lard applied in this method was home made, obtained from the fresh pig's meat.

The antioxidant activity index (AI) was calculated from the measured induction times, according to the following formula by Forster *et al.* (*27*):

AI=Induction time of lard oxidation with antioxidant/
Induction time of lard oxidation without antioxidant

Results and Discussion

Identification of total phenols, flavonoids, catechins and anthocyanins in aqueous tea infusions from oregano, thyme and wild thyme

The results of the determination of total phenolics, flavonoids, non-flavonoids, catechins and anthocyanins from the investigated aqueous tea infusions are presented in Table 1. In oregano aqueous tea infusion, a very high content of total phenols (12 500 mg/L GAE) and flavonoids (9000 mg/L GAE) was determined. Cervato *et al.* (*13*) also demonstrated that the oregano extracts (aqueous and methanol) have very high polyphenol content. Aqueous tea infusions from thyme and wild thyme are less rich source of total phenols (2000 mg/L in thyme, 4000 mg/L in wild thyme) and flavonoids (1500 mg/L in thyme, 3100 mg/L in wild thyme).

Anthocyanins and catechins represent a smaller amount of total polyphenol content in aqueous tea infusions from oregano (2600 mg/L anthocyanins, 50 mg/L catechins), thyme (400 mg/L anthocyanins, 70 mg/L catechins) and wild thyme (1310 mg/L anthocyanins, 36 mg/L catechins)[1].

HPLC analysis of aqueous tea infusions from oregano, thyme and wild thyme

The results of HPLC-PDA analysis for aqueous tea infusions from oregano, thyme and wild thyme are presented in Table 2. The chromatogram of HPLC analysis is presented in Fig. 1. As can be observed, rosmarinic acid is a dominant component detected in all aqueous tea infusions (123.22 mg/g=12 % dry mass in oregano, 17.45 mg/g=1.7 % dry mass in thyme, 93.13 mg/g=9.3 % dry mass in wild thyme). These results are in accordance with numerous studies that confirmed plants from *Lamiaceae* as a good source of rosmarinic acid (*11–14*).

Other significant components of the investigated aqueous tea infusions are eriocitrin (17.20 mg/g in oregano, 10.26 mg/g in wild thyme), apigenin-7-*O*-glucoside (5.97 mg/g in oregano) and luteolin-7-*O*-glucoside (3.89 mg/g in oregano, 10.37 mg/g in wild thyme).

Caffeic acid (0.02 mg/g in oregano and thyme, 0.03 mg/g in wild thyme), quercetin (0.7 mg/g in oregano, 0.16 mg/g in thyme, 0.31 mg/g in wild thyme), luteolin (0.61 mg/g in oregano, 0.41 mg/g in thyme, 0.25 mg/g in wild thyme) and apigenin (0.03 mg/g in oregano, 0.05 mg/g in thyme, 0.44 mg/g in wild thyme) were detected only in traces.

Antioxidant activity of aqueous tea infusions from oregano, thyme and wild thyme

The use of simplified model systems, which mimic the main features of a given food system, or antioxidant assays for quantifying the antioxidant action can be very helpful in clarifying the action of potential antioxidants (*28–31*). In the present study, four different antioxidative methods were used to examine the activity of aqueous tea infusions from oregano, thyme and wild thyme: the β-carotene bleaching (BCB) method, the DPPH radical scavenging method, the thiobarbituric acid reactive species (TBARS) assay and the induction period of lard oxidation (Rancimat assay).

Table 1. The polyphenol content in aqueous tea infusions (60 g/L concentration) from oregano, thyme and wild thyme

Herb	Total phenols	Flavonoids	Non-flavonoids	Catechins	Anthocyanins
	γ(GAE)/(mg/L)	γ(GAE)/(mg/L)	γ(GAE)/(mg/L)	mg/L	mg/L
Oregano	12500	9000	3500	50	2600
Thyme	2000	1500	500	70	400
Wild thyme	4400	3100	1300	26	1300

Table 2. Qualitative and quantitative data for aqueous tea infusions from oregano, thyme and wild thyme

Identified compounds	*m*(sample)=1 g		
	Oregano	Thyme	Wild thyme
	m/mg	*m*/mg	*m*/mg
p-hydroxybenzoic acid	n.d.	0.05±0.01	n.d.
Caffeic acid	0.02±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

Values represent average of triplicates ± standard deviation
n.d.=not detected

[1] The same samples were used for the investigation of inhibition of the copper-induced oxidation of human low-density lipoproteins (Kulisic *et al.*: The effects of essential oils and aqueous tea infusions of oregano (*Origanum vulgare* L. ssp. *hirtum*), thyme (*Thymus vulgaris* L.) and wild thyme (*Thymus serpyllum* L.) on the copper-induced oxidation of human low-density lipoproteins; submitted to *Int. J. Food Sci. Nutr.*).

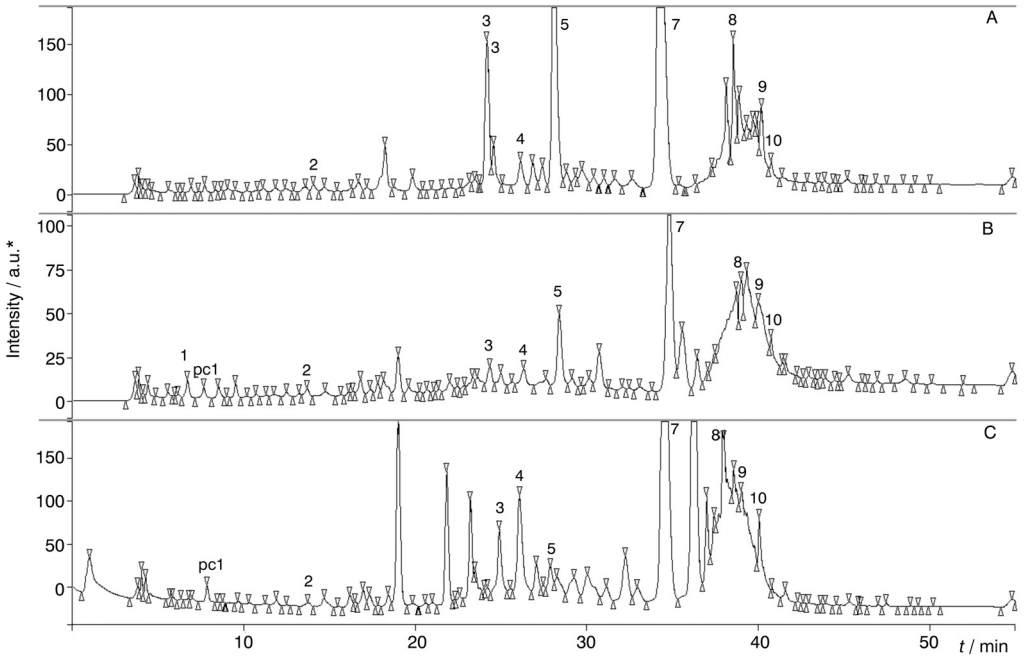

Fig 1. HPLC-PDA analyses of aqueous tea infusions from oregano (A), thyme (B), wild thyme (C) with detector responses at 278 nm. Peaks identification: 1, *p*-hydroxybenzoic acid; pc, unidentified procyanidin; 2, caffeic acid; 3, eriocitrin; 4, luteolin-7-*O*-glucoside; 5, apigenin-7-*O*-glucoside; 7, rosmarinic acid; 8, quercetin; 9, luteolin; 10, apigenin
*a.u.=arbitrary units

β-carotene bleaching (BCB) method

The BCB method is usually used to evaluate the antioxidant activity of compounds in emulsions, accompanied with the coupled oxidation of β-carotene and linoleic acid. Table 3 shows the antioxidant activity coefficients (AAC) of two different concentrations of the investigated aqueous tea infusions, ascorbic acid, α-tocopherol, BHT and BHA as determined with β-carotene bleaching method. The AAC values calculated from the formula given in experimental part facilitate comparisons of the relative activity of different antioxidants. The antioxidant activity expressed as AAC values decreased in the order BHA>BHT>α-tocopherol>oregano tea infusion>thyme tea infusion>wild thyme tea infusion. The antioxidant activity of these samples was dose-dependent.

In spite of the fact that ascorbic acid, as polar compound, is a well known antioxidant, its antioxidant activity was not proved by this method. This can be explained by a phenomenon formulated as the »polar paradox«: non-polar antioxidants exhibit stronger antioxidative activities in emulsions because they concentrate at the lipid/air surface, thus ensuring high protection of the emulsion itself (*32–34*). Generally, antioxidative behaviour of compounds in emulsions has not yet been completely explained (*35*).

Von Gadow *et al.* (*36*) reported the antioxidant activity of flavonoids isolated from rooibos tea (*Aspalathus linearis*). However, their results also showed that reference antioxidants like α-tocopherol, BHT and BHA were better inhibitors of β-carotene bleaching than phenolic acids (caffeic acid and *p*-hydroxybenzoic acid) and flavonoids (quercetin and luteolin). Koleva *et al.* (*34*) reported about high antioxidant activity of rosmarinic acid using BCB method. Its AAC value was even higher than that of BHT.

Aqueous tea infusions from oregano, thyme and wild thyme represent a mixture of different, mainly polar compounds, which complicates the detailed explanation of their antioxidant activity by this method.

DPPH radical scavenging method

In contrast to the BCB method, DPPH method is independent of the substrate polarity. This method is based on the reduction of alcoholic DPPH solutions at 517 nm in the presence of an antioxidant that donate hydrogen or electron. Non-radical form DPPH-H is formed (*34,36*). Evaluation of the antioxidant activity of aqueous tea infusions from oregano, thyme and wild thyme was done in comparison with those of ascorbic acid, α-tocopherol, BHT and BHA (Table 3). Their concentrations needed for 50 % inhibition of DPPH radical were several times lower than those of the investigated aqueous tea infusions. Oregano aqueous tea infusion needed the lowest concentration (0.07 g/L) to reach 50 % of DPPH inhibition in comparison with thyme (0.30 g/L) and wild thyme (0.45 g/L) aqueous tea infusions (Table 3). At the same concentration in reacting system (0.5 g/L), the wild thyme aqueous tea infusions reached 91.55 % inhibition of DPPH, while oregano and thyme aqueous tea infusions reached 85.00 and 69.05 % inhibition (Table 3).

Several authors (*34,36*) confirmed high radical scavenging ability of rosmarinic acid, the major constituent of aqueous tea infusions, and its relatively rapid kinetic behaviour with DPPH radical. They also proved high scavenging ability of phenolic acids (caffeic acid, *p*-hydroxybenzoic acid) and flavonoids (apigenin, luteolin, quercetin). However, the chemical diversity of aqueous tea infusions from oregano, thyme and wild thyme in-

Table 3. Antioxidant activity of aqueous tea infusions from oregano, thyme and wild thyme, BHT, BHA, α-tocopherol and ascorbic acid determined with β-carotene bleaching method, DPPH method, TBARS assay and Rancimat method

Antioxidant	β-carotene bleaching		DPPH scavenging test			TBARS			Rancimat assay
	AAC		Inhibition of DPPH/%**		IC_{50}	AI/%			AI
	0.161 g/L*	1.6 g/L*	0.05 g/L*	0.5 g/L*		100 ppm*	500 ppm*	1000 ppm*	
BHT	851	1945			$1.80 \cdot 10^{-2}$	37.50	64.04	68.60	3.79
BHA	972	2038			$5.40 \cdot 10^{-3}$	38.48	78.93	88.76	7.25
α-tocopherol	741	1671			$8.60 \cdot 10^{-3}$	72.60	79.49	90.00	6.78
Ascorbic acid	–	–			$4.40 \cdot 10^{-3}$	12.50	5.30	2.70	4.31
Oregano infusion	558	1462	46.41	85.00	0.07	–	–	13.40	2.67
Thyme infusion	373	1124	12.90	69.05	0.30	–	16.55	31.39	2.10
Wild thyme infusion	170	897	15.68	91.55	0.45	–	–	10.00	1.90

*Antioxidant concentration in reacting systems

**At these concentrations, kinetic behaviour of BHT, BHA, α-tocopherol and ascorbic acid is so fast that it immediately reaches 100 % of DPPH inhibition

AAC – the antioxidant activity coefficient calculated (as described in experimental part)

IC_{50} – concentration (g/L) for a 50 % inhibition

AI/% – values represent average of triplicates in the presence of 1000 ppm of antioxidants

AI – antioxidant activity index for Rancimat method

cludes the compounds with different abilities to donate hydrogen or an electron. Also, the activity of active substances in aqueous tea infusions is decreased because of the dilution with other components of the infusions. Due to this, it is very difficult to compare the radical scavenging activity between pure compounds and the complex systems like aqueous tea infusions.

TBARS

Lipid peroxides, derived from polyunsaturated fatty acids, are unstable and decompose to form a complex series of compounds. These include reactive carbonyl compounds. The most abundant among them is malondialdehyde (MDA), one of the secondary lipid peroxidation products. Table 3 shows the antioxidant activity expressed as antioxidant index (AI/%) of aqueous tea infusions from oregano, thyme and wild thyme in comparison with those of BHT, BHA, α-tocopherol and ascorbic acid. Investigated aqueous tea infusions did not show protective antioxidant activity even at the highest concentration (1000 ppm), while α-tocopherol and BHA showed high protective antioxidant activity at the lowest concentration. Contrary to α-tocopherol, ascorbic acid did not prove its antioxidant activity by this method. Low antioxidant activity of ascorbic acid and aqueous tea infusions could be explained by their preparation in water, which could provoke the already mentioned polar paradox (the reacting media in this method is egg yolk water emulsion). Because of polar paradox, water-soluble phenolic compounds from aqueous tea infusions did not show the expected antioxidant activity. In our previous study, high antioxidant activity of liposoluble phenolic compounds from oregano essential oil was proved (*14*).

In spite of its simplicity at first sight, this method could not offer completely explained and logical results, not only because of the polar paradox, but also because of the structural features of the antioxidant as well as chemical complexity of this method (*37*).

Rancimat assay

Table 3 gives the induction times and related antioxidant activity index (AI) of lard with and without the addition of antioxidants. The higher induction period of the lard with the antioxidant added, compared to the control (pure lard), the better the antioxidant activity of that compound (*35*). Antioxidant activity as determined with the Rancimat method decreased in the order BHA> α-tocopherol>ascorbic acid>BHT>oregano tea infusion> thyme tea infusion>wild thyme tea infusion. Aqueous tea infusion from oregano, thyme and wild thyme showed antioxidant activity by this method (AI=1.90–2.67), but it is not comparable enough with the activity of natural (ascorbic acid, AI=4.31, and α-tocopherol, AI=6.78) and synthetic (BHT, AI=3.79 and BHA, AI=7.21) antioxidants. Antioxidant activity of oregano extracts using Rancimat method is reported by Aruoma and Halliwell (*38*) and Aruoma *et al.* (*39*). Contrary to our research, Gordon and Kourimska (*40*) observed that BHA and BHT had no antioxidant activity using Rancimat method, explaining that the activity was low due to their high volatile compounds. Von Gadow *et al*. (*36*) reported good antioxidant activity of polyphenolic compounds from rooibos tea extracts, but their research included pure compounds. Aqueous tea infusions of oregano, thyme and wild thyme include many different polyphenolic compounds and it is very difficult to prove the ability of some compound to act as chain-breaking antioxidant and its reaction with peroxyl radicals.

Conclusions

Investigated aqueous tea infusions showed antioxidant activity by three used methods (the β-carotene bleaching, DPPH radical scavenging and TBARS). However, the chemical complexity and the dilution of the active substances in the presence of the other, less active or inactive, compounds from aqueous tea infusions make the comparison with the pure compounds (commercial antioxidants) more complicated. Aqueous tea infusions from oregano, thyme and wild thyme represent a good source of the compounds with significant antioxidant activity.

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