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REGULAR ARTICLE

ANTIMICROBIAL AND ANTIRADICALS ACTIVITY OF *ORIGANUM VULGARE* L. AND *THYMUS VULGARIS* ESSENTIAL OILS

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ABSTRACT

The objective of our study were antioxidant properties of oregano and thyme essential oil by testing their scavenging effect on DPPH radicals activities and antibacterial activities against one Gram-positive strain (*Bacillus cereus* CCM 2010) and two Gram-negative strains (*Pseudomonas aeruginosa* CCM 1960; *Escherichia coli* CCM 3988) was also performed. The thyme EOs showed strong antibacterial activity against *Escherichia coli* CCM 3988 in 0.75

and 0.375 ml.ml^{-1} concentration of EOs. Very strong antibacterial activity was found in thyme and oregano EOs against *Bacillus cereus* CCM 2010 in 0.75, 0.375, 0.188 and 0.094 ml.ml^{-1} concentration of EOs. In comparison to BHT (5.60 µg.ml^{-1} after 30 min; 2.82 µg.ml^{-1} after 60 min) and ascorbic acid (7.48 µg.ml^{-1} after 30 min; 4.79 µg.ml^{-1} after 60 min), *O. vulgare* oil shows significantly higher DPPH activity (2.99 µl.ml^{-1} after 30 min; 2.02 µl.ml^{-1} after 60 min). From the other side, *T. vulgaris* essential (9.69 µl.ml^{-1} after 30 min; 5.84 µl.ml^{-1} after 60 min) oil shows lower antiradical activity in comparison to BHT, and higher activity in comparison to ascorbic acid.

Keywords: antimicrobial activity, DPPH assay, essential oils, oregano, thyme

INTRODUCTION

Essential oils are complex mixtures of numerous compounds from various parts of the plants. Some of the main groups of found in essential oils include alcohols, aldehydes, esters, ethers, ketones, phenols and terpenes. Each of the group consists of numerous compounds. For example, terpenes include monoterpenes, diterpenes, sesquiterpenes, sesquiterpene lactones, etc, which are an important class of volatile constituents and may have bioactive properties (**Orav, 2001**).

Aromatic and medicinal plants have been extensively studied for their antimicrobial and antioxidant activities. Because of their radical scavenging activities and their lipophilic nature, essential oils have a potential to be used in small amounts in fat and in fat-containing food systems to prevent or delay some chemical deteriorations occurring during the storage of these products. This application is especially of interest for those manufacturers and consumers who prefer natural preservatives instead of artificial ones (**Puertas-Mejía, 2002**).

Antimicrobial activities of essential oils have been recognized for many years and recently have been extensively researched (**Elgayyar et al., 2001; Daferera et al., 2003**). However, the most of the studies have focused on the activity *in vitro*, and only very few authors have documented their antimicrobial activity on food products (**Bajpai et al., 2009**).

Ground oregano (*Oreganum vulgare* subsp. *hirtum*) and thyme (*Thymus vulgaris* L), which are both herbs of the *Labiatae* family have long been used as flavouring agents in various food products. Both oregano and thyme EOs possess considerable antibacterial properties due primarily to their carvacrol and thymol content (**Burt, 2004; Govaris et al.,**

2011). The antibacterial activity of the EOs of oregano and thyme against food-borne pathogens has been examined extensively in many in vitro studies. The antimicrobial properties of volatile aromatic oils and medium-chain fatty acids derived from edible plants have been recognized since antiquity. Origanum oil, used as food- flavouring agent, possesses a broad spectrum of antimicrobial activity due, at least in part, to its high content of phenolic derivatives, such as carvacrol and thymol (**Preuss et al., 2005**). In the literature, there are many reports relating the chemical composition and the antimicrobial properties of the essential oils of various origanum species, and their application in various commercial preparations, as antimicrobials and antioxidants (**Baydar et al., 2004; Kulisic et al., 2004**).

Cervato et al. 2000 have found some antiradical activity in aqueous and methanolic extracts of oregano leaves and **Bendini et al. 2002** reported that ethanolic extracts under selected conditions showed antioxidant activity. In other spices, such as rosemary, antioxidant activity has been attributed to phenolic compounds like carnosic acid, rosmanol and rosmarinic acid so as to flavonoids. Phenolic compounds and flavonoids such as luteolin, hispidulin, apigenin, acacetin, diosmetin, herbacetin, quercetin, naringin, among others, had also been described in oregano extracts. Other compounds as, for example, rosmarinic acid have also been identified in oregano. Even though a variety of flavonoids are known there is no correlation between compositional data and antioxidant activity.

The objective of our study were antioxidant properties of oregano and thyme essential oil by testing their reducing power and scavenging effect on DPPH radicals activities and antibacterial activities against one Gram-positive strain (*Bacillus cereus* CCM 2010) and two Gram-negative strains (*Pseudomonas aeruginosa* CCM 1960; *Escherichia coli* CCM 3988) was also performed.

MATERIALS AND METHODS

Essential oils samples

The original *Origanum vulgare l.* and *Thymus vulgaris* essential oils samples produced in Slovakia (Calendula a.s., Nova Lubovna) were obtained. The samples were stored in the dark at a temperature of 4 °C.

Antimicrobial activity

The antimicrobial activity against bacteria was determined by employing the standard discs diffusion technique. Antibacterial activity was assessed on the *Bacillus cereus* CCM 2010, *Escherichia coli* CCM 3988 and *Pseudomonas aeroginasa* CCM 1960. Cultures of each bacteria were set up 24 h before the assays in order to reach the stationary phase of growth. The tests were assessed by inoculating Petri dishes from the cultures with proper sterile media, with the aim of obtaining the microorganism concentration of 10^5 colony forming units CFU.ml^{-1}. An aliquot of dimethylsulfoxide (DMSO; Sigma–Aldrich) was added to the essential oils in order to obtain a 0.0235 - 0.75 ml.ml^{-1} concentration range. Serial dilutions of the DMSO (70%)/essential oil solution were deposited on sterile paper discs (6 mm diameter, Difco) which were subsequently placed in the centre of the inoculated Petri dishes. Therefore, the Petri dishes were then incubated at 37 °C for 24 h and the growth inhibition zone diameter (IZD) was measured to the nearest mm. Controls were set up with DMSO (70%) in amounts corresponding to the highest quantity present in the test solution.

DPPH free radical scavenging activity

Free radical scavenging activities of the *Origanum vulgare l.* and *Thymus vulgaris* essential oils were evaluated with some modification in accordance with method of **Takao *et al.* (1994)**. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) (8 mg) was dissolved in absolute methanol (100 ml) to obtain a concentration of 80 mg.ml^{-1}. Diluted solutions of oil samples (50 µl.ml^{-1} – 0.39 ml.ml^{-1}) as well as BHT (butylated hydroxytoluene) and ascorbic as standards (50 µg.ml^{-1} – 0.39 µg.ml^{-1}), 2 ml each in absolute methanol) were mixed with DPPH (2 ml) and allowed to stand for 30 and 60 min for any reaction to occur. Absorbance was recorded at 517 nm using T80 UV/Vis Double Beam Spectrophotometer.

DPPH radical scavenging activities of standard antioxidants, BHT and ascorbic acid were also assayed for comparison.

Antioxidant activity was expressed as percentage (%) of scavenging activity and IC_{50} value as concentration of oil or standard compound which produces decrease of concentration of DPPH radicals by 50 %:

$$\% = [(A_{DPPH} - A_{sample}) / A_{DPPH}] \times 100$$

IC_{50} values for oils and standards (after 30 min and 60 min) were determined from values of percentage of scavenging of DPPH radicals by using linear regression analysis.

RESULTS AND DISCUSSION

The mechanism of action of EOs and their components as antimicrobials has not been fully elucidated. This is complicated by the fact that there are a large number of chemical compounds present in EOs and often they are all needed for antibacterial activity and the EOs does not seem to have a specific cellular target. Thus the antimicrobial mechanism of EOs may not be attributable to one specific mechanism, but rather there may be several targets in the cell. Most of the focus on antimicrobial mechanisms for EOs has been on the cell membrane and targets interconnected with the membrane. For bioactivity, the EOs pass through the cell wall and cytoplasmic membrane **(Bakkali et al. 2008),** disrupt the structure of different layers of polysaccharides, fatty acids and phospholipids and permeabilize them **(Chaieb et al., 2007).**

Antibacterial activity of essential oils analyzed was according to the disk diffusion method, and the results are shown in Tables 1. The thyme EOs showed strong antibacterial activity against *Escherichia coli* CCM 3988 in 0.75 and 0.375 $ml.ml^{-1}$ concentration of EOs. Very strong antibacterial activity was found in thyme and oregano EOs against *Bacillus cereus* CCM 2010 in 0.75, 0.375, 0.188 and 0.094 $ml.ml^{-1}$ concentration of EOs.

Thyme essential oil had strong antimicrobial activity against *E. coli* and *E. coli* 157:H7. *E. coli* was also significantly inhibited by oregano essential oil. Dependent antimicrobial activity of oregano and thyme essential oils against *E. coli* O157:H7 was also reported by **Sağdıç (2003), Dadalioglu and Evrendilek (2004)** and **Burt (2004).**

Among the bacteria tested, *P. aeruginosa* was the third resistant bacterium against oregano and thyme essential oils after *B. cereus*. Gram-negative *P. aeruginosa* is known to possess a high level of intrinsic resistance to most of the antimicrobial agents due to a very restrictive outer membrane barrier (**Mann et al., 2000**). Resistance of *P. aeruginosa* to essential oils was also reported by other researchers. **Cosentino et al. (1999)** assessed antimicrobial activity of Sardinian thymus essential oils and their components against some spoilage and pathogenic bacteria including *P. aureginosa* isolated from food products, and reported that among the reference strains tested, *P. aureginosa* was the least sensitive both to growth inhibition and lethal effect.

Table 1 Inhibition of the growth of different strains of bacteria by essential oils (in mm)

CO	*E. coli* CCM 3988		*P. aeruginosa* CCM 1960		*B. cereus* CCM 2010	
	TEO	OEO	TEO	OEO	TEO	OEO
0.750	I	10.50±0.58	9.00±0.00	5.75±0.87	I	I
0.375	I	9.00±0.00	8.25±1.44	5.50±0.58	I	I
0.188	14.00±0.00	9.25±0.87	7.75±0.87	5.75±0.87	I	I
0.094	8.50±2.89	5.50±0.58	7.25±0.29	4.75±2.02	I	I
0.047	4.75±0.29	4.75±0.29	3.75±1.44	3.00±0.00	1.25±1.44	5.00±3.46
0.0235	4.75±0.29	3.50±0.00	2.75±0.87	2.50±1.15	2.25±0.87	4.00±1.15
C	NI	NI	NI	NI	NI	NI

CO-concentration of essential oil (ml.ml^{-1}); TEO-Thyme essential oil; OEO-Oregano essential oil; NI-no inhibition; I-inhibition (very strong); C-control

Preliminary screening of the *in vitro* antimicrobial activity of orange, lemon and mandarin EOs was carried out against 6 spoiling and pathogenic microorganisms using the filter paper disc agar diffusion technique. In this respect, lemon and orange EOs showed no inhibition against the 6 microorganisms tested. Mandarin EO showed a wide spectrum of antimicrobial activity, being moderately active against the three Grampositive bacterial strains assayed and strongly active against two Gram negative bacterial strains (*E. coli* O157:H7 and *Salmonella* Enteritidis). Under the treatment conditions assayed, *P. aeruginosa* was not susceptible to the presence of any EOs assayed (**Espina et al., 2011**).

Table 2 Percentage of scavenging activity of DPPH radicals induced by various concentrations of *O.vulgare* and *T. vulgaris* essential oils

	Concentration of essential oil in absolute methanol (µl.ml^{-1})							
	50	25	12.5	6.25	3.13	1.56	0.78	0.39
	percentage (%) of scavenging activity of *O. vulgare* oil							
after 30 min	93.00	92.78	92.24	73.26	59.28	41.49	28.93	19.62
after 60 min	97.20	95.65	93.56	85.63	72.53	52.05	37.24	24.73
	percentage (%) of scavenging activity of *T. vulgaris* oil							
after 30 min	86.8	74.42	57.14	39.71	26.35	17.39	12.27	5.88
after 60 min	97.12	91.77	78.53	59.27	42.57	29.35	20.41	8.76

Table 3 Concentrations of *O. vulgare* and *T. vulgaris* essential oils which produced decrease of DPPH concentration by 50 %

	IC_{50} (**µl.ml^{-1}**)		IC_{50} (**µg.ml^{-1}**)	
	O. vulgare oil	*T. vulgaris* oil	BHT	Ascorbic acid
after 30 min	2.99	9.69	5.60	7.48
after 60 min	2.02	5.84	2.82	4.79

It is clearly seen in Table 2 that, unlike *T. vulgaris* oil, essential oil of *O. vulgare* retains strong DPPH activity up to concentration of 1.56 µl.ml^{-1}. As a result, after measurements in 30 min and 60 min, *O. vulgare* oil shows significantly lower IC_{50} values. Obtained results are in accordance with those previously published showing a high level of phenolic components like carvacrol and thymol, found in *O. vulgare* oil (**Preuss et al., 2005**). When compared to standard antioxidants (BHT and ascorbic acid), *O. vulgare* oil shows significantly higher antiradical activity (Table 3), while oil isolated from *T. vulgaris* shows lower activity in comparison to BHT, but higher in comparison to ascorbic acid.

CONCLUSION

To the best of our knowledge, this is the study providing data on antibacterial and antioxidant activities of the essential oil of *Origanum vulgare* L. and *Thyme vulgaris* from Slovakia. The oil obtained from investigated *Origanum vulgare* L. and *Thyme vulgaris* are quite interesting from a pharmaceutical standpoint because of its antimicrobial properties. Further studies are needed to evaluate the *in vivo* potential of these oils in animal models.

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