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BASIL ESSENTIAL OIL (*OCIMUM BASILICUM*): *IN VITRO* ANTIFUNGAL PROPERTIES AND ANTIOXIDANT ACTIVITY

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Abstract: The purpose of the present study was to evaluate the antioxidant and *in vitro* antifungal properties of commercial basil (*Ocimum basilicum*) essential oil (BEO). The antioxidant activity of BEO was estimated by DPPH free radical scavenging ability. The antifungal activity of the EO was tested against three pathogenic *Penicillium* (*P.*) spp. (*P. expansum*, *P. citrinum*, *P. crustosum*) using the disc diffusion method (concentrations: 12.5 $\mu L.L^{-1}$, 25 $\mu L.L^{-1}$, 50 $\mu L.L^{-1}$, and 100 $\mu L.L^{-1}$). From the results it is clearly evident that *Ocimum basilicum* EO showed a strong antioxidant activity with the value of 86.20 ± 0.15% for inhibition. The highest concentration (100 $\mu L.L^{-1}$) of BEO exhibited the strongest antifungal activity manifested by the highest diameters (5.33 ± 0.58 mm, 4.33 ± 0.58 mm, 3.33 ± 0.58 mm) of inhibition zones against all three fungi strains (*P. crustosum*, *P. citrinum* and *P. expansum*, respectively). These findings show that the BEO represents a good source of biologically active substances that could have potential applications in the food and pharmaceutical industries.

Keywords: basil, essential oil, disc diffusion method, DPPH assay

INTRODUCTION

Currently, the efforts of consumers for a healthy lifestyle and also well-known increasing resistance of microorganisms to synthetic antifungal substances has supported the search for new types of effective and non-toxic antifungal substances among natural sources (Roller et al., 2009). One of the possible solutions to this problem is the application of plant essential oils (EOs; Ba-Hambad et al., 2014).

Generally, EOs are products obtained from diverse parts of herbs, routinely isolated using the steam distillation method (Sahraoui et al., 2008). These natural substances are usually composed of secondary metabolites of aromatic plants with oxygenated structures (e.g. alcohols, ketones, aldehydes, and esters), characterized by significant biological properties, including antibacterial, antifungal and antioxidant activities (Babtista-Silva et al., 2020). In total, about 3,000 types of EOs are known, of which about 300 are also used commercially in the food, pharmaceutical and cosmetic industries (Shaaban et al., 2012).

Aromatic plants belonging to the genus *Ocimum* from the Lamiaceae family are also considered to be a rich source of EOs (Avetisyan et al., 2017), from which basil (*Ocimum basilicum* L.) is the most common species. Consumption of this herb has an anti-inflammatory, antimicrobial, antiviral (Martinec, 2012) and also strong antiseptic effect (Bozin et al., 2006) on human health. Moreover, a number of proven biological properties are dominated by its antifungal (Oxenham et al. 2005), antibacterial, repellent and high antioxidant potential (Bunrathep et al., 2007; Carović-Stanko et al., 2010).

Methyl chavicol (45.8%) and linalool (24.2%), the most abundant components in the concept of basil essential oil (BEO), are responsible for these biological effects (Bozin et al., 2006). Regarding these properties, the effect of BEO as a growth inhibitor of microorganisms in selected food models has been documented in several studies (Suppakul et al., 2003; Hemalatha et al., 2017; Amor et al., 2021).

Therefore, the aim of our study was to determine antioxidant and *in vitro* antifungal activity of BEO to assess its potential as an agent used in food or pharmaceutical industries.

MATERIALS AND METHODS

Essential oil

For all determinations, a commercial *Ocimum basilicum* essential oil (BEO) possessing methyl chavicol (≥ 65%), linalool, and eugenol as major compounds (declared by the manufacturer) was applied. The EO was obtained by the steam distillation of fresh stalks of basil growing in Vietnam (Hanus Company, Nitra, Slovakia).

Fungal strains

Three *Penicillium* (*P.*) strains (*P. crustosum*, *P. citrinum*, and *P. expansum*) were isolated from berry samples of *Vitis vinifera* and consequently classified using a reference based MALDI-TOF MS Biotyper. The obtained results were also validated by comparison with the taxonomic identification obtained by 16S rRNA sequences analysis.

DPPH assay

The antioxidant activity of the BEO was assessed on the basis of the scavenging activity of the stable radicals 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the methodology used in the study Valková et al. (2021).

Disc diffusion method

The evaluation of the antifungal activity of the BEO was performed using the agar disc diffusion method. For this purpose, there was an aliquot of 0.1 mL of fungal suspension in distilled water inoculated on Sabouraud Dextrose Agar (SDA; Merck, Gernsheim, Germany).

Subsequently, the discs of filter paper (6 mm) were impregnated with 10 µL of the analyzed BEO samples (in four

concentrations: 12.5 μL.L^{-1}, 25 μL.L^{-1}, 50 μL.L^{-1}, and 100 μL.L^{-1}), and then applied on the SDA surfaces.

The fungi were incubated aerobically at 25 °C for 5 days. The diameters of the inhibition zones were measured in mm after incubation. Each test was repeated three times (one repetition reflected one separate plate). The values for inhibitory activity increased in the following manner: weak antifungal activity (5 – 10 mm) < moderate antifungal activity (10 – 15 mm) < very strong antifungal activity (zone > 15 mm).

Statistical analysis

The data from the analyses were statistically evaluated using Prism 8.0.1 (GraphPad Software, San Diego, CA, USA). One-way analysis of variance (ANOVA) followed by Tukey's test were used to evaluate the statistical significance of differences between the analyzed groups of samples.

RESULTS AND DISCUSSIONS

Antioxidant activity of BEO

The antioxidant potential of the BEO was estimated in terms of the multiple radical scavenging abilities (Alara et al., 2019). Generally, it is known that DPPH radical is a stable free radical that can donate hydrogen when reacts with antioxidant constituents, and it is reduced to diphenyl picryl hydrazine (Thaipong et al., 2006), which has the ability to neutralize free radicals of extracts that possess unpaired electrons (Atangwho et al., 2013).

Our results showed that the BEO had a strong antioxidant activity with the value for inhibition of 86.20 ± 0.15%. In agreement with our study, Bozin et al. (2006) reported strong antioxidant activity of basil EO containing methyl chavicol (45.8%) and linalool (24.2%) as the main EO components. On the other hand, Mahmoud (2013) found that methyl chavicol had only moderate antioxidant activity.

The study by Dawidowicz and Olszowy (2014) even showed no antioxidant properties of methyl chavicol. Therefore, we assume that the main component of EO does not have to determine its antioxidant activity. Indeed, it is possible that the constituents present only in lower concentrations may contribute to some type of synergic interactions with other active compounds to enhance their antioxidant properties.

Antifungal properties of BEO

Results from the inhibitory effects of the BEO on growth of three tested *Penicillium* spp. fungi (*P. crustosum*, *P. citrinum*, and *P. expansum*) assessed by disc diffusion method are shown in Tables 1-3. Our findings showed that the growth inhibition of *Penicillium* strains depends on the concentration of the BEO applied; whereas the highest growth inhibition ($P < 0.05$) was recorded in all three analyzed strains in the highest BEO concentration (100 μL.L^{-1}) used.

On the other hand, the lowest concentration of the BEO (12.5 μL.L^{-1}) tested had no (*P. crustosum* and *P. expansum*) or only very weak inhibitory efficacy (*P. citrinum*) against the growth of microscopic filamentous fungi.

Table 1. Antifungal activity of BEO against P. crustosum growth.

Fungal strain	Concentration of BEO (μL.L^{-1})			
	12.5	25	50	100
P. crustosum	0.00 ± 0.00^{a}	1.00 ± 0.00^{b}	2.33 ± 0.58^{c}	$5.33 \pm 0.58^{d*}$

Notes: Means ± standard deviation. Values followed by different superscripts within the same row are significantly different ($P < 0.05$). 0.00 – no efficacy. * Weak antifungal activity (5 – 10 mm).

Table 2. Antifungal activity of BEO against P. citrinum growth.

Fungal strain	Concentration of BEO (μL.L^{-1})			
	12.5	25	50	100
P. citrinum	0.67 ± 0.58^{ab}	1.33 ± 0.58^{a}	2.33 ± 0.58^{b}	4.33 ± 0.58^{c}

Notes: Means ± standard deviation. Values followed by different superscripts within the same row are significantly different ($P < 0.05$).

Table 3. Antifungal activity of BEO against P. expansum growth.

Fungal strain	Concentration of BEO (μL.L^{-1})			
	12.5	25	50	100
P. expansum	0.00 ± 0.00^{a}	1.67 ± 0.58^{b}	2.67 ± 0.58^{bc}	3.33 ± 0.58^{c}

Notes: Means ± standard deviation. Values followed by different superscripts within the same row are significantly different ($P < 0.05$). 0.00 – no efficacy.

Generally, microscopic filamentous fungi possess a great ability to colonize many kinds of substrates, and grow even under extreme conditions. Among them, *Penicillium* spp. are the most important species producing the spoilage of food products (Groot et al., 2019); therefore, these species were also selected in our research for analyses.

Our results are in agreement with the study of Saggiorato et al. (2009) who observed that BEO inhibited the growth of *Penicillium* spp. (isolated from an industrial environment), depending on the concentrations used. The weaker antifungal potential of our BEO in lower concentrations can be attributed to the lower presence of methyl chavicol (as the most abundant compound in the EO) that in earlier studies showed variable activity depending on the analyzed microorganisms (Stević et al., 2014). Tadtong et al. (2009) found a moderate to weak antimicrobial activity of EO comprising the highest amount of this substance. Our evaluation of the antifungal activity of the BEO using the disc diffusion method showed promising results. In view of this fact, the study focused on the application of BEO on selected food models in order to determine its effective concentration inhibiting the fungi growth in food products is our next challenge.

CONCLUSIONS

Findings obtained from the study have revealed the antioxidant and *in vitro* antifungal properties of the BEO. From the results it is clearly evident that the BEO showed a remarkable value (86.20 ± 0.15%) for antioxidant activity. Further, all the tested *Penicillium* spp. (*P. crustosum*, *P. citrinum*, and *P. expansum*) were the most sensitive to the BEO in the highest concentration (100 μL.L^{-1}). Thus, our data confirm the possibility of the application of the BEO in the higher

concentration (≥ 100 µL.L^{-1}) as an alternative to traditional medicine, and also as a natural agent applied for food preservation. These data also complement our previous research providing an extensive overview of the biological functions of several commercial EOs purchased from the Hanus Company.

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