Chemical Composition and Antifungal Activity of Lemongrass Essential Oil

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Abstract
Essential oils (EOs) are liquid extracts from aromatic plants with many applications in diverse industries. Lemongrass (*Cymbopogon nardus* Rendle) belongs to plants known for their many biological properties. The present study was designed to evaluate commercial lemongrass essential oil (LGEO) in relationship to its chemical composition and *in vitro* antifungal activity against four filamentous fungi of the genus *Penicillium* (*P.*) spp. (*P. expansum, P. italicum, P. aurantiogriseum,* and *P. chrysogenum*). For these purposes, gas chromatography-mass spectrometry and disc diffusion methods were used. The results from the volatile profile determination showed that citronellal (35.3%), geraniol (23.4%), β-citronellol (11.7%), citronellyl acetate (3.9%) and α-limonene (3.8%) were the major components of the EO chemical constitution. Lemongrass EO at the highest concentration (500 µL/L) exhibited the most effective ($P < 0.05$) inhibitory action (inhibition zones: 6.17 ± 0.27 mm, 4.27 ± 0.25 mm, 6.90 ± 0.36 mm, 5.90 ± 0.36 mm, respectively) against the growth of all fungi strains (*P. expansum*, *P. italicum*, *P. aurantiogriseum*, and *P. chrysogenum*) investigated. Based on the above-mentioned findings it can be seen that LGEO appears to be a promising natural agent with an inhibitory effectiveness on the *Penicillium* spp. growth and thus, it can find an application in the food industry.

Keywords: Lemongrass essential oils, antifungal properties, disc diffusion method, volatile compounds

1. Introduction

Medicinal plants have been used for many years for various applications, such as the treatment of diseases [1], perfumery and food preservation [2]. Due to the growing antibiotic resistance of microorganisms, as well as the emergence of new types of diseases, the scientific community is currently focusing on the development of new and effective natural drug / preservative alternatives [3]. Herbs represent an invaluable source of antimicrobial compounds; therefore, they appear to be a promising option to solve this problem [4]. Due to a number of biological functions, essential oils (EOs) are among the most widely used plant metabolites [5].

Generally, plant EOs are complex mixtures of natural compounds, both polar and non-polar [6]. According to International Organization for Standardization (ISO; ISO/D1S9235.2), EOs are characterized as substances produced by

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distillation (water/steam/dry) or by mechanical processing of natural materials; in terms that substances are safer to consumers, as well as to animals and to the whole environment [7]. In total, approximately 3,000 types of these aromatic substances are known in the world, of which 300 are of commercial interest in various industries [8]. Currently, there is a great demand for EOs obtained from various species of *Cymbopogon* [9]. Lemongrass (*Cymbopogan nardus*) belongs to the Poaceae family which is well-known besides others as a source of cellulose, hemicellulose and lignin [10]. Lemongrass EO (LGEO) is most often extracted by steam distillation from fresh or dried leaves of the plant [11]. On a large scale, it is applied in commercial production, mainly in the food (fragrances or flavors) and pharmaceutical industries [12]. In addition, this volatile oil is widely used in the perfumes and cosmetics manufacturing sector [13]. Scientific research has shown that the chemical composition of LGEO exerts different biological functions including antibacterial, antifungal [14], antioxidant, anti-inflammatory, anxiolytic [15], and antinociceptive [16] activities.

Therefore, in the present study, an effort was made to determine the chemical profile and inhibitory effectiveness of LGEO against the growth of different *Penicillium* (*P.*) fungal strains (*P. expansum*, *P. italicum*, *P. aurantiogriseum*, and *P. chrysogenum*) as a potential preservative used in the food sector or alternative for synthetic antimicrobial agents employed in pharmaceuticals.

2. Materials and methods

2.1. Essential oil

Lemongrass essential oil (LGEO; *Cymbopogon nardus* Rendle) was obtained from a commercial company (Hanus Ltd, Nitra, Slovakia).

2.2. Strains of microscopic filamentous fungi

Four strains of microscopic filamentous fungi of the genus *Penicillium* were used for analyzes (*P. expansum*, *P. italicum*, *P. aurantiogriseum*, *P. chrysogenum*). The fungi were isolated from a variety of materials usable in the food sector, and were subsequently identified using macro- and micro-morphological characteristics based on mycological keys [17-19].

2.3. Determination of volatile substances

The chemical profile of LGEO was performed using an Agilent 6890N gas chromatograph coupled to quadrupole mass spectrometer 5975B as reported by Valková et al. [20]. The individual peaks of samples were identified based on their retention indices [21], and they were compared with the library mass spectral database (Wiley and NIST databases), as well. The retention indices were experimentally determined using the standard method [22] which included retention times of n-alkanes (C6-C34), injected under the same chromatographic conditions. The percentage composition of compounds (relative quantity; amounts higher than 0.1%) were derived from their GC peak areas.

2.4. *In vitro* antifungal activity by disc diffusion assay

Testing of the antifungal properties of LGEO was performed using the agar disc diffusion method according to the Valková et al. [20] with minor modifications. For this purpose, an aliquot of 0.1 mL of fungal strains in distilled water was inoculated on Sabouraud dextrose agar (SDA). Subsequently, the discs of filter paper (6 mm) were impregnated with 10 μL of LGEO samples (in concentrations: 62.5, 125, 250, and 500 $\mu L.L^{-1}$), then applied on the SDA surface, and incubated at 25 °C for 5 days. The discs impregnated with ethanol served as negative controls. After incubation, the diameters of the inhibition zones (mm) were measured. 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 (5 – 10 mm) < very strong antifungal activity (zone > 15 mm).

2.5. Statistical analysis

Statistical analyses were performed using program Prism 8.0.1 (GraphPad Software, San Diego, California, USA). Means and standard deviations were calculated for each measurement. One-way analysis of variance (ANOVA) followed by

Tukey's test were used to evaluate the significance of differences between analyzed groups of samples. Probability values less than 0.05 ($P < 0.05$) were accepted as significant.

3. Results and discussion

3.1. Chemical composition of LGEO

Determination of EOs chemical composition is a crucial step needed to understand their biological activities [23]. Therefore, the chemical compounds of our LGEO were detected; those representing more than 0.1% of total EO are listed in Table 1. From the findings it can be seen that 26 different volatile components were detected in LGEO, comprising 99.2% of the total EO volatile constituents. The EO was particularly rich in citronellal (35.3%) followed by geraniol (23.4%), and β-citronellol (11.7%).

According to Nhu-Trang et al. [24] citronellal, as one of the important monoterpene aldehydes (causing the intense aroma of EOs), is a main component of lemongrass. Similar to our finding, the concentrations of citronellal (35.5 and 37.8%), as the major compound of LGEO, were detected in the studies performed by Koba et al. [25], and Trindade et al. [26], respectively. Lower concentration of citronellal (29.6%) has been reported by Wei and Wee [27]. Inconsistently with our research, citral (43.8%), z-citral (18.9%), geranyl acetate (5.3%) and trans-geraniol (3.7%) have been found to be the main constituents in the EO of lemongrass employed in the research of Pandey et al. [28]. We propose that the above mentioned differences in chemical compositions of these LGEO used in the reports may be associated with various factors such as genetic diversity, habitat, agronomic practices and also geoclimatic conditions [29] which must be kept in mind in such comparisons.

Other frequently isolated components of LGEO such as β-ocimene, linalool, citronellol, neral, and geranial [30, 31] were also identified in the composition of our LGEO.

3.2. *In vitro* antifungal activity of LGEO

The inhibitory effects of increasing LGEO concentrations (from 62.5 to 500 $\mu L.L^{-1}$) on the growth of *P. chrysogenum*, *P. aurantiogriseum, P. expansum* and *P. italicum* have been revealed in the present study. As summarized in Table 2, the growth of these four fungi was influenced by the EO in a concentration-dependent manner. Indeed, the dramatically strongest inhibitory activity ($P < 0.05$) was induced by the highest concentration (500 $\mu L.L^{-1}$) of LGEO which was manifested by the following inhibition zones: 5.90 ± 0.36 mm in *P. chrysogenum*, 6.90 ± 0.36 mm in *P. aurantiogriseum*, 6.17 ± 0.27 mm in *P. expansum*, and 4.27 ± 0.25 mm in *P. italicum*.

Although the antifungal activity of LGEO was reported several times (largely against phytopathogens and dermatophytes), its activities against food spoilage fungi strains were only slightly investigated [32]. Many of these studies showed that *Cymbopogon* EOs is able to reduce or completely inhibit the growth of different type of microorganisms depending on their concentrations [33-35].

In agreement with our study, Baratta et al. [36] reported 91% growth inhibition of A*spergillus niger* due to the antifungal action of lemongrass EO (1000 $\mu L.L^{-1}$). Also, Mishra and Dubey [37] found that LGEO in concentrations of 500 and 1000 $\mu L.L^{-1}$ reduced the growth of *Fusarium verticillioide*s by 90 and 100%, respectively.

Table 1. Chemical composition of Lemongrass EO

Compound[1]	%[2]	Compound[1]	%[2]	Compound[1]	%[2]	Compound[1]	%[2]
citronellal	35.3	δ-cadinene	1.7	(*E*)-caryophyllene	0.5	(*E*)-β-ocimene	0.2
geraniol	23.4	germacrene D-4-ol	1.3	α-muurolene	0.4	n-decanal	0.2
β-citronellol	11.7	isopulegol	1.3	methyl isoeugenol	0.3	germacrene A	0.2
citronellyl acetate	3.9	eudesm-7(11)-en-4-ol	1.0	(*E,E*)-α-farnesene	0.3	α-eudesmol	0.2
α-limonene	3.8	linalool	0.9	α-cadinol	0.5	germacrene D	1.9
geranyl acetate	3.4	α-amorphene	0.9	neral	0.5	β-elemene	1.7
		geranial	0.8	elemol	2.9	TOTAL	99.2

[1]Identified compounds.

[2]Percentage of compounds in amounts more than 0.1 %.

Table 2. *In vitro* antifungal activity of Lemongrass EO

	Lemongrass EO (µL.L^{-1})			
Fungi strains	62.5	125	250	500
P. chrysogenum	2.20 ± 0.26 ^{a}	3.17 ± 0.29 ^{b}	4.10 ± 0.36 ^{c}	5.90 ± 0.36 * ^{d}
P. aurantiogriseum	1.80 ± 0.26 ^{a}	2.73 ± 0.25 ^{b}	4.50 ± 0.50 ^{c}	6.90 ± 0.36 * ^{d}
P. expansum	1.17 ± 0.29 ^{a}	2.33 ± 0.58 ^{b}	4.00 ± 0.50 ^{c}	6.17 ± 0.27 * ^{d}
P. italicum	2.03 ± 0.15 ^{a}	2.10 ± 0.10 ^{a}	2.17 ± 0.29 ^{a}	4.27 ± 0.25 ^{b}

Means ± standard deviation.
Values followed by different superscripts within the same row are significantly different ($p< 0.05$). * Weak antifungal activity (zone 5–10 mm). ** Moderate inhibitory activity (zone > 10 mm). *** Very strong inhibitory activity (zone > 15 mm).

The antifungal activity against the growth of *Candida* (*C.*) *albicans* and *C. krusei* was attributed to citronellal contained in LGEO in the study by Toledo et al. [38], i.e., to the major compounds of our EO. Moreover, the second most abundant constituent detected in our LGEO, geraniol, is also involved in LGEO antifungal efficacy [32]

4. Conclusions

The current study has revealed the volatile fraction and *in vitro* antifungal properties of commercial LGEO. Regarding the chemical composition, the major compounds of LGEO were found to be citronellal (35.3%), geraniol (23.4%), β-citronellol (11.7%), citronellyl acetate (3.9%) and α-limonene (3.8%). All the tested *Penicillium* spp. strains (*P. expansum*, *P. italicum, P. aurantiogriseum*, and *P. chrysogenum*) were the most sensitive to LGEO in the highest concentration (500 µL.L^{-1}). Hence, our data confirm the possibility of the application of *Cymbopogon nardus* EO as an alternative to synthetic agents applied for food preservation to extend their shelf life.

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