Antifungal activity of the essential oil from Artemisia santonicum and its constituent isogeranic acid

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This study represents a continuation of exploring of the biological activities of the Artemisia santonicum essential oil. The previous investigation was focused on the antibacterial, antifungal, and antifungal activities of A. santonicum essential oil and isogeranic acid as the main antibacterial constituent. The present study describes its antifungal activity. The antifungal activity of the A. santonicum essential oil was tested against eight fungi isolates, whereas antifungal effects of isogeranic acid were studied using four fungi species, because of the limited quantities of the isolated compound. The results were compared to the commercial antifungicides, bifonazole and ketoconazole. Antifungal activity of isogeranic acid against all tested fungi was significantly higher in comparison to the essential oil and the both controls.

Key words: antifungal activity, essential oil, isogeranic acid

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1. INTRODUCTION

Artemisia santonicum, also known as “saline wormwood” is the perennial bushy herb able to form its own saline steppe vegetation type (Dajić Stevanović et al., 2016) (Figure 1). The species grows on dry and alkaline places and deserts, with preference of conditions of increased soil salinity. Apart from variations in chemical composition of the volatiles of EO were studied using four fungi species, because of the limited quantities of the isolated compound. The results were compared to the commercial antifungicides, bifonazole and ketoconazole. Antifungal activity of isogeranic acid against all tested fungi was significantly higher in comparison to the essential oil and the both controls.

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compounds were reported as the most dominant: camphor (18.2 %), 1,8-cineole (7.5 %), α-terpineol (4.1 %) and borneol (4.0 %) (i.e. oxygenated monoterpenes (50.9 %) (Kordali et al., 2005a), α-thujone (44.8 %) (Badea and Delian, 2014), and camphor (20.11 %), cis-verbenol (19.85 %) and eucalyptol (18.26 %) (Burzo et al., 2008). Some other species of the Artemisia genus exhibited similar EO chemical profiles, regarding to the presence of its main constituents. For example, in A. distans (Konakchiev et al., 2011) 1,8-cineole (16.8 %), β-thujone (9.8 %), sabinene (8.2 %), borneol (7.5 %), β-pinene (6.5 %) and camphor (5.8 %) were found; high content of 1,8-cineole (21.5–27.6 %) and camphor (15.9–37.3 %) was found in A. cana, A. frigida, A. longifolia and A. ludoviciana (Lopes-Lutz et al., 2008). Additionally, the major compounds in EO of A. gorgonum were camphor (28.7 %) and chrysanthene (10.8 %) (Ortet et al., 2010), whereas in A. fragans, chrysanthene (23.8 %), and 1,8-cineole (23.7 %) were the most dominant (Shafaghat et al., 2009). There are numerous reports about biological activities of different Artemisia species such as antioxidant, antibacterial, antifungal, antimalarial, and anti-diabetic activity (Dadaoglu et al., 2015). Additionally, EO of A. santonicum, together with selected extracts, was investigated on selected enzymes (cholinesterase, tyrosinase a-amylase, and α-glucosidase) as
well as their antioxidant and pharmacological effects (Ferrante et al., 2019). The aim of the present investigation was to determine the potential antifungal activity of the A. santonicum EO and of its individual component, isogeranic acid (IA) as a continuation of the previous work and antibacterial investigation (Stanković et al., 2019).

2. MATERIALS AND METHODS

2.1. Plant material, isolation and chemical analysis of the essential oil
The data about plant material, isolation of the A. santonicum EO and its chemical analysis were described in the previous study of Stanković et al. (2019).

2.2. Isolation and identification of isogeranic acid
The A. santonicum EO fractionation, isolation of isogeranic acid and structure elucidation were also described in the previous study of Stanković et al. (2019).

2.3. Antifungal activity of the essential oil and isogeranic acid
Antifungal activity of the EO was studied using the following fungal species: Aspergillus niger (ATCC 6275), Aspergillus ochraceus (ATCC 12066), Aspergillus fumigates (ATCC 9197), Aspergillus versicolor (ATCC 11730), Penicillium funiculosum (ATCC 36839), Penicillium ochrochloron (ATCC 9112), Trichoderma viride (IAM 5061) and Penicillium verrucosum var. cyclopium (food isolate). Only four fungal species A. niger, A. fumigatus, P. ochrochloron and P. verrucosum were selected for the antifungal activity of IA. The reason was the limited quantity of isolated IA. The selection of particular fungi was made upon previous information on their high sensitivity to IA. Fungal species were obtained from the Mycological Laboratory, Department of Plant Physiology, Institute for Biological Research “Siniša Stankovic”, Belgrade, Serbia. The micromycetes were maintained on malt agar and the cultures stored at 4 °C and sub-cultured once a month (Booth, 1971). The antifungal assay was carried out by modified microdilution method (Espinel-Ingroff, 2001) using procedure described by Rashed et al. (2014). Ranges of concentrations of the EO and IA were 3-12 and 0.05-0.075 mg/mL, respectively. The fungicides bifonazole and ketoconazole were used as positive controls (1-3500 g/mL). Three independent experiments were performed in duplicate.

3. RESULTS AND DISCUSSION
In the previous investigation, the chemical composition of the EO isolated from the aerial parts of A. santonicum was established by GC-MS/FID and 75 components were identified (Stanković et al., 2019). The major group consisted of the oxygenated monoterpenes (67.5 %) and the most abundant compounds were 1,8-cineole (18.8 %), chrysanthenone (13.3 %), cis-thujone (8.4 %), trans-sabinyl acetate (3.3 %), camphor (3.0 %) and trans-sabinol (2.9 %). In addition to the oxygenated monoterpenes, the monoterpene hydrocarbons accounted for 8.2 %, sesquiterpene hydrocarbons participated with 5.6 %, oxygenated sesquiterpenes with 3.3 %, whereas nonmonoterpenes, homononoterpenes and aromatics with 2.6 %, 1.0 % and 1.9 %, respectively.

The antifungal activity of the EO, IA and known antimycotics bifonazole and ketoconazole used as controls are presented in Table 1 and Figure 2. So far, the antifungal activity of A. santonicum EO was tested against A. niger only, and moderate activity was determined (Kordali et al., 2005b). This study represents the first report on antifungal activity of A. santonicum EO against A. ochraceus, A. fumigates, A. versicolor, P. funiculosum, P. ochrochloron, T. viride and P. verrucosum. EO showed moderate antifungal activity in the range of 3-12 mg/mL for MIC, with the most pronounced effect on A. ochraceus, T. viride, P. funiculosum and P. verrucosum. Compared to bifonazole and ketoconazole, known antifungal standards, EO exhibited lower activity. Pure IA exhibited strong antifungal activity against tested fungi. MIC for A. fumigatus and P. ochrochloron was 0.05 mg/mL and for A. niger and P. verrucosum was 0.075 mg/mL. IA exhibited stronger antifungal activity not only compared to the EO, but also higher than the both antimycotic standards, bifonazole and ketoconazole (in most cases 2-4 times, Figure 2). These results are in agreement with the findings of Kordali et al. (2005a) that the main components, camphor and 1,8-cineole, are not responsible for the antifungal activity of Artemisia oils and that antifungal activity of these oils can be attributed to some minor components, like IA.

In the previous studies on antifungal activity of the A. santonicum EO, Kordali et al. (2005a) reported the chemical composition, antifungal and antibacterial activities of the EO obtained from four Turkish Artemisia species, A. dracunculus, A. absinthium, A. santonicum and A. spicigera. The main components of these EOs were camphor (1.4–34.9 %), 1,8-cineole (1.5–9.5 %), chamazulene (n.d.–17.8 %), nucleifer propionate (n.d.–51.1 %), nucleifer butanoate (n.d.–8.2 %), carophyllene oxide (1.7–4.3 %), etc. The antifungal activities of these EOs were tested against eleven plant fungi, compared to commercial antimycotic benzoyl, and the results showed that all tested oils have potent inhibitory effects against almost all of the tested fungi. Pure camphor and 1,8-cineole, which are the major components of the oils, showed antifungal activity against some of

Fig. 1. Artemisia santonicum L. (Slano Kopovo, July 2016.)
Table 1. Minimum inhibitory (MIC) and fungicidal concentration (MFC) of Artemisia santonicum essential oil, isogeranic acid and commercial antibiotics

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Method</th>
<th>Essential oil [mg/mL]</th>
<th>Isogeranic acid [mg/mL]</th>
<th>Bifonazole [mg/mL]</th>
<th>Ketoconazole [mg/mL]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillus fumigatus</td>
<td>MIC</td>
<td>6±0.3</td>
<td>0.05</td>
<td>0.15±0.004</td>
<td>0.20±0.006</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
<td>12±0.2</td>
<td>0.1</td>
<td>0.20±0.005</td>
<td>0.50±0.005</td>
</tr>
<tr>
<td>Aspergillus versicolor</td>
<td>MIC</td>
<td>6±0.3</td>
<td>-</td>
<td>0.10±0.006</td>
<td>0.20±0.003</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
<td>12±0.4</td>
<td>-</td>
<td>0.20±0.005</td>
<td>0.50±0.005</td>
</tr>
<tr>
<td>Aspergillus ochraceus</td>
<td>MIC</td>
<td>3±0.6</td>
<td>-</td>
<td>0.15±0.008</td>
<td>1.50±0.050</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
<td>6±0.5</td>
<td>-</td>
<td>0.20±0.009</td>
<td>2.00±0.060</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>MIC</td>
<td>12±3</td>
<td>0.075</td>
<td>0.15±0.006</td>
<td>0.20±0.004</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
<td>25±4</td>
<td>0.1</td>
<td>0.20±0.007</td>
<td>0.50±0.006</td>
</tr>
<tr>
<td>Trichoderma viride</td>
<td>MIC</td>
<td>3±0.4</td>
<td>-</td>
<td>0.15±0.009</td>
<td>1.00±0.050</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
<td>6±0.6</td>
<td>-</td>
<td>0.20±0.003</td>
<td>1.00±0.060</td>
</tr>
<tr>
<td>Penicillium funiculosum</td>
<td>MIC</td>
<td>3±0.3</td>
<td>-</td>
<td>0.20±0.004</td>
<td>0.20±0.004</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
<td>9±0.4</td>
<td>-</td>
<td>0.25±0.005</td>
<td>0.50±0.003</td>
</tr>
<tr>
<td>Penicillium ochrochloron</td>
<td>MIC</td>
<td>5±0.8</td>
<td>0.05</td>
<td>0.20±0.006</td>
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<tr>
<td></td>
<td>MFC</td>
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<td>0.1</td>
<td>0.25±0.006</td>
<td>3.50±0.300</td>
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<tr>
<td>Penicillium verrucosum</td>
<td>MIC</td>
<td>3±0.2</td>
<td>0.075</td>
<td>0.10±0.006</td>
<td>0.20±0.005</td>
</tr>
<tr>
<td></td>
<td>MFC</td>
<td>9±0.3</td>
<td>0.1</td>
<td>0.20±0.008</td>
<td>0.30±0.003</td>
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</tbody>
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Fig. 2. Minimum inhibitory (A) and fungicidal concentration (B) of A. santonicum essential oil, isogeranic acid, bifonazole and ketoconazole.

the fungal species and had weaker effects than essential oils of investigated Artemisia species (Kordali et al., 2005a) showing that antifungal activity of the oil do not arise only from the main constituents.

In further investigation, antibacterial and antifungal activities of the EOs isolated from A. dracunculus, A. absinthium, A. santonicum, and A. spicigera, the antifungal activity was tested against 34 fungal species revealing the potent antifungal activity, similar to effects of the standard antymycotic compound, benomyl. Among the tested oils, the weakest antifungal activity showed the EO of the A. dracunculus. In most cases, the oils of A. absinthium, A. santonicum, and A. spicigera completely inhibited the growth of some fungal species (Kordali et al., 2005b). In the work of Badea and Delian (2014), EOs of ten Artemisia species including A. santonicum, were studied against fungal pathogen Sclerotinia sclerotiorum. In conclusion of this investigation, Artemisia oils exhibited significant activity and were proposed for use as botanical fungicides and green pesticides (Badea and Delian, 2014).

At the end, after checking all of the literature data, antifungal activity of A. santonicum against seven not investigated fungal strains up to now was here confirmed, fulfilling present antifungal data of the A. santonicum EO. Isogeranic acid was found for the first time in these oils and its strong antifungal action against four fungal strains was reported for the first time. According to these results, an extended investigation of the antifungal activity of this compound is needed.

CONCLUSION

In this work, the antifungal activity of A. santonicum essential oil and isogeranic acid as an active component is presented. For the first time, the antifungal activity of A. santonicum essential oil was investigated against A. ochraceus, A. fumigates, A. versicolor, P. funiculosum, P. ochrochloron, T. viride and P. verrucosum. The essential oil showed moderate antifungal activity, whereas isogeranic acid, which is present in essential oil in small quantity of only 0.2 %, exhibited strong antifungal action against A. fumigatus, P. ochrochloron, A. niger and P. verrucosum. Moreover, isogeranic acid showed 2-4 times stronger effects than both antymycotic standards bifonazole and ketoconazole. These results suggest that minor components, like isogeranic
acid, might be responsible for the antifungal activity, and further research on its biological activity and mechanisms of antifungal action could be recommended.

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REFERENCES


