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, antibiofilm, and antiquorum activities of *A. santonicum* essential oil and isogeranic acid as the main antibacterial constituent. The present study describes their 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 antimycotics, 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 habitats of the central and southeast Europe, the species occurs in the Caspian region, in the mid Asia towards Mongolia. In Serbia, *A. santonicum* was recorded only in the northern part of the country, in the Vojvodina province, within mosaic-form patches exposed to increased soil pH and salinity (Gajić, 1975).

The chemical composition of essential oils (EOs) of the genus *Artemisia* was investigated in several species of different distribution and varied among the species. In some cases, the variations in chemical composition of the volatiles of EO were a consequence of different collecting time, i.e. were ontogenesis–dependent. In addition to collecting period, the quality of EO was affected by genotypic variations, plant part used, and the habitat conditions, mainly soil pH. The applied agricultural and postharvest practices, such as fertilization and drying conditions, as well as the applied extraction method, have all affected the EO composition (Abad et al., 2012). In the previous investigations on the *A. santonicum* EO, different 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 chrysanthenone (10.8 %) (Ortet et al., 2010), whereas in *A. fragans*, chrysanthenone (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 antidiabetic activity (Dadasoglu et al., 2015). Additionally, EO of *A. santonicum*, together with selected extracts, was investigated on selected enzymes (cholinesterase, tyrosinase α-amylase, and α-glucosidase) as well as their antioxidant and pharmacological effects (Ferrante

Fig. 1. *Artemisia santonicum* (Slano Kopovo, july 2016.)

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 Stankovic 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 normonoterpenes, homomonoterpenes 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. verrucous*. 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 %), nuciferol propionate (n.d.-5.1 %), nuciferol butanoate (n.d.-8.2 %), caryophyllene oxide (1.7–4.3 %), etc. The antifungal activities of these EOs were tested against eleven plant fungi, compared to commercial antimycotic benomyl, 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

Table 1. Minimum inhibitory (MIC) and fungicidal concentration (MFC) of *Artemisia santonicum* essential oil, isogeranic acid and commercial antibiotics

Fungi	Method	Essential oil [mg/mL]	Isogeranic acid [mg/mL]	Bifonazole [mg/mL]	Ketoconazole [mg/mL]
Aspergillus fumigatus	MIC	6±0.3	0.05	0.15±0.004	0.20±0.006
	MFC	12±0.2	0.1	0.20±0.005	0.50±0.005
Aspergillus versicolor	MIC	6±0.3	-	0.10±0.006	0.20±0.003
	MFC	12±0.4	-	0.20±0.005	0.50±0.005
Aspergillus ochraceus	MIC	3±0.6	-	0.15±0.008	1.50±0.050
	MFC	6±0,5	-	0.20±0.009	2.00±0.060
Aspergillus niger	MIC	12±3	0.075	0.15±0.006	0.20±0.004
	MFC	25±4	0.1	0.20±0.007	0.50±0.006
Trichoderma viride	MIC	3±0.4	-	0.15±0.009	1.00±0.050
	MFC	6±0.6	-	0.20±0.003	1.00±0.060
Penicillium funiculosum	MIC	3±0.3	-	0.20±0.004	0.20±0.004
	MFC	9±0.4	-	0.25±0.005	0.50±0.003
Penicillium ochrochloron	MIC	5±0.8	0.05	0.20±0.006	2.50±0.200
	MFC	6±0.6	0.1	0.25±0.006	3.50±0.300
Penicillium verrucosum	MIC	3±0.2	0.075	0.10±0.006	0.20±0.005
	MFC	9±0.3	0.1	0.20±0.008	0.30±0.003

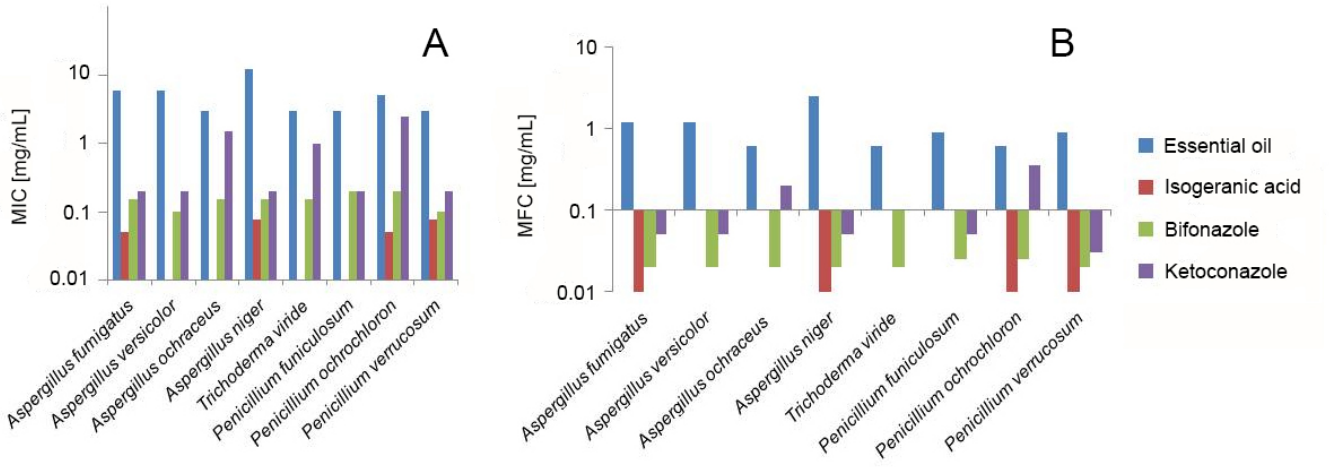

Fig. 2. Minimum inhibitory (A) and fungicidal concentration (B) of *A. santonicum* essential oil, isogeranic acid, bifonazole and ketoconazole.

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 antimycotic 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 activity 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. verrucous*. 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 antimycotic 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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