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Chemical characterisation and antibacterial activity of the essential oil of wild *Angelica* seeds

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ABSTRACT: The aim of the present study was to clarify the chemical composition of essential oil from seeds of *Angelica* (*Angelica sylvestris*) plants growing wild in Serbia. In the essential oil, a total of 27 compounds were detected, among which 22 compounds were identified (comprising 97.9% of all compounds), while five were unidentified (comprising 2.1%). The main components were limonene (66.6%) and α-pinene (19.0%), followed by camphene (1.9%), α-phellandrene (1.6%), bornyl acetate (1.6%), and *trans*-caryophyllene (1.0%), while all other compounds were present in amounts of less than 1.0%. The antibacterial effect of the essential oil of wild *Angelica* seeds was tested against two pathogenic bacteria that cause food poisoning, , viz., *Staphylococcus aureus* and *Escherichia coli.* According to the obtained results, *S. aureus* was more sensitive to *A. sylvestris* seed oil than *E. coli*. The minimal inhibitory concentrations were 28.40 μL/mL and 56.81 μL/mL, respectively, confirming a good antibacterial activity potential of the essential oil against *E. coli* and *S. aureus*, and indicating possibilities for its application in the food and pharmaceutical industries.

Keywords: *Angelica sylvestris* L., seeds, essential oil, chemical composition, antibacterial activity

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INTRODUCTION

Angelica sylvestris L., wild or woodland angelica, is a perennial herb from the family Apiaceae native in northern and eastern parts of continental Europe. It grows on base-enriched soils in a wide variety of habitats, including damp woods, damp neutral grassland, marshes, mires, swamps and tall-herb fens, sea-cliffs, ungrazed montane grassland, and mountain ledges (Preston *et al.* 2002).

This species is herbaceous, non-odorant, with long, thick, tapering branches that are externally brown and internally white. Leaves are pinnated, composed of ovate serrated equal pinnae, with an odd one at the end. During the second year, a stout stem grows from the leaf rosette. It is an erect flower stem, hollow, furrowed, and up to over 2 m tall. Flowers are white or slightly pink, arranged in large compound umbels. The fruit is brownish in colour, with wide lateral wings, six secretory channels visible externally, high dorsal ribs, a subterminal rib, and connate pericarp containing the seed (Woodville 1810; Dihoru *et al.* 2011; Niemirski & Zych 2011; Stpiczyńska *et al.* 2015).

Even though *A. sylvestris* is widespread throughout Europe, it is not an official drug in the European Pharmacopoeia. It is used traditionally for the treatment of gastrointestinal and respiratory tract disorders, as well as against fever, infections, and the flu. The root and seed are mainly used for these purposes, rarely the aerial parts. Moreover, the seeds of *A. sylvestris* have been used to make flavoured wine, while the root can be candied (Sarker *et al.* 2005).

Due to the increasing prevalence of resistance to antibiotics and negative side effects of synthetic reme-

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dies, the usefulness of compounds from plants as natural active compounds against bacteria has become a very popular topic. That is the reason why there is such great interest among scientists to investigate the chemical composition and medical properties of wild growing and traditionally used plants.

Investigations of the *A. sylvestris* root showed the presence of glycosides (Lemmich *et al.* 1983) and essential oil with nonane as the main component (Bernard & Clair 1997). It has been shown that the root extract possesses antimicrobial activity against *Enterococcus faecium*, *Listeria monocytogenes*, *Bacillus subtilis*, *Staphylococcus epidermidis*, and *S. aureus* (Canli *et al.* 2016).

Analysis of the bioactivity of combined n-hexane and dichloromethane extracts of *A. sylvestris* seeds revealed high cytotoxic as well as antibacterial activity. The MICs were between 1.5×10^{-3} and 1.5×10^{-2} mg/mL in action exerted against *Citrobacter freundii*, *Escherichia coli*, and *S. aureus,* and a component called umbelliprenin, a sesquiterpenyl coumarin, was identified as responsible for the antimicrobial activity (Sarker *et al.* 2005). Moreover, the seeds contain 17.3% fatty acids, with oleic and linoleic acids as dominant (Hilditch & Jones 1928), as well as essential oil with limonene and α-pinene (Aćimović *et al.* 2016) as the major constituents.

Aerial parts of *A. sylvestris* contain only 0.05% of essential oil, with limonene (75.3 %) and α-pinene (9.5 %) as the major compounds (Simonović *et al.* 2013). Investigations also show that the methanol extract of aerial parts possesses good antioxidative and neurobiological activity (Orhan *et al.* 2016).

The aim of the present study was to evaluate chemical composition of the essential oil of *A. sylvestris* seeds. Investigation of the antibacterial effect of the oil against two pathogenic bacteria that cause food poisoning, viz., *Staphylococcus aureus* and *Escherichia coli*, was another purpose of this study.

MATERIAL AND METHODS

Plant material. *Angelica sylvestris* seeds were collected from plants growing wild at the locality of Borkovac in Serbia. A voucher specimen (No. 2-1538) was confirmed and deposited in the herbarium of the University of Novi Sad (BUNS). The seeds were ground in a mill (Bosch, MKM 6003) and subjected to hydro-distillation using an all-glass Clevenger-type apparatus to extract essential oils according to the method outlined by European Pharmacopoeia (2004). The content of essential oil in *A. sylvestris* seeds was 0.47%.

GC-MS analysis. The oil chemical composition was assessed by combined gas chromatography and mass spectrometry (GC-MS) using an Agilent 6890 gas chromatograph together with an Agilent 5973 Network mass selective detector (MSD) in the positive ion electron impact (EI) mode. Separation was achieved using an Agilent 19091S-433 HP-5MS fused silica capillary column (30 m × 0.25 mm i.d., 0.25μm film thickness). The GC oven temperature was programmed from 60 to 285°C at a rate of 4.3°C/min. Helium was used as the carrier gas; inlet pressure was 25 kPa; and linear velocity was 1mL/min at 210°C. The injector temperature was 250°C, the injection mode splitless. The MS scan conditions were as follows: source temperature, 200°C; interface temperature, 250°C; energy, 70 eV; and mass scan range, 40-350 amu. Components were identified by applying the retention index (RI) and retention times (RT), and by means of comparison with reference spectra (Wiley 7 and NIST 11 databases).

Antimicrobial activity. Antimicrobial activity of the tested sample was evaluated using laboratory control bacterial strains obtained from the American Type Culture Collection: the Gram-negative *Escherichia coli* (ATCC 8739) and Gram-positive *Staphylococcus aureus* (ATCC 25923). Antimicrobial activity of *A. sylvestris* seed essential oil was tested by a modified broth microdilution method according to the National Committee for Clinical Laboratory Standards (NCCLS 2002). Suspensions of the microbial strains were prepared from overnight broth cultures and were adjusted to 0.5 McFarland standard turbidity (corresponding to 1×10^{8} CFU/mL) using a DEN-1 densitometer (Biosan, Riga, Latvia). Serially doubling dilutions of the tested essential oil were prepared in a 96-well microtitre plate over the range of 454.4-0.22 μL/mL in inoculated Mueller-Hinton broth (MHB, HiMedia). From the last well in the row, 100μL of the mixture was discharged. The test was performed in a total volume of 110 μL/mL with a final microbial concentration 10^{6} CFU/mL per well. The plate was incubated for 24 h at 37°C. The same tests were performed simultaneously for the growth control (MHB + test organism), sterility control (MHB + test oil), and positive control (MHB + gentamicin + test organism). Gentamicin was prepared in sterile water and diluted in MHB to obtain concentrations in a range of from 16 to 0.016 μg/mL. Additionally, susceptibility to gentamicin was confirmed using a quantitative assay for determining the MIC value [the Gentamicin MIC Test Strip (Liofilchem®)] according to the manufacturer's instructions. Microbial growth was determined by adding 10μl of a 0.01% resazurin (7-hydroxy-3*H*-phenoxazin-3-one 10-oxide, HiMedia) aqueous solution. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the samples inhibiting visible growth (judged from appearance of a blue colour of the pellet on the bottom of wells after the addition of resazurin). To determine the minimal bactericidal concentration (MBC), the broth was taken from each well without visible growth and inoculated in Mueller-Hinton agar (MHA) for 24 h at 37°C. The MBC was defined as the lowest concentration of samples killing 99.9% of bacterial cells.

Table 1. Chemical composition of the essential oil of *A. sylvestris* seeds.

No	Compound name	RI_a	RI_b	%
1	Tricyclene	919	921	Tr
2	*α*-pinene	930	932	19.0
3	Camphene	945	946	1.9
4	Sabinene	970	969	0.3
5	*β*-pinene	974	974	0.9
6	Myrcene	988	988	0.8
7	*α*-phellandrene	1003	1002	1.6
8	*p*-cymene	1022	1020	0.4
9	Limonene	1027	1024	66.6
10	NI	1073	/	0.3
11	NI	1088	/	0.2
12	NI	1166	/	1.2
13	NI	1172	/	0.3
14	Carvone	1240	1239	0.7
15	bornyl acetate	1283	1287	1.6
16	Daucene	1377	1380	0.2
17	*trans*-caryophyllene	1419	1417	1.0
18	*trans*-*α*-bergamotene	1434	1432	0.1
19	*α*-humulene	1452	1452	0.1
20	*trans*-*β*-farnesene	1456	1454	0.4
21	*γ*-muurolene	1480	1478	0.1
22	Isodaucene	1498	1500	0.3
23	*β*-bisabolene	1508	1505	0.1
24	*β-sesquiphellandrene*	1518	1521	0.1
25	*γ*-bisabolene	1542	1538	0.8
26	*epi*-*α*-bisabolol	1685	1686	0.9
27	NI	1909	/	0.1
	Total			100

tr-compound present in traces (less than 0.1%), NI-compound not identified,
RI_a - experimental retention indices: retention indices on HP-5MS (from temperature programming, using the definition of Van den Dool & Kratz (1963), RI_b - retention indices from the literature, viz., Adams (2007).

RESULTS AND DISCUSSION

A total of 27 compounds were detected in the essential oil of *A. sylvestris* seeds, of which 22 were identified (compromising 97.9%), while five were unidentified. The main components in the essential oil were limonene (66.6%) and *α*-pinene (19.0%), followed by camphene, *α*-phellandrene, bornyl acetate, and *trans*-caryophyllene (Table 1).

Different isolation techniques were used to obtain essential oil from the seed of *A. sylvestris* var. *sylvestris* originating from Turkey. It was concluded that composition of the oil depends on the isolation method employed. When hydrodistillation was used, 62 constitu-

ents were detected, comprising 93.5% of the total amount of essential oil, while monoterpenes comprised 64.8% of the oil. Monoterpene hydrocarbons were found to be the predominant group (51.6%), with α-pinene (25.6%) and β-phellandrene (9.1%) as the main representatives present in the oil. They were followed by limonene (5.6%), myrcene (4.4%), and camphene (3.9%) (Ozek *et al.* 2008). The oil of *A. sylvestris* seeds in our study also contained monoterpene hydrocarbons as the predominant group, but α-pinene was present in a smaller amount, while β-phellandrene was not detected. This difference could be a result of the effect exerted by origin and chemotype.

In a previous study of ours, volatiles obtained from *A. sylvestris* seeds using the headspace method consisted of 22 compounds, of which limonene (62.7%) and *α*-pinene (28.0%) were dominant, followed by camphene (2.6%) and *b*-pinene (1.0%) (Aćimović *et al.* 2016). As can be seen, the main compounds from the same plant material are limonene and α-pinene regardless of the method used (headspace or hydrodistillation), but in different percentages. This indicates that the extraction technique influences the relative share of compounds in the total.

Resazurin is an oxidation-reduction indicator used for estimation of cell viability. It is a blue non-fluorescent and non-toxic dye that becomes pink and fluorescent when reduced to resorufin by the oxidoreductase enzymes within viable cells. Resorufin can be further reduced to hydroresorufin, which is colourless and non-fluorescent. In a previous study, antifungal activity was assessed on the basis of the MIC, which was defined as the lowest concentration at which a substance prevents the occurrence of a change in colour (Ivanova *et al.* 2013).

A microtitre plate-based antibacterial assay was performed for *E. coli* and *S. aureus* according to a slightly modified version of the CLSI protocol using resazurin to determine the MIC value. According to the assay, *S. aureus* was more sensitive to the essential oil of *A. sylvestris* seeds than was *E. coli*. The minimal inhibitory concentrations were 28.40 μL/mL and 56.81 μL/mL, respectively.

The assayed bacteria showed susceptibility to gentamicin with MIC/MBC values of 0.25/0.75 μg/mL for *S. aureus* and 0.50/1.0 μg/mL for *E coli*. Determination of MIC using the Gentamicin MIC Test Strip (Liofilchem®) also provided a MIC value of 0.25 μg/mL for *S. aureus* and 0.5 μg/mL for *E. coli*.

The results are in accordance with previous studies which showed that Gram-positive bacteria are more susceptible to the influence of external agents in comparison with Gram-negative bacteria (Hussain *et al.* 2011). This is explained by the fact that Gram-negative bacteria possess another membrane around part of the peptidoglycan cell wall, which reduces the diffusion of hydrophobic components through its lipopolysaccharide layer (Zhang *et al.* 2016).

CONCLUSIONS

Angelica sylvestris is widespread throughout Europe. Although not an official drug, it has been used in traditional medicine in many European countries. The obtained results confirm that the essential oil of *A. sylvestris* seeds possesses good antibacterial activity against *E. coli* and *S. aureus*, indicating the expediency of conducting further research on the plant for possible utilisation in the food and pharmaceutical industries.

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REZIME

Hemijska karakterizacija i antibakterijska aktivnost etarskog ulja semena divlje anđelike

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Cilj ovog istraživanja je bio da se utvrdi hemijski sastav etarskog ulja divlje anđelike (*Angelica sylvestris*) dobijenog hidrodestilacijom semena samoniklih biljaka iz Srbije. U etarskom ulju utvrđeno je 27 komponenti (koje čine 97.9%), među kojima je pet nedeterminisanih komponenti (koje čine 2.1%). Dominantne komponente su limonen (66.6%) i α-pinen (19.0%), a potom slede kamfen (1.9%), α-felandren (1.6%), bornil acetat (1.6%) i *trans*-kariofilen (1.0%), dok su ostale komponente bile prisutne sa manje od 1.0%. Takođe, cilj ispitivanja je bio i antibakterijski efekat ovog ulja na dve patogene bakterije koje su uzročnici trovanja hranom: *Staphylococcus aureus* i *Escherichia coli*. Na osnovu rezultata, utvrđeno je da je *S. aureus* značajno osetljiviji na uticaj etarskog ulja semena *A. sylvestris* u poređenju sa *E. coli*. Minimalna inhibitorna koncentracija je bila 28.40 μL/mL i 56.81 μL/mL, što potvrđuje dobru antibakterijsku aktivnost na obe bakterije, kao i na mogućnost njegove primene u prehrambenoj i farmaceutskoj industriji.

Ključne reči: *Angelica sylvestris* L. seme, etarsko ulje, hemijski sastav, antibakterijska aktivnost