NPC Natural Product Communications

2017
Vol. 12
No. 2
205 - 206

Chemical Composition and Antibacterial Activity of *Angelica archangelica* Root Essential Oil

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Received: May20th, 2016; Accepted: August 7th, 2016

Roots of wild growing *Angelica archangelica* L. from Mt. Ozren (Serbia) were subjected to hydrodistillation and GC-MS analysis. The roots contained 0.10% of essential oil with α-pinene (29.7%), δ-3-carene (14.2%), and a mixture of β-phellandrene and limonene (13.2%) as main compounds. The modified resazurin microtiter-plate assay was used to evaluate the antibacterial activity of the essential oil against *Staphylococcus aureus* and *Escherichia coli*. The minimum inhibitory concentration (MIC) values were 14.2 µL/mL for *S. aureus* and 28.4 µL/mL for *E. coli*, while the minimum bactericidal concentrations (MBC) were 56.8 µL/mL and 113.6 µL/mL, respectively. According to the obtained results, the angelica root essential oil can be applied as a natural preservative in food and as a natural antibiotic for the treatment of several infectious diseases caused by these two bacteria.

Keywords: Angelica, *Staphylococcus aureus*, *Escherichia coli*, MIC, MBC.

Angelica archangelica L. (syn. *A. officinalis* Hoffm.),Apiaceae, is distributed throughout Northern Europe and Eastern Siberia, and is cultivated in Europe. The wild grown type is rare in the Serbian flora and there are attempts at its cultivation in the mountainous regions of central Serbia [1, 2]. Angelica has been used in folk medicine and as a food ingredient. The rhizome with roots is used for treatment of gastrointestinal problems [3]. However, it is established that angelica also possesses anxiolytic, hepatoprotective, antimicrobial and antioxidant effects [4].

Essential oils possess different biological properties due to their chemical diversity. The aim of our investigation was to determine the chemical composition and antibacterial activity of root essential oil of a population of *A. archangelica* from Serbia. A total of 59 compounds were detected in the essential oil (AREO) (99.3% of the total oil), including 15 that were unidentified (3.6%). The main components were α-pinene (29.7%), δ-3-carene (14.2%), and a mixture of β-phellandrene and limonene (13.2%). Other important compounds were sabinene, α-phellandrene, myrcene, *p*-cymene and *trans*-β-ocimene. All other compounds constituted less than 2% (Table 1).

Essential oil composition varies depending on many factors, including origin and variety. The AREO from France contained α-pinene (32.2%) and δ-3-carene (16.2%) as the main compounds [5], whereas in Italy they wereα-pinene (21.3%) and δ-3-carene (16.5%), followed by limonene (16.4%) and α-phellandrene (8.7%) [6]. The dominant compounds in the AREO from Siberia were β-phellandrene (30.5%) and α-pinene (23.6%) [7]. According to [8], there are two chemotypes of AREO, differing mainly in either the absence or presence of β-phellandrene.

The antibacterial activity was assessed by the MIC using the resazurin assay [9]. *S. aureus* was more sensitive to AREO than *E. coli*. The oil at a concentration of 14.20 µL/mL inhibited growth of *S. aureus*, while the MIC for *E. coli* was higher, 28.4 µL/mL (Table 2).

Table 1: Angelica root essential oil composition.

No	Compound*	Rt	RI	%
1	α-Thujene	5.651	927	0.5
2	α -Pinene	5.844	934	29.7
3	Camphene	6.227	948	1.1
4	Thuja-2,4(10)-diene	6.373	954	0.1
5	Sabinene	6.898	973	6.1
6	β-Pinene	7.005	977	1.8
7	Myrcene	7.385	990	4.1
8	δ-2-Carene	7.723	1002	tr
9	α-Phellandrene	7.840	1005	5.7
10	δ-3-Carene	8.038	1011	14.2
11	α-Terpinene	8.248	1017	0.5
12	*p*-Cymene	8.502	1024	3.8
13,14	β-Phellandrene + Limonene	8.670	1028	13.2
15	*cis*-β-Ocimene	8.945	1036	1.4
16	*Trans*-β-Ocimene	9.318	1046	3.6
17	γ-Terpinene	9.729	1058	1.0
18	Terpinolene	10.858	1086	1.2
24	*p*-Mentha-1,5-dien-8-ol	14.081	1165	0.3
25	Terpinen-4-ol	14.518	1175	1.1
27	Cryptone	14.922	1184	0.3
30	Bornyl acetate	19.299	1283	0.4
32	Cyclosativene	22.935	1364	tr
33	Cubenene	23.118	1374	tr
34	α-Copaene	23.312	1378	0.7
35	β-Elemene	24.038	1391	tr
36	*trans*–Caryophyllene	25.198	1418	0.1
37	β-Copaene	25.631	1429	0.1
38	β-Barbatene	26.165	1441	0.1
39	α-Humulene	26.653	1453	0.6
40	*Trans*-Muurola-4(14),5-diene	27.845	1481	0.2
41	α-Muurolene	28.662	1501	0.3
42	Cuparene	28.868	1506	0.1
43	β-Bisabolene	29.033	1510	0.3
45	δ-Cadinene	29.625	1524	0.2
46	α-Copaene-11-ol	30.317	1540	1.3
48	β-Gemacrene	30.979	1558	0.1
49	β-Copaene-4-alpha-ol	31.198	1562	0.4
50	Spathulenol	31.817	1577	0.1
54	Humulene epoxide II	33.072	1608	0.3
55,56	Oxacyclotetradecane-2-one + NI	33.777	1626	0.5
57	β-Eudesmol	34.679	1650	0.1
58	Cyclopentadecanolide	41.271	1830	0.2
59	Osthole	51.751	2150	0.1

*Compounds listed in order of elution on a HP-5MS column (Rt - retention time, RI - retention index), tr- compound present less than 0.1%, NI – Unidentified compound.

Table 2: The minimum inhibitory concentration (MIC) of angelica root essential oil*.

Essential oil concentration (µL/mL)	454.40	227.25	113.62	56.81	28.40	14.20	7.10	3.55	1.77	0.88	0.44	0.22
Escherichia coli												
Staphylococcus aureus												

*Plates after 24 h in modified resazurin assay (grey color indicates growth and white means inhibition of growth)

According to published data, the principal constituents in our AREO, α-pinene, and limonene showed considerable activities against *E. coli* and *S. aureus* [10, 11] while δ-3-carene was inactive [11]. The AREO from central Italy, similar in composition to our sample, possesses activity against *Clostridium difficile*, *C. perfringens*, *Enterococcus faecalis*, *Eubacterium limosum*, *Peptostreptococcus anaerobius* and *Candida albicans*, with MIC values of 0.25, 0.25, 0.13, 0.25, 2.25, and 0.50 v/v, respectively [6]. The MBC values in our study were slightly higher than the MIC ones. The lowest concentration of AREO which reduced the viability of the initial bacterial inoculums for *S. aureus* was 56.8 µL/mL, while for *E. coli* the MBC was 113.6 µL/mL. As a positive control, gentamicin was used with MIC/MBC of 0.25/0.75 µg/mL for *S. aureus* and 0.50/1 µg/mL for *E coli*. Determination of MIC by 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.*

Experimental

Plant material: Roots of *A. archangelica* were collected from wild plants near Aleksinac at Mt. Ozren (Serbia). A voucher specimen (No 2-1575) were confirmed and deposited at the BUNS Herbarium, University of Novi Sad. Roots were dried and ground. The powdered material was subjected to distillation in a Clevenger apparatus; the yield of essential oil was 0.10%.

GC-FID and GC-MS analyses were carried out with an Agilent 7890A apparatus equipped with a 5975C mass-selective detector, a flame ionization detector, and a HP-5MS fused-silica capillary column (30 m x 0.25 mm i.d., 0.25µm film thickness). Temperature program: 60°C to 285°C at a rate of 4.3°C/min. Carrier gas He; inlet pressure 25 kPa; linear velocity 1 mL/min at 210°C. Injector temperature: 250°C; splitless. MS conditions: source temperature, 200°C; interface temperature, 250°C; energy, 70 eV; mass scan range, 40-350 amu. Compound identification was made based on retention index, retention times, and by comparison with reference spectra (Wiley and NIST databases). The percentage of each compound was calculated from peak area obtained by FID.

The antimicrobial activity was evaluated using control strains obtained from the American Type Culture Collection: *Escherichia coli* (ATCC 8739) and *Staphylococcus aureus* (ATCC 25923). The activity was tested by a modified broth microdilution method according to the National Committee for Clinical Laboratory Standards [12]. A serial doubling dilution of the tested essential oil was prepared in a 96/well microtiter plate over the range of 454.4-0.22 µL/mL in inoculated Mueller-Hinton broth (MHB, HiMedia). The mixture was discharged from the last well in row 100 µL. The test was performed in a total volume of 110 µL/mL with 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 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 16 to 0.016 µg/mL. Additionally, susceptibility to gentamicin was confirmed using a quantitative assay for determining the MIC (gentamicin MIC Test Strip (Liofilchem®) according to the manufacturer's instructions. Microbial growth was determined by adding 10 µL of 0.01% resazurin (7-Hydroxy-3*H*-phenoxazin-3-one 10-oxide, HiMedia) aqueous solution. The MIC was defined as the lowest concentration of the samples inhibiting visible growth (blue colored pellet on the bottom of the wells after the addition of resazurin). To determine the 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 samples concentration killing 99.9% of bacterial cells.

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