ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIV, N 1, 3 SECTIO DDD 2011

[1]Department of Pharmacognosy with Medicinal Plant Laboratory,
Medical University of Lublin, Poland
[2]Department of Pharmaceutical Microbiology, Medical University of Lublin, Poland
[3]Main Pharmaceutical Inspectorate, Warsaw, Poland

KRYSTYNA SKALICKA-WOŹNIAK[1], RENATA ŁOŚ[2],
ZOFIA ULZ[3], KAZIMIERZ GŁOWNIAK[1], ANNA MALM[2]

Essential oil from fruits of Angelica officinalis and its antibacterial activity

Olejek eteryczny z owoców *Angelica officinalis* i jego aktywność przeciwbakteryjna

INTRODUCTION

The genus *Angelica* L. belongs to the family *Apiaceae* (*alt. Umbelliferae*), commonly known as parsley family, and comprises more than 60 species of medicinally important biennial or perennial herbs [15]. *Angelica officinalis* is a biennial plant, native of Northern Europe westwards to the Nederlands and Iceland and southwards to Central Ukraine. It is also often cultivated elsewhere for its aromatic petioles. The leaves are large, bipinnate, with acute, lance-ovate, serrate leaflets. The greenish-white flowers are arranged in umbels which in turn are made up of semiglobular umbellets. Fruits have rather thick, corky wings [20].

Reports on the traditional uses of *Angelica* species can be found in various ancient literatures, and the folklore of all North European countries. It is known that the genus *Angelica* still plays an important role in the Chinese traditional medicine systems. Many species of this genus have traditionally been used as a potent drug for numbers of diseases [15].

The European species *A. officinalis* is believed to have 'angelic' healing power. This plant used traditionally as a cure for nervous headaches, fever, skin rashes, rheumatism, digestive problems as stimulating gastric and pancreatic secretions. It acts as expectorans, diaphoretic, carminative and antibacterial agent. The stems and seeds of *A. officinalis* are used as flavouring in confectionery and liqueurs [15].

Numbers of coumarins were investigated in fruits of *A. officinalis* such as bergapten, izopimpinelin, imperatorin, phellopterin, xanthotoxin, archangelicin, osthol, oxypeucedanin and its derivatives [5,11,14,21]. They were tested for anti-inflammatory activity in terms of in vitro cyclooxygenase-1 (COX-1) and 5-lipoxygenase (5-LO) inhibition. They were found to be not active

in the COX-1 assay. Concerning the 5-LO inhibition osthol and oxypeucedanin hydrate isovalerate turned out to be active, whereas the other observed effects were only weak [14]. Imperatorin isolated from fruits of *A. officinalis*, in a dose-dependent manner, increased the threshold for electroconvulsions in mice and at subthreshold doses enhanced the anticonvulsant effects of known antileptic drugs [9,10]. Ethanolic extract from fruits of *A. officinalis*, containing mostly coumarins, was investigated for its apoptotic activities. The extract showed out strong apoptotic effect in two lines: J45 and C8166 (92 and 88% of apoptotic cells, respectively) [3]. Crude alcohol extracts have displaced nicotine binding to nicotine receptors in a concentration-dependent manner and inhibited AChE activity *in vitro* [7].

Second important group of active compounds are essential oils. The essential oils from the fruits of *A. officinalis* growing in Iceland prepared by steam distillation were examined in PANC-1 human pancreas cancer cells and Crl mouse breast cancer cells in concentrations ranging from 10–400 μg/ml. The ED_{50} of the oils ranged from 48.6 μg/ml to 108.3 μg/ml for PANC-1 and 48.0 μg/ml to 91.8 μg/ml for Crl cells. Results showed that cytotoxic activity of the essential oils was independent of the quantity of their main components [17].

Many essential oils are known to exert antimicrobial activity. The inhibitory effect of essential oils obtained from *A. officinalis* against a broad spectrum of microorganisms including bacteria, yeast, molds, and two bacteriophage has been studied and this oil was found to be moderately active against a number of bacteria but had no effect on fungi [15].

The aim of our study was to identify the composition of essential oil obtained from hydrodistillation of *A. officinalis* fruits collected in Botanical Garden in Poland. As a part of our research programme focused on searching for potential antibacterial agents naturally occurring, we also determined *in vitro* activity of the essential oil against the panel of reference bacterial strains.

MATERIAL AND METHODS

Plant material. Fruits of *A. officinalis* were collected in The Medicinal Plant Garden, Department of Pharmacognosy with Medicinal Plant Unit, Medical University in Lublin, Poland. The taxonomic identification was confirmed by botany specialists. The voucher specimens are deposited in the Herbarium of Pharmacognosy Department, Medical University, Lublin, Poland. Fruits were air dried at room temperature and powdered.

Hydrodistillation (HD). The homogenized fruits (100 g) of the plants were subjected to hydrodistillation for 3 h using a Deryng apparatus as described in Polish Pharmacopoea VI [13]. The oil was collected into the small vial, dried over anhydrous sodium sulphate and storage in 4°C until the moment of analysis.

GC – MS analysis. GC-MS GCQ (Thermo-Finnigan, USA) equipped with a RT-5 (Resteck) capillary column (20 m length, 0.18 mm ID) with 0.2 μm film thickness was used. The temperature programme for analysis of essential oil was: 50°C kept for 1 min, then increasing to 320°C with a heating rate 4°/min. A split injection with a ratio 1 : 50 was used and the volume of injected sample was 1 μl. The flaw rate of the carrier gas (He) was 0.5 ml/min. The mass spectrometer was fitted with EI source operated 70 eV and mass spectra were recorded in the range m/z 35-500 a.m.u. in the full-scan acquisition mode.

The identifications of the components were based on the comparison of their mass spectra with those of NIST library data (National Institute of Standards and Technology, Gaithersburg, MD, USA), own library and literature data [1], as well as by comparison of their retention indices, retention time and mass spectra with those of the authentic samples.

Bacterial strains. The following reference strains were used: belonging to Gram-positive bacterial species – *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6633, *Micrococcus luteus* ATCC 10240 and belonging to Gram-negative bacteria – *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 9027, *Proteus mirabilis* ATCC 12453. All strains were acquired from the American Type Culture Collection and stored at –70°C in Nutrient broth (Biocorp, Poland) containing 16% (v/v) glycerol until used. Before the experiments, each bacterial strain was passaged onto fresh Nutrient agar (Biocorp, Poland) at 35°C for 24 h. Inocula (0.5 McFarland standard scale – approximately 150 x 10^6 CFU/ml) were prepared using sterile physiological saline.

Determination of antibacterial activity. Power of antibacterial activity of the essential oil obtained from the fruits of *A. officinalis* was determined by the broth microdilution method according to Skalicka-Woźniak et al. [18]. The stock solution of tested essential oil was prepared in DMSO (10 mg/ml). Then, the two-fold dilutions of this stock solution (ranged from 0.156 to 5 mg/ml) in Mueller-Hinton broth (Biocorp, Poland) were made and dispensed into the sterile 96-well polystyrene plates (200 µl per well). 2 µl of the bacterial inocula were added to all wells. After incubation (35°C for 24 h) the minimal inhibitory concentrations (MICs) were assessed visually as the lowest extract concentration showing complete bacterial growth inhibition. Appropriate DMSO, growth and sterile controls were carried out. Gentamicin was used as a reference compound. Following the MIC assay, minimal bactericidal concentrations (MBCs) were determined by plating 10 µl from each well that showed complete growth inhibition onto Mueller-Hinton agar plates (Biocorp, Poland). After the incubation (35°C for 24 h), the MBC values were defined as the lowest concentration of the essential oil at which there was no bacterial growth. The experiment was carried out in triplicates. Representative data are presented.

RESULTS AND DISCUSSION

The composition of essential oil obtained by hydrodistillation from fruits of *A. officinalis* growing in the botanical garden is presented in Table 1. Volatile compounds were identified by GC–MS. A total of 30 compounds were extracted and identified. As main compound of the essential oil (calculated as percentage peak area of GC analyses) β-phellandrene was examined. The content of α-pinene (5.4%), cryptone (4.4%), p-cymene (3.3%), α-copaene (1.8%), α-humulene (1.5%), α-phellandrene (1.4%), β-myrcen (1.1%) were higher than 1%. Our results are in agreement with the published data. β-phellandrene was dominant in rape fruits of *A. officinalis* [12] and essential oils obtained from fruits collected in different parts of the world in different experiments were examined [2,4,6,8].

It is known that essential oils act as strong antimicrobial agents. Not always the dominant compounds are responsible for the antimicrobial activity, but there is some evidence that minor components have a critical part to play in this activity, possibly by producing a synergistic effect between

other components [4]. In this study, the antibacterial properties of essential oil were examined *in vitro* by the determination of MIC and MBC against eight reference bacterial strains. According to our data (Table 2), the tested extract showed a similar level of an inhibitory effect against all strains. However, Gram-positive bacteria were more sensitive than Gram-negative ones with MIC ranged between 0.62–1.25 mg/ml and 1.25–2.5 mg/ml, respectively. The low MBC/MIC ratios indicate bactericidal activity of the tested oil. The experiment confirms our previous results where Gram-negative bacteria were generally less sensitive to the essential oils obtained from plants belonging to *Apiaceae* family [19].

Table 1. Chemical composition of the volatile compounds of *A. officinalis* fruits obtained by hydrodistillation

Compounds	**RI**	**RT**	**%Area**
α-pinene	932	5.82	5.4
camphene	948	6.25	0.2
sabinene	974	6.97	0.3
β-pinene	976	7.03	0.8
β-myrcene	993	7.51	1.1
α-phellandrene	1005	7.86	1.4
Δ3-carene	1012	8.08	0.3
p-cymene	1026	8.54	3.3
β-phellandrene	**1031**	**8.69**	**73.0**
2-methylbutyl 2-methylbutyrate	1113	11.39	0.1
cryptone	1189	13.98	4.4
cuminyl aldehyde	1249	16	0.4
α-terpine-7-al	1295	17.52	0.2
myrtenylacetate	1337	18.91	0.4
cyclosativene	1368	19.9	0.1
α-ylangene	1373	20.05	0.1
α-copaene	1377	20.18	1.8
β-bourbonene	1386	20.48	0.2
β-elemene	1393	20.72	0.3
β-caryophyllene	1421	21.57	0.1
β-copaene	1431	21.89	0.2
γ-elemene	1436	22.03	0.4
α-humulene	1455	22.63	1.5
germacrene D	1483	23.5	0.3
α-curcumene	1486	23.57	0.5
α-amorphene	1503	24.1	0.2
β-bisabolene	1512	24.35	0.6
δ-cadinene	1527	24.81	0.3
germacrene B	1560	25.77	0.3
1,2-humulenepoxide	1613	27.31	0.2

Compounds listed in order of elution from column. RI – retention index, RT – retention time

Table 2. Antibacterial activity (in mg/ml) of essential oil obtained from *A. officinalis*

Species	MIC	MBC	MBC/MIC
Staphylococcus epidermidis ATCC 12228	0.62	1.25	2
Staphylococcus aureus ATCC 25923	1.25	2.5	2
Bacillus subtilis ATCC 6633	0.62	2.5	4
Micrococcus luteus ATCC 10240	0.62	1.25	2
Escherichia coli ATCC 25922	2.5	2.5	1
Klebsiella pneumoniae ATCC 13883	2.5	5.0	2
Pseudomonas aeruginosa ATCC 9027	1.25	2.5	2
Proteus mirabilis ATCC 12453	2.5	5.0	2

ATCC – American Type Culture Collection; MIC – Minimal Inhibitory Concentration; MBC – Minimal Bactericidal Concentration

MICs of gentamicin ranged from 0.03–0.12 x 10^{-3} mg/ml and 0.25–1.0 x 10^{-3} mg/ml for Gram-positive and Gram-negative bacterial strains, respectively.

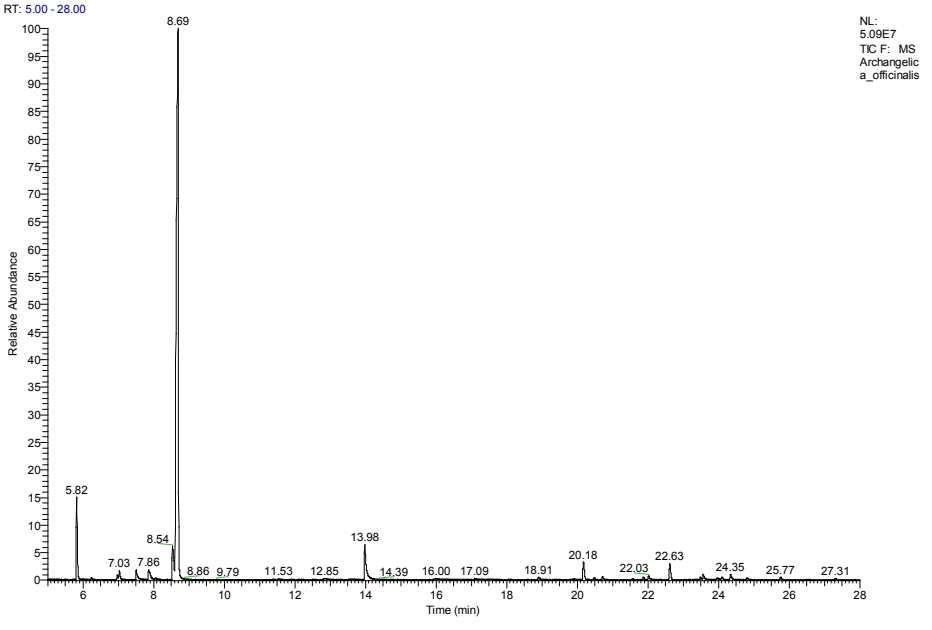

Fig. 1. GC-MS chromatogram of essential oil obtained from fruits of *A. officinalis*.

REFERENCES

1. Adams R.P.: Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy' Allured Publishing Corp., Carol Stream, IL, USA 2001.
2. Bernard C.: Essential oils of three *Angelica* L. species growing in France. Part II: Fruit oils. J. Essent. Oil, 13, 260, 2001.

3. Bogucka-Kocka A., Smolarz H.D., Kocki J.: Apoptotic activities of ethanol extracts from some *Apiaceae* on human leukaemia cell lines. Fitoterapia, 79, 487, 2008.
4. Burt S.: Essential oils: their antibacterial properties and potential applications in foods—a review. Intern. J. Food Microbiol., 94, 223, 2004.
5. Glowniak K., Gawron A., Kwietniewska B.: Investigations on coumarins of *Archangelica officinalis* Hoffm. fruits.: Annales UMCS Sect. D Medicina, 31, 349, 1976.
6. Holm Y., Vuorela P., Hiltunen R.: Enantiomeric composition of monoterpene hydrocarbons in n-hexane extracts of *Angelica archangelica* L. roots and seeds. Flav. Frag. J., 12, 397, 1997.
7. Howes M.R., Perry S.L., Houghton P.J.: Plants with traditional uses and activities, relevant to the management of Alzheimer's disease and other cognitive disorders. Phytother. Res., 17, 1, 2003.
8. Lopes D., Strobl H., Kolodziejczyk P.: 14-Methylpentadecano-15-lactone (muscolide): a new macrocyclic lactone from the oil of *Angelica archangelica* L. Chem. Biodivr., 1, 1880, 2004.
9. Luszczki J.J., Głowniak K., Czuczwar S.J.: Imperatorin enhances the protective activity of conventional antileptic drugs against maximal electroshock-induced seizures in mice. Eur. J. Pharmacol., 574, 133, 2007.
10. Luszczki J.J., Głowniak K., Czuczwar S.J.: Time-course and dose-response relationships of imperatorin in the mouse maximal electroshock seizure threshold model. Neurosci. Res., 59, 18, 2007.
11. Muller M. et al.: 2D NMR spectroscopic analyses of archangelicin from the seeds of *Angelica archangelica*. Acta Pharm., 54, 277, 2004.
12. Nivinskiene O., Butkiene R., Mockute D.: The seed (fruit) essential oils of *Angelica archangelica* L. growing wild in Lithuania. J. Essent. Oil, 19, 477, 2007.
13. Polish Pharmacopoea VI, Polish Pharmaceutical Society, Warsaw 2002.
14. Roos G. et al.: Isolation, identification and screening for COX-1- and 5-LO-inhibition of coumarins from *Angelica archangelica*. Pharm. Pharmacol. Lett., 7, 157, 1997.
15. Sarker S.D., Nahar L.: Natural Medicine: The Genus *Angelica*. Curr. Med. Chem., 11, 1479, 2004.
16. Schelz Z., Molnar J., Hohmann J.: Antimicrobial and antiplasmid activities of essential oils. Fitoterapia, 77, 279, 2006.
17. Sigurdsson S., Ögmundsdottir H.M., Gudbjarnason S.: The cytotoxic effect of two chemotypes of essential oils from the fruits of *Angelica archangelica* L. Anticancer Res., 25, 1877, 2005.
18. Skalicka-Woźniak K. et al.: Antimicrobial activity of fatty acids from fruits of *Peucedanum cervaria* and *P. alsaticum*. Chem. Biodiv., 7, 2748, 2010.
19. Skalicka-Woźniak K. et al.: Comparison of hydrodistillation and headspace solid-phase microextraction techniques for antibacterial volatile compounds from the fruits of *Seseli libanotis*. Nat. Prod. Commun., 5, 1427, 2010.
20. Tutin T.G. et al.: Flora Europaea, *Rosaceae* to *Umbelliferae*. Cambridge Univeristy Press, II, 1968, page 357.
21. Waksmundzka–Hajnos M. et al: Effect of extraction method on the yield of furanocoumarins from fruits of *Archangelica officinalis* Hoffm. Phytochem. Anal., 15, 1, 2004.

SUMMARY

The essential oil from the fruits of *Angelica officinalis*, collected in the botanical garden in Poland, was obtained by hydrodistillation (HD) techniques and analyzed using GC-MS. A total of 30 components were identified. The most abundant compound was β-phellandrene (73%). The minimal inhibitory concentration of essential oil, defined as the lowest concentration able to inhibit visible microbial growth, and the minimal bactericidal concentration, the lowest concentration required to kill of the bacteria, were determined using the broth microdilution method and plating on agar. Essential oil showed moderate antibacterial activity against all reference strains. There was only a small difference between power of activity against Gram-positive and Gram-negative bacteria with MICs ranging from 0.62 to 1.25 mg/ml and 1.25 to 2.5 mg/ml, respectively.

Keywords: Angelica officinalis, essential oil, antibacterial activity, GC-MS

STRESZCZENIE

Olejek eteryczny otrzymany przez destylację z parą wodną owoców *Angelica officinalis*, zebranych w ogrodzie botanicznym w Polsce, poddano analizie metodą GC-MS. Zidentyfikowano 30 składników olejku, spośród których dominującym był β-felandren (73%). Stosując metodę mikrorozcieńczeń w podłożu bulionowym oznaczono najmniejsze stężenie olejku hamujące namnażanie bakterii (MIC), a następnie najmniejsze stężenie bakteriobójcze (MBC). Badany olejek eteryczny wykazał umiarkowane działanie przeciwbakteryjne w kierunku testowanych szczepów. Zauważono niewielką różnicę w sile działania wobec bakterii Gram-dodatnich i Gram-ujemnych – zakres wartości MIC odpowiednio 0.62 do 1.25 mg/ml oraz 1.25 do 2.5 mg/ml.

Słowa kluczowe: Angelica officinalis, olejek eteryczny, aktywność przeciwbakteryjna, GC-MS