ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIV, N 2, 13 SECTIO DDD 2011

Department of Pharmacognosy, Medical University of Lublin, Poland

ELWIRA SIENIAWSKA, KAZIMIERZ GŁOWNIAK

Identification of the coumarin compounds in the Mutellina purpurea extracts

Identyfikacja związków kumarynowych w ekstraktach z marchwicy pospolitej

INTRODUCTION

Mutellina purpurea (Poir.) Thell. is a perennial herb growing on the alpine pastures, among the mountain pine or on the glades. It is the herb typical of Carpathian Mountains and of Polish Tatra Mountains. It has an erected, branched and bare stem growing up to 50 cm, which is wrapped with brown fibers in the base. The flowers are small, white, pink or purplish red, collected in the umbels. The fruits are oval, ribbed, 5-6 mm long [20, 32]. In traditional medicine *M. purpurea* was used for the substitution of calcium and potassium. Since mineral balance is disturbed during the cancer preventing diet excluding proteins. The tea made from *M. mutellina* herb supplements deficiency of calcium and potassium [16]. *M. purpurea* belongs to Apiaceae family and as other plants in this group produces coumarins, phenolic acids, flawonoids and volatile compunds [5, 24] The essential oil obtained from roots of different collections of *L. mutellina* contains ligustylid as the one of the major constituents. Ligustilide suppreses reactive oxygen species production and extracellular signal-related kinases. Thus, ligustilide contributes to be the effective agent in preventing cardiovascular diseases and cancer [24].

Coumarins are typical of Apiaceae. Furanocoumarins were found in the leaves mainly of Angelica, *Peucedanum* and *Seseli* species but a survey of seeds of 130 species showed that these compounds were widespread in the all family [7, 22]. Biological activity of coumarins is manifold. Xanthotoxin and other furanocoumarins play an important defensive role in the plants. They are phytoalexins which possess antifungal and antibacterial properties [1, 14]. Heraclenol, isoimperatorin, imperatorin, phellopterin, byakangelicin, scopoletin and psoralen derivatives are active antimicrobial agents [17]. Girennavar et al. described that dihydrobergamottin and bergamottin inhibit biofilm formation [9]. Coumarins are active anticoagulants [4, 21]. Osthole causes hypotension *in vivo*, inhibits platelets aggregation and smoothes muscle contraction in vitro, probable by preventing thromboxane generation. [11]. Furocoumarins are widely used as photosensitizers in photochemical therapies against different skin disorders such as psoriasis and vitiligo [29]. Among them 8-hydroxybergapten and alloisoimperatorin show the highest free radical scavenging properties [27]. Pellopterin, strongly inhibits the binding of [3H]diazepam to central nervous system benzodiazepine receptors in vitro [2, 8]. Imperatorin

has hepatoprotective activity [12] and induces vasodilatation via voltage dependent calcium channel inhibition and receptor-mediated Ca2+ influx and release [10]. Osthol may also have antiproliferative, vasorelaxant, antihepatitis, anti-inflammatory, antimicrobacterial and antiallergic effects [6].

MATERIALS AND METHODS

Chemical reagents of high purity and standards (xanthotoxin, imperatorin, osthole, visnadine) were purchased from Sigma–Aldrich Chemie GmbH (Munich, Germany) and Merck (Darmstadt, Germany). The plant material was collected in the vegetative season in 2009 in the Botanical Garden of Medical University in Lublin, Poland. Flowering herb of *Mutellina purpurea*, herb afetr flowering, fruits and rots were collected in June, July, Augest, and September respectivelly. Plants were dried at room temperature, powdered and extracted.

Extraction. Accelerated solvent extraction was performed with Dionex ASE 100 instrument (Dionex, Sunnyvale, CA, USA). The plant material (1g) was placed into a 10ml stainless steel extraction cell. Extraction procedure was performed at 60 bar pressure in the temperature of 100ºC for 10 min. After the extraction process, the extraction cell content was flushed using the same extractant in the amount equal to 60% of the extraction cell volume. The optimisation of the extraction procedure was done using petroleum ether, methanol and 80% methanol as the extracting solvents. The highest coumarin content in the petroleum ether extract was detected, therefore the petroleum ether was chosen as the best extractant. Obtained extracts were evaporated to dryness, dissolved in methanol, transferred into 10 ml volumetric flask, filled up to its volume with methanol and subjected to SPE purification.

Samples were cleaned on BakerBond octadecyl SPE microcolumns (500 mg, 3 mL; J.T. Baker, Phillipsburg, NJ, USA) previously activated with 10 mL f methanol then 10 mL of water and 10ml of 80% methanol. Each extract was mixed with water to furnish 80% (v/v) aqueous solutions of the methanol extracts and these (10 mL) were then filtered through the columns under reduced pressure (SPE-12G chamber; J.T. Baker, Groß-Gerau, Germany) [36]. The elutes obtained were free from ballast compounds.

HPLC analysis. HPLC analysis was performed on an Agilent 1100 HPLC system (Agilent, USA) equipped with an automatic degasser, a quaternary pump, a column thermostat, an autosampler and DAD detector. Chromatographic separation was carried out on an Zorbax Eclipse XDB-C18 column (250mm×4.6mm, 5µm) at 25 ºC. The flow rate of mobile phase was maintained at 1ml/min and the injection volume was 10µl. Compounds were separated by use of a stepwise mobile phase gradient prepared from methanol (A) and water (B). The gradient was: 50:50 (A:B, v/v) for 5 min., 40:60 (A:B, v/v) for 20 min., 20:80 (A:B, v/v) for 7 min. [30]. All compounds were detected at λ= 254 nm and λ= 320 nm. The LC pumps, autosampler, column oven, and DAD were monitored and controlled by use of HP Chem Station ver. 10.0 software (Agilent).

The HPLC method was validated. Linearity as a calibration curve was determined. Concentration of standards was ploted against the peak area and calculated in investigated material by means of calibration curve. Every point of calibration curve was estimated in triplicate in the range 0.01-1.0 mg/10ml for xanthotoxin and imperatorin, 0.1-1.5 mg/10ml for osthole and 0.1-10.0 mg/10ml for visnadine. The linearity was kept in the range of used concentrations. The precision was estimated as a recovery after SPE procedure. The known concentrations of the standard solutions and fortified samples were subjested to SPE purification and then to the HPLC analysis. Concentration of coumarins in the investigated material was measured in triplicate.

RESULTS AND DISCUSSION

The most studied family concerning furanocoumarins is undoubtedly Apiaceae family, with more than 19 genus investigated for their production. Psoralen, bergapten, xanthotoxin and isopimpinellin are present in almost all species of this family [3, 19, 25]. To the best of our knowledge, according to a literature survey there is no report on the phytochemical constituents of *M. purpurea* with the exception of root essential oil study. In this work, we report for the first time the occurence of coumarin compounds in the ether extract from the aerial parts and root of *M. purpurea.*

Table 1. The concentration of the identified coumarin compounds in the different plant organs

Compound	Content [mg/g]	Flowering herb	Herb after flowering	Fruits	Root
1. Xanthotoxin y = 5002,4x + 11,392 R^2 = 0,9998 t_R =6,562 min. Λ=254 nm	C SD RSD	ND	0.189 ± 0.001 ± 0.005	ND	0.030 ± 0.002 ± 0.068
2. Imperatorin y = 3153,6x + 42,213 R^2 = 0,9995 t_R =14,842 min. Λ=254 nm	C SD RSD	ND	0.261 ± 0.002 ± 0.008	ND	ND
3. Visnadine derivative y = 371,55x - 261,45 R2 = 0,9929 t_R =16,971 min. Λ=320 nm	C SD RSD	ND	9.782 ± 0.002 ± 2.044	3.546 ± 0.044 ± 0.012	4.092 ± 0.008 ± 0.002
4. Visnadine derivative y = 371,55x - 261,45 R^2 = 0,9929 t_R =17,894 min. Λ=320 nm	C SD RSD	6.044 ± 0.001 ± 1.655	ND	8.053 ± 0.156 ± 0.019	27.770 ± 0.002 ± 7.202
5. Osthol y = 892,21x - 578,24 R^2 = 0,9963 t_R =18,731 min. Λ=320 nm	C SD RSD	3,483 ± 0.002 ± 5.742	3,327 ± 0.002 ± 6.011	1,740 ± 0.001 ± 5.747	2,569 ± 0.007 ± 0.002
6. Visnadine derivative y = 371,55x - 261,45 R^2 = 0,9929 t_R =21,056 min. Λ=320 nm	C SD RSD	4.778 ± 0.002 ± 4.186	3.876 ± 0.002 ± 5.160	21.470 ± 0.056 ± 0.003	6.204 ± 0.002 ± 3.224
7. Visnadine y = 371,55x - 261,45 R^2 = 0,9929 t_R=21,999 min. Λ=320 nm	C SD RSD	6.630 ± 0.003 ± 4.525	4.907 ± 0.001 ± 2.038	5.220 ± 0.017 ± 0.003	7.026 ± 0.001 ± 1.423

C – concentration, SD – standard deviation, RSD – relative standard deviation, ND – not detected, t_R – retention time, R^2 – regression coefficient

The SPE recovery was on a level of 85% (85,56% for the 0.25mg/ml and 85.90% for the 0.75mg/ml concentration respectively).The coumarin content in the different stages of vegetation and in the different organs of investigated plant material is presented in the Tab. 1. The most of all coumarin compounds are present in the herb after flowering. Xanthotoxin, imperatorin, osthol, visnadin and its derivatives were detected. The osthole was present in the all samples. Xanthotoxin was determined in the herb after flowering and in the root, whereas imperatorin only in the herb after flowering. The most abundant in the visnadin derivatives were fruits and root. The visnadin quantity in the all investigated material was comparable and amounted to 6.630, 4.907, 5.220 and 7.026 (mg per g of the dry plant material) in the flowering herb, herb after flowering, fruits and root respectively. The highest coumarin amount detected was compound with visnadine type UV spectrum (27.770 mg/g) present in the root, with retention time t_R=17.894min. The similar result (21.470 mg/g) was obtained for the compound, t_R=21.056 detected in the fruits. The xanthotoxin amount detected in the *M. purpurea* samples is quit low comparing to other Apiaceae species.

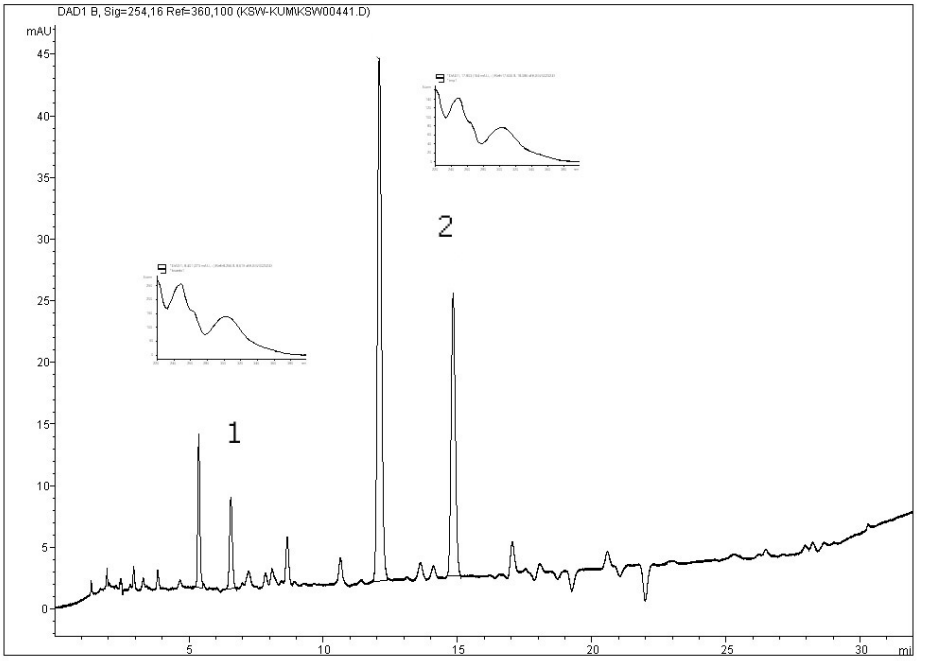

Fig. 1. HPLC analyses of the coumarin fraction of *Mutellina purpurea* herb after flowering. HPLC conditions: Zorbax Eclipse XDB-C18 column (250mm×4.6mm, 5µm) at 25 ºC. Mobile phase: methanol (A) and water (B): 50:50 (A:B, v/v) for 5 min., 40:60 (A:B, v/v) for 20 min., 20:80 (A:B, v/v) for 7 min., flow-rate: 1.0 ml/min, monitored at 254 nm by DAD; 1 = xanthotoxin, 2 = imperatorin

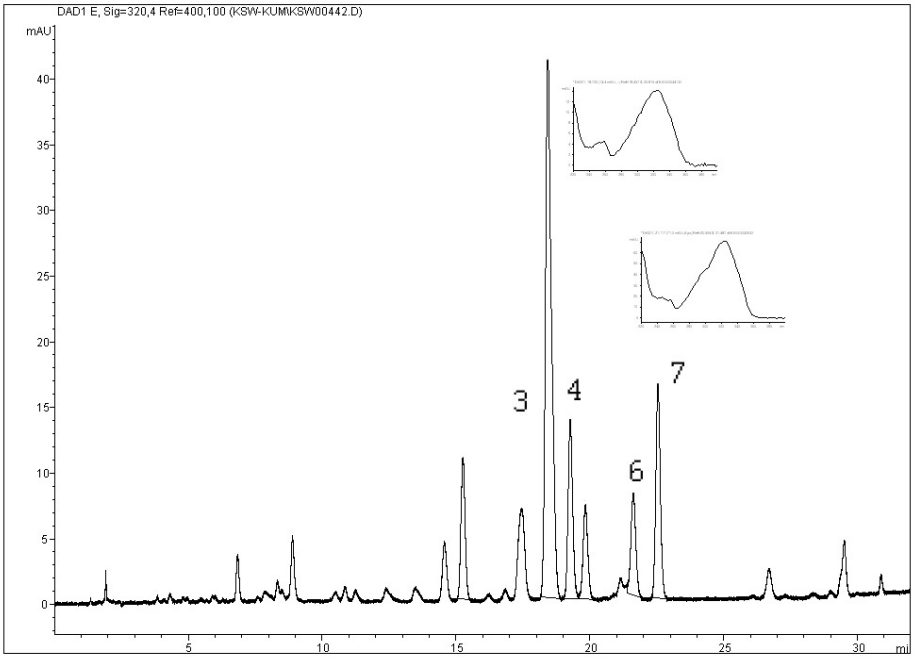

Fig. 2. HPLC analyses of the coumarin fraction of Mutellina purpurea herb after flowering. HPLC conditions: see Fig. 1, monitored at 320 nm by DAD; 3, 4 = visnadine derivatives, 6 = osthol, 7 = visnadine

The highest concentrations of xanthotoxin were detected in *Heracleum mantegazzianum*, with about 4 mg/g DW [28]. This concentration is comparable or slightly higher than the one measured in *Ruta* species [19]. The petroleum ether extract of *Pastinaca sativa* fruits obtained by accelerated solvent extraction yielded 2.501 mg/g of xanthotoxin and 11.258 mg/g of imperatorin [33], whereas homogenisation of the plant material combined with the ultrasound agitated extraction yielded 1.809 mg/g of xanthotoxin from *Pastinaca sativa and* 2.251 mg/g of xanthotoxin from *Archangelica officinalis* in the petroleum ether extract. The sum of imperatorin and bergapten in the *Archangelica officinalis and Pastinaca sativa* fruits was calculated as 25.007 mg/g and 3.311 mg/g respectively [15]. Long-Hu Wang described imperatorin content in the ethanolic extract from the *Angelica dahurica* root as 1.2 mg/g [34]. It is quite low amount resulting from the solvent used to the plant material extraction. Determination of the imperatorin content was done also in the *Angelica dahurica* fruits from the different habitats. The results varied from 6.98 mg/g to 17.22 mg/g depending on the place of harvest [35]. The comparison of the imperatorin content in the the tetraploidy radix *Angelicae Dahuricae* and the original diplontic varietas has been done by Peng F. et al. The chromatographic investigation revealed 4,6mg/g and 2.25mg/g of imperatorin in the tetraploidy and in the diplontic species respectively [26]. Determination of furanochromones and pyranocoumarins gave 4.5mg/g of visnadin in the *Ammi visnaga* fruit extracts [36].

Angelica Dahuricae radix ethyl acetate extract containing furocoumarins inhibits lipopolysaccharide-induced NO, TNF-α and PGE2 production as well as NOS and COX-2 expression in macrophage. It may represent a potential new source of drugs for the treatment against inflammatory diseases [13]. Xanthotoxin possesses antileukodermal activity and antitumor properties. It is also active, inhibiting the growth of HeLa cells [1]. Imperatorin is active against *Bacillus subtilis, Escherichia coli, Cladosporium herbarum and Aspergillus candidus* [17]. Luszczki et al. studied that imperatorin increased in a dose-dependent manner the threshold for electroconvulsions in mice [18]. Imperatorin has also hepatoprotective activity [12] and induces vasodilatation via voltage dependent calcium channel inhibition and receptor-mediated Ca2+ influx and release [10]. In the tests osthol induced a significant increase in hepatic HMG-CoA reductase mRNA expression, which may lead to decrease in hepatic cholesterol. Osthol may accelerate oxidation of hepatic fatty acids. These beneficial effects of osthol could be useful for both prevention of atherosclerosis and suppression of hepatic lipid accumulation [23, 31]. Osthol may also have antiproliferative, vasorelaxant, antihepatitis, anti-inflammatory, antimicrobacterial and antiallergic effects [6]. Because determined in the plant material coumarin compounds posses beneficial activities for the human health, *Mutellina purpurea* is a useful plant and can be a potential source of this compounds. To the best of our knowledge this is the first time the qualitqtive and quantitative analysis of coumarins in *M.purpurea* was done.

Acknowledgments – Thiswork was financially supported by grant No. N N405 091540 from the Polish Ministry of Science and Higher Education.

REFERENCES

1. Abdel Hafez O.M., Amin K.M., Abdel-Latif N.A. et al.: Synthesis and antitumor activity of some new xanthotoxin derivatives. Eur. J. Med. Chem., 44, 2967, 2009.
2. Bergendorff O., Dekermendjian K., Nielsen M. et al.: Furanocoumarins with affinity to brain benzodiazepine receptors in vitro. Phytochem., 44, 1121, 1997.
3. Ceska O., Chaudhary S.K., Warrington P.J. et al.: Photoactive furocoumarins in fruits of some umbellifers. Phytochem., 26, 165, 1987.
4. Chen I.S., Chang C.T., Sheen W.S. et al.: Coumarins and antiplatelet aggregation constituents from formosan Peucedanum japonicum. Phytochem., 41 525, 1996.
5. Chen X., Kong L., Su X.Y. et al.: Separation and identification of compounds in Ligusticum Chuanxiong by comprehensive two-dimensional liquid chromatography coupled to mass spectrometry. J. Chromatogr. A, 1040, 169, 2004.
6. Chiu P.R., Lee W.T., Chu Y.T. et al.: Effect of the chinese herb extract osthol on IL-4-induced eotaxin expression in BEAS-2B. Cells. Pediatr. Neonatol., 49, 135, 2008.
7. Crowden K., Harborne J.B., Heywood V.H.: Chemosystematics of the umbellifereae – a general survey. Phytochem., 8, 1963, 1969.
8. Dekermendjian K., Jinglu Ai., Nielsen M. et al.: Characterisation of the furanocoumarin phellopterin as a rat brain benzodiazepine receptor partial agonist in vitro. Neurosci. Let., 219, 151, 1996.
9. Girennavar B., Cepeda M.L., Soni K.A. et al.: Grapefruit juice and its furocoumarins inhibits autoinducer signaling and biofilm formation in bacteria. Int. J. Food Microbiol., 125, 204, 2008.

10. He J.H., Zhang W., He L.Ch. et al.: Imperatorin induces vasodilatation possibly via inhibiting voltage dependent calcium channel and receptor-mediated Ca2+ influx and release. Eur. J. Pharmacol., 573, 170, 2007.
11. Hoult J.R.S., Paya M.: Pharmacolgical and biochemical actions of simple coumarins: Natural products with therapeutic potential. Gen, Pharmac., 27, 713, 1996.
12. Jagiełło-Wójtowicz E., Chodkowska A., Madej A. et al.: Comparison of CNS activity of imperatorine with fraction of furanocoumarins from Angelica archangelica fruit in mice. Herba Pol., 50, 106, 2004.
13. Kang O.H., Lee G.H., Choi H.J. et al.: Ethyl acetate extract from Angelica dahuricae radix inhibits lipopolysaccharide-induced production of nitric oxide, prostaglandin E2 and tumor necrosis factor-α via mitogen-activated protein kinases and nuclear factor-α B in macrophages. Pharmacol. Res., 55, 263, 2007.
14. Khatunea N.A., Islama M.E., Haquea M.E. et al.: Antibacterial compounds from the seeds of Psoralea corylifolia. Fitoterapia, 75, 228, 2004.
15. Komaszewska E., Mucha K., Baj T. et al.: Comparison of classic and modern methods of furocoumarins extraction from Angelica officinalis Hoffm. and Pastinaca sativa L. fruits. Annales UMCS, sectio DDD, 22, 93, 2009.
16. Krebs B.R.: Leukämie und andere scheinbar unheilbare Krankheiten mit natürlichen Mitteln heilbar. Mebus, 1990.
17. Kwon Y.S., Kobayashi A., Kajiyama S.I. et al.: Antimicrobial constituents of Angelica dahurica roots, Phytochem., 44, 885, 1997.
18. Luszczki J.J., Glowniak 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.
19. Masuda T., Takasugi M., Anetai M.: Psoralen and other linear furanocoumarins as phytoalexins in Glehnia littoralis. Phytochem., 47, 13, 1998.
20. Mirek Z., Piękoś-Mirkowa H.: Kwiaty Tatr. Przewodnik kieszonkowy. Warszawa: MULTICO Oficyna Wyd., 2003.
21. Monti M., Pinotti M., Appendino G. et al.: Characterization of anti-coagulant properties of prenylated coumarin ferulenol. Biochim. Biophys. Acta., 1770, 1437, 2007.
22. Murphy E.M., Nahar L., Byres M. et al.: Coumarins from the seeds of Angelica sylvestris (Apiaceae) and their distribution within the genus Angelica. Biochem. Syst. Ecol. 32 203, 2004.
23. Ogawa H., Sasai N., Kamisako T. et al.: Effects of osthol on blood pressure and lipid metabolism in stroke-prone spontaneously hypertensive rats. J. Ethnopharmacol., 112, 26, 2007.
24. Passreiter C.M., Akhtar Y., Isman M.B.: Insecticidal activity of the essential oil of Ligusticum mutellina roots. Z. Naturforsch C, 60, 411, 2005.
25. Pathak M.A., Farrington D., Fitzpatrick T.B.: The presently known distribution of furocoumarins (psoralens) in plants. J. Investig. Dermatol., 39, 225, 1962.
26. Peng F., Yhang S., Liu T.S. et al.: Determination of imperatorin in tetraploidy radix Angelicae dahuricae. Zhongguo Zhong Yao Za Zhi, 459, 426, 2002.
27. Piao X.L., Park I.H., Baek S.H. et al.: Antioxidative activity of furanocoumarins isolated from Angelicae dahuricae, J. Ethnopharmacol., 93. 243, 2004.

28. Pira E., Romano C., Sulotto F. et al.: Heracleum mantegazzianum growth phases and furocoumarin content. Contact. Dermat., 21, 300, 1989.
29. Serrano-Perez J.J., Gonzalez-Luque R., Merchan M. et al.: The family of furocoumarins: Looking for the best photosensitizer for phototherapy, J. Photochem. Photobiol. A, 199, 34, 2008.
30. Skalicka-Woźniak K., Mroczek T., Garrard I. et al.: Isolation and purification of new minor dihydropyranochromone and furanocoumarin from fruits of Peucedanum alsaticum L. by high-speed counter-current chromatography. J. Chromatogr. A, 1216, 5669, 2009.
31. Sun F., Xie M., Xue J. et al.: Osthol regulates hepatic PPARa-mediated lipogenic gene expression in alcoholic fatty liver murine. Phytomedicine, 17, 669, 2010.
32. Szafer W., Kulczyński S.: Rośliny polskie. Warszawa: PWN, 1953.
33. Waksmundzka-Hajnos M., Petruczynik A., Dragan A. et al.: Influence of the extraction mode on the yield of some furanocoumarins from Pastinaca sativa fruits, J. Chromatogr. B, 800, 181, 2004.
34. Wang L.H., Mei Y.H., Wang F. et al.: A novel and efficient method combining SFE and liquid–liquid extraction for separation of coumarins from Angelica dahurica. Sep. Pur. Technol., 77, 397, 2011.
35. Jiang X., Wang P., Wang Y. et al.: Content determination of imperatorin in Angelica dahurica(Fisch.) Bench. et Hook. seeds from different habitats by HPLC, Chemistry and Industry of Forest Products, 3, 25, 2008.
36. Zgorka G., Dragan T., Głowniak K. et al.: Determination of furanochromones and pyranocoumarins in drugsn and Ammi visnaga fruits by combined solid-phase extraction–highperformance liquid chromatography and thin-layer chromatography– high-performance liquid chromatography, J. Chromatogr. A, 797, 305, 1998.

SUMMARY

Mutellina purpurea belongs to Apiaceae plants. The coumain compounds are typical of this family. In the investigated extracts obtained from the flowering herb, as well as the herb after flowering, fruits and root, the identified compounds were xanthotoxin, imperatorin, osthol, wisnadin and it`s derivatives. The qualitative and quantitative analysis was done by means of HPLC-DAD technique. To the best of our knowledge this is the first report of the coumarins determination in the *Mutellina purpurea* extracts.

Keywords: Mutellina purpurea, Apiaceae, coumarins, furocoumarins

STRESZCZENIE

Marchwica pospolita jest rośliną z rodziny Apiaceae. Cechą charakterystyczną dla tej rodziny jest obecność w materiale roślinnym związków kumarynowych. W badanych ekstraktach sporządzonych z kwitnącego ziela, ziela po kwitnieniu, owoców i korzenia stwierdzono imperatorynę, ksantotoksynę, ostol, wisnadynę i jej pochodne. Analizę wykonano techniką HPLC-DAD. Zgodnie z dostępnymi danymi literaturowymi po raz pierwszy oznaczono jakościowo i ilościowo związki kumarynowe w badanym gatunku.

Słowa kluczowe: kumaryny, furanokumaryny, *Mutellina purpurea,* Apiaceae,