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Phytochemical investigation of *Pimpinella serbica*

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Abstract: The plant species *Pimpinella serbica* (Vis.) Drude, endemic to West Balkans, belongs to the genus *Pimpinella* L. (Apiaceae) according to newer botanical classification. Initially, the plant was described as *Pancicia serbica* Vis. and long considered as a monotypic genus. Previous phytochemical investigations of this plant were limited to the essential oil analysis by the GC–MS method. This is first study that includes LC-DAD–MS screening of the extracts of the roots and aerial parts, followed by isolation of secondary metabolites using chromatographic techniques. Five compounds belonging to phenylpropanoids and polyacetylenes, well known for their bioactivities, were isolated and structurally determined by combined spectroscopic methods (UV, NMR, MS). Dillapiole (**1**), nothoapiole (**2**) and oplopantriol A 18-acetate (**4**) were found in both extracts, while falcarindiol (**3**) was isolated from the roots and dendrotrifidol (**5**) from the aerial parts only. The phytochemical profile of *P. serbica* L. supports its position in the *Pimpinella* L. genus.

Keywords: Apiaceae (Umbelliferae); phenylpropanoids; polyacetylenes.

INTRODUCTION

The Apiaceae (formerly Umbelliferae) family contains around 420 genera with 3100 species, found throughout most of the world, but mainly in northern temperate regions. This large family contains many important food plants, *e.g.*, *Daucus* (carrot), *Pastinaca* (parsnip), and *Apium* (celery), while others are very poisonous, *e.g. Oenanthe* (water dropwort), or are used medicinally.[1] *Pimpinella* L. is one of the largest genera of the Apiaceae family and comprises 170–180

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species.[2] This genus is well known for a number of medicinally and pharmaceutically important species, *Pimpinella anisum* (anise) being the most notable one.[3] *Pimpinella serbica* (Vis.) Drude is a species endemic to the West Balkans, limited to Serbia, Bosnia and Herzegovina, Montenegro, North Macedonia, and Albania.[4] The plant has been described as *Pancicia serbica* Vis. and has long been considered as monotypic genus named after Serbian botanist Josif Pančić.[5] In light of modern nomenclature *P. serbica* is included in the genus *Pimpinella*.[4]

It is a glabrous perennial herb up to 50 cm tall; the rhizome is vertical, thick; stems are straight, striate, rounded, branched at the top. The leaves are alternate, markedly heteromorphic: the lower ones are long petiolate, simple, cordate, serrate, the middle ones are shortly petiolate, deeply pinnately lobed, the upper ones are very short petiolate, palmate, with setaceous lobes. The umbel consists of 10––15 rays; bracts 5–8 linear, scarbid bracteoles 5, the flowers are white, rarely light pink, small, 5-numerous, with obcordate petals. The fruit is glabrous, ovoid, 3–4 mm long, with 5 narrowly winged ridges, slightly laterally compressed.[5,6]

Previous phytochemical investigations of *P. serbica* were limited to GC–MS analysis of the essential oils obtained from the fruits and aerial parts of the plant.[7,8] Here for the first time, an investigation of chemical constituents of *P. serbica* extracts, obtained from the roots and aerial parts of the plant, is reported.

EXPERIMENTAL

General

The NMR spectra were acquired on a Bruker Avance DRX 500 MHz instrument with a 5 mm inverse detection probe, in $CDCl_3$ as the solvent, at 298 K. The spectra were referenced to tetramethylsilane (TMS), chemical shifts are given in ppm (δ / ppm), and coupling constants are reported in Hz (J / Hz).

The GC and GC–MS analyses were performed on an Agilent 7890A GC equipped with a 5975C inert XL EI/CI mass selective detector (MSD) and a flame ionization detector (FID) connected by a capillary flow technology two-way splitter with make-up gas. An HP-5MSI capillary column (30 m×0.25 mm×0.25 μm) was used. The temperature of the GC oven was programmed from 60 to 300 °C at 3 °C min^{-1} and held for 10 min. Helium was used as the carrier gas at 20.343 psi (constant pressure mode). The injection volume was 1 μL with split ratio 10:1. The FID temperature was 300 °C. The MS data was acquired in the EI mode, with a scan range 30–550 m/z; the source temperature was 230 °C and the quadrupole temperature was 150 °C. The solvent delay was 3 min.

The mass spectra were obtained on an Agilent Technologies 6210 time-of-flight LC–MS system with electrospray (ESI) ion source. The separation was performed with an LC apparatus (1200 Series Agilent Technologies) comprising an on-line degasser, binary pump, auto injector, column oven and diode array (DAD) detector, equipped with analytical Zorbax Eclipse Plus C18 column (150 mm×4.6 mm, 1.8 μm ID) maintained at 40 °C at a constant flow-rate of 1.4 mL min^{-1}. The mobile phase was a mixture of solvent A (5 mM ammonium formate in H_2O) and solvent B (acetonitrile) according to a combination of gradient and isocratic modes: 95 % A, 0–1.5 min; 95–5 % A, 1.5–26 min; 5 % A, 26–35 min; 5–95 % A, 35––36 min, 95 % A, 36–41 min. The UV spectra were recorded in the 190–450 nm range, and ESI MS spectra in m/z 100–2000 Da range. ESI-TOF-MS conditions: drying gas (N_2) flow 12

L min^{-1}, nebulizer pressure = 310 kPa, drying gas temperature = 350 °C, capillary voltage = 4000 V, fragmentor voltage = 140 V, skimmer = 60 V, Oct RF voltage = 250 V, positive mode, 10,000 transients/scan. A personal computer system running MassHunter Workstation software was used for data acquisition and processing.

Optical rotation measurements were performed on a Rudolph Research Analytical Autopol IV automatic polarimeter.

UV spectra were recorded on a GBC Cintra 40 UV–Vis spectrometer and analytical LC--DAD system Agilent Technologies 6210.

Silica gel (0.063–0.200 mm) was used for column chromatography (CC). Silica gel G and silica gel F-254 were used for analytical (0.25 mm) and preparative (0.75 mm) thin layer chromatography (TLC).

Plant material

The plant material was collected during the flowering season in June 2011 at Mokra Planina near Čakor (Montenegro). The plant was identified by one of the authors (D. Stešević), and voucher specimen was deposited at the herbarium collection of the Faculty of Sciences (TGU), University of Montenegro (Voucher Code 1737603).

Extraction and isolation

Herbal material was air-dried in shade to yield 8 g of the roots and 80 g of aerial parts. Powdered roots and aerial parts were extracted with dichloromethane. The solvent was removed by vacuum evaporation; the yields the extracts were 100 mg of the roots and 960 mg of the aerial parts.

The extracts were first analyzed by LC-DAD–MS chromatography. Four major compounds were detected in the root extract (**1**–**4**), and four in the aerial parts extract (**1**, **2**, **4** and **5**). These compounds were then isolated using CC and preparative TLC.

The root extract was subjected to CC on silica gel, starting the elution with *n*-hexane/ /acetone 9/1 and increasing the polarity by adding acetone to 50 %. Fractions **4** and **5** were combined and purified by preperative TLC (*n*-hexane/acetone 8/2) to yield 3 mg of dillapiole (**1**) and 6 mg of nothoapiole (**2**). Fraction 12 contained 6 mg of falcarindiol (**3**), and fractions 17–19 gave 3 mg of oplopantriol A 18-acetate (**4**).

The extract of the aerial parts was fractionated by CC on silica with petroleum ether/ /Et_2O/ MeOH with increasing polarity to yield 50 fractions. Fraction 8, purified by preperative TLC (petroleum ether/acetone 8/2), gave 18 mg of dillapiole (**1**). Fractions 42–47 were combined and re-chromatographed by CC using dichloro methane/methanol with increasing polarity to collect 38 subfractions. Subfractions 13–15 contained 3.6 mg of dendrotrifidiol (**5**).

Analytical and spectral data are given in Supplementary material to this paper.

RESULTS

The roots and aerial parts extracts of *P. serbica* were analysed by LC-DAD– –MS chromatography prior to purification and isolation of the components. In this way, four major peaks were identified in the root extract (compounds **1**–**4**, Figs. S-1 and S-2 of the Supplementary material). In the LC-DAD chromatogram of the extract of the aerial parts, three peaks were observed (Fig. S-3). The first two peaks were identical to those detected in the root extract (compounds **1** and **4**). Careful analysis of the MS data revealed that the third peak consisted of two

unresolved components (Fig. S-4). The first was identical to compound **2** in the root extract, and the other one was an additional compound **5**.

Compound **1** exhibited the molecular ion [M+H$^+$] at *m*/*z* 223.0963 (Fig. S-2), compatible with a molecular formula $C_{12}H_{14}O_4$. The ^{1}H-NMR spectrum (Fig. S-5) suggested the phenylpropanoid structure. Specifically, the signals at δ 5.91 *ddt*, 5.05 *ddq*, 5.04 *ddq* and 3.30 ppm *dt* (each 1H) corresponded to the allyl group, and the singlet at δ 5.88 ppm (2H) indicates the presence of a methylenedioxy group. In addition, the signals at δ 4.01 (3H, *s*) and 3.75 ppm (3H, *s*) are attributed to methoxy groups. These data are compatible with the structures of apiole and dillapiole, which were identified as the major components in previous investigations of the essential oils of the plant.[7,8] By comparing the ^{1}H-NMR spectrum of compound **1** to the spectrum of apiole isolated in a study of *Malabaila aurea* essential oils,[9] it is clear that they are different compounds (Fig. S-8). At the same time, the NMR data of compound **1** matched well with published data for dillapiole.[10] GC–MS data of compound **1**, including the EI-MS spectrum and Kovats index (Fig. S-9) are also in accordance with the structure of dillapiole. While the MS spectra of dillapiole and apiole are similar, they can be distinguished by different retention times and Kovats indices on a DB-5 column (Figs. S-9 and S-10). Thus, compound **1** was unequivocally identified as dillapiole (1-allyl-2,3-dimethoxy-4,5-(methylenedioxy)benzene).

The molecular formula of compound **2** can be deduced as $C_{13}H_{16}O_5$, based on the [M+H$^+$] ion at *m*/*z* 253.1082. Its ^{1}H and ^{13}C spectra (Figs. S-11 and S-12) were similar to those of **1**, except that in spectra of compound **3**, there are three signals corresponding to methoxy groups, and the aromatic proton at C-6 is missing. Thus, compound **3** was identified as nothoapiole (1-allyl-2,3,6-trimethoxy--4,5-(methylenedioxy)bezene). Both the NMR and GC–MS data of compound **2** are in good accordance with literature values.[10]

The molecular formula of compound **3** was determined to be $C_{17}H_{24}O_2$ from the ions [M+H$^+$-H_2O] at *m*/*z* 243.1742 and [M+H$^+$-2H_2O] at *m*/*z* 225.1634. The UV spectrum (Fig. S-14) showed an absorption pattern typical for polyacetylenes: one large and intense band with a maximum around 200 nm, and three smaller bands with maxima at 230, 245 and 260 nm.[11] The ^{13}C-NMR spectrum (Fig. S-16) is in accordance with a C-17 polyacetylene structure, showing 4 sp, 4 sp^2 and 7 sp^3 hybridized carbons, with two of them oxygenated. Based on this evidence, compound **3** was identified as falcarindiol. This was confirmed by comparison to literature data.[12] Since its spectral data, including optical rotation, were identical to falcarinol isolated in a previous study of *Seseli annuum* roots,[13] its absolute configuration was assigned as (3*R*,8*S*).[12,14]

The ESI MS spectrum of compound **4** showed multiple ions and they all point to the molecular formula $C_{20}H_{28}O_4$. The UV spectrum (Fig. S-17) was compatible with a polyacetylene structure. By comparing its ^{1}H- and ^{13}C-NMR data (Figs.

S-18 and S-19) to those of falcarindiol, it could be deduced that **4** is trioxygenated C-18 polyacetylene with the acetyl group at the terminal carbon. This corresponds with the structure of oplopantriol A 18-acetate, which is supported by literature data.[15] Due to the oxygenation at the terminal position, the numbering of this compound should start from the opposite side comparing to falcarindiol. This makes comparison of NMR data to falcarindiol difficult. For this reason, and assumed common biosynthetic origin, it was decided to use the same numbering system for these compounds. The absolute configuration of **4** was accordingly assigned as (3*R*,8*S*).

From the ions at *m*/*z* 257.1900, *m*/*z* 292.2271 and *m*/*z* 297.1822, the molecular formula of compound **5** was established as $C_{18}H_{26}O_2$. The ^{1}H- and ^{13}C--NMR spectra (Figs. S-18 and S-19) suggested dioxigenated C-18 polyacetylene. The signals at δ 4.91 (1H, *br d*) 3.64 (2H, *t*) and 3.03 ppm (2H, *br d*) showed that the positions of oxigenation were 3 and 18. This led to the structure of dendrotrifidol, which is confirmed by comparison to literature data.[16] The numbering and absolute configuration were aligned to those of falcarinol.

The structures of isolated compounds are presented in Fig. 1, and their NMR data are summarized in Tables S-I and S-II of the Supplementary material.

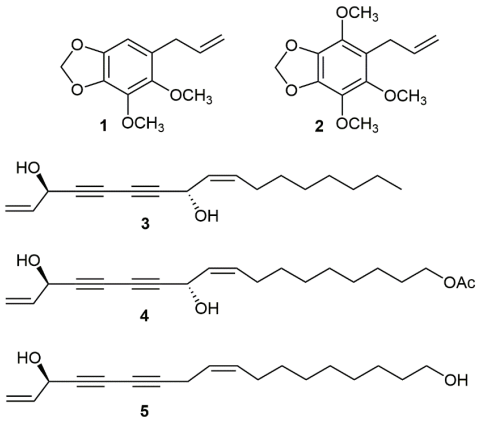

Fig. 1. The structures of the isolated compounds.

DISCUSSION

In a previous study of the essential oil obtained from the fruits of *P. serbica*, dillapiole (35.1 %) and nothoapiole (9.5 %) were among the most abundant components.[7] This is in line with the present findings, even though the plant material was collected at different localities (Kopaonik, Kodža Balkan and Zlatar). On the other hand, in the study of the essential oil from the aerial parts of the plant, apiole was major component (76.8 %); dillapiole was a minor component (0.8 %) and nothoapiole was not reported.[8] This was puzzling given that plant material was collected at one of the localities mentioned in the first study (Kopaonik). Further investigation is required to determine whether this difference can be attributed to

the existence of different chemotypes and adaptation to particular habitats. In general, the phytochemical profile of *P. serbica* is dominated by phenylpropanoids, which aligns well with the other *Pimpinella* species[17] and therefore supports its botanical classification to this genus.

Compounds **1**–**5** are known for their bioactivities. Specifically, dillapiole showed significant anti-inflammatory[18] and gastroprotective activity.[19] Nothoapiole exhibited antibacterial and antifungal activity.[20] The bioactivity of polyacetylenes (falcarinol, falcarindiol and related compounds) is also well documented, including but not limited to antifungal activity, allergenicity and cytotoxicity.[11] This makes *P. serbica* an important natural source of biologically relevant chemicals. Due to its protected status, bringing *P. serbica* into cultivation is a reasonable way to harness its potential as medicinal plant.

SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11073, or from the corresponding author on request.

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И З В О Д

ФИТОХЕМИЈСКО ИСПИТИВАЊЕ ВРСТЕ *Pimpinella serbica*

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Ендемска врста Западног Балкана *Pimpinella serbica* (Vis.) Drude према последњој ботаничкој класификацији припада роду *Pimpinella* L. (Apiaceae), мада је најпре била описана као *Pancicia serbica* Vis. и дуго је сматрана једином припадајућом врстом овог рода. Претходна фитохемијска испитивања ове врсте обухватају само анализу етарског уља GC–MS техником, тако да је ово прво детаљно испитивање биљке које укључује LC-DAD–MS анализу праћену изоловањем секундарних метаболита биљке применом хроматографских техника. Пет једињења која припадају групи фенилпропаноида и полиацетилена, добро познатих по својим биолошким активностима, изоловано је из екстраката надземног дела и корена биљке. Њихова структура је одређена применом спектроскопских метода (UV, NMR, MS). Дилапиол (**1**), нотоапиол (**2**) и оплопантриол A 18-ацетат (**4**) нађени су у оба екстракта, док је фалкариндиол (**3**) изолован само из екстракта корена, а дендротрифидол (**5**) само из екстракта надземног дела биљке. Фитохемијски профил биљке *P. serbica* подржава њену припадност роду *Pimpinella* L.

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REFERENCES

1. *Apiaceae*, in *A Dictionary of Plant Sciences* (4th ed.), M. Allaby Ed., Oxford University Press, New York, 2019 (https://doi.org/10.1093/acref/9780198833338.001.0001)
2. M. G. Pimenov, M. V. Leonov, *The Genera of the Umbelliferae,* Royal Botanic Gardens, Kew, 1993, p. 156 (ISBN 0-947643-58-3)
3. *Assessment report on Pimpinella anisum L*., Doc. Ref: EMEA/HMPC/137421/2006 (https://www.ema.europa.eu/en/documents/herbal-report/draft-assessment-report-pimpinella-anisum-l_en.pdf, accessed August 2021)
4. *Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity* (http://ww2.bgbm.org/EuroPlusMed/, accessed August 2021)
5. V. Nikolić in *Flora SR Srbije V*, M. Josifović, Ed., SASA, Belgrade, 1975, p. 229 (in Serbian)
6. T. G. Tutin, *Genus Pimpinella*, in *Flora Europaea, Vol 2*, T. G. Tutin, V. H. Heywood, N. A. Burges, D. M. Moore, D. H. Valentine, S. M. Walters, D. A. Webb, Eds., Cambridge University Press, Cambridge, 1968, p. 332 (ISBN 9780521066624)
7. R. Ivanic, K. Savin, F. V. Robinson, *Planta Med.* **48** (1983) 60 (https://doi.org/10.1055/s-2007-969883)
8. C. Kapetanos, A. Kariot, S. Bojovic, P. Marin, M. Velic, H. Skaltsa, *Chem. Biodivers.* **5** (2008) 101 (https://doi.org/10.1002/cbdv.200890000)
9. I. Vučković, L. Vujisić, M. Todosijević, D. Stešević, S. Milosavljević, S. Trifunović, *Rec. Nat. Prod.* **8** (2014) 148 (https://www.acgpubs.org/doc/2018080912225120-RNP-EO_1205-040.pdf)
10. A. R. S. Chauhan, M.C. Nautiyal, R. Cecotti, M. Mella, A. Tava, *Ind. Crops Product.* **94** (2016) 401 (https://doi.org/10.1016/j.indcrop.2016.08.044)
11. L. P. Christensen, K. Brandt, *J. Pharm. Biomed. Anal*. **41** (2006) 683 (https://doi.org/10.1016/j.jpba.2006.01.057)
12. C. Seger, M. Godejohann, M. Spraul, H. Stuppner, F. Hadacek, *J. Chromatogr., A* **1136** (2006) 82 (https://doi.org/10.1016/j.chroma.2006.09.058)
13. I. Vucković, V. Vajs, M. Stanković, V. Tesević, S. Milosavljević, *Chem. Biodivers*. **7** (2010) 698 (https://doi.org/10.1002/cbdv.200900067)
14. E. Lemmich, *Phytochemistry* **20** (1981) 1419 (https://doi.org/10.1016/0031-9422(81)80053-2)
15. M. Kobaisy, Z. Abramowski, L. Lermer, G. Saxena, R. E. Hancock, G. H. Towers, D. Doxsee, R. W. Stokes, *J. Nat. Prod.* **60** (1997) 1210 (https://doi.org/10.1021/np970182j)
16. A. Kobayashi, K. Tamagawa, K. Yamashita, *Agric. Biol. Chem.* **41** (1977) 691 (https://doi.org/10.1080/00021369.1977.10862563)
17. N. Tabanca, G. Ma, D. S. Pasco, E. Bedir, N. Kirimer, K. H. Can Baser, I. A. Khan, S. I. Khan, *Phytother. Res.* **21** (2007) 741 (https://doi.org/10.1002/ptr.2154)
18. R. Parise-Filho, M. Pastrello, C. E. Pereira Camerlingo, G. J. Silva, L. A. Agostinho, T. de Souza, F. M. Motter Magri, R. R. Ribeiro, C. A. Brandt, M. C. Polli, *Pharm. Biol.* **49** (2011) 1173 (https://doi.org/10.3109/13880209.2011.575793)
19. R. Rojas-Martínez, J. Arrieta, L. Cruz-Antonio, D. Arrieta-Baez, A. M. Velázquez-Méndez, M. E. Sánchez-Mendoza, *Molecules* **18** (2013) 11327 (https://doi.org/10.3390/molecules180911327)
20. H. Laouer, E. K. Meriem, S. Prado, N. Baldovini, *Phytother. Res.* **23** (2009) 1726 (https://doi.org/10.1002/ptr.2820).