FACTA UNIVERSITATIS
Series: **Physics, Chemistry and Technology** Vol. 21, Nº 1, 2023, pp. 39 - 46
https://doi.org/10.2298/FUPCT2301039J

Original scientific paper

QUANTITATIVE ANALYSIS OF LIGNANS FROM THE FRUITS OF WILD CHERVIL (*ANTHRISCUS SYLVESTRIS* (L.) HOFFM.)

UDC 547.9 : 582.893.6

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Abstract. *Anthriscus sylvestris (L.) Hoffm., known as wild chervil, is a perennial widespread Apiaceae species. Being rich in lignans, it was used in traditional medicine to treat headaches, as analgesic, antipyretic, diuretic, antitussive, antihypertensive etc. Quantitative studies of lignans in Anthriscus sylvestris are limited to a few dominant lignans in roots and herbs. Hereby, the HPLC-MS method was used to quantify 14 lignans in fruit extract of A. sylvestris. A much higher content of lignans (970.2 mg/g) was found, compared to the previously analyzed root and herb extracts (4.3–66 mg/g) from the same location. The three most abundant lignans were found to be deoxypodophyllotoxin (867 mg/g), yatein (61.0 mg/g) and dimethylmatairesinol (27.7 mg/g). A significant amount of deoxypicropodophyllotoxin was also detected, but not quantified due to lack of reference standard. Due to previously observed spontaneous interconversion of cis/trans isomers, nemerosin and isochaihulactone, as well kaerophyllin and isokaerophyllin, were quantified together. Based on the obtained results, the fruits of A. sylvestris could be used as an industrial raw material for obtaining deoxypodophyllotoxin. This is also supported by a significantly simpler lignan profile of the fruit extract.*

Key words: *Anthriscus sylvestris, Apiaceae, ESI-MS, HPLC, lignan, quantification.*

Received: November 20th, 2023; accepted: December 24th, 2023
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1. INTRODUCTION

Anthriscus sylvestris (L.) Hoffm. is an herbaceous plant widespread in most temperate regions. Also known as wild chervil or cow parsley, it is a species belonging to the Scandiceae tribe of the Apiaceae family (Olaru et al., 2016). Members of Apiaceae family have a wide range of uses – some of them are traditionally used in food (carrots, celery, parsley, anise, coriander), others as ornamental or medicinal herbs (wild chervil), while some are characterized by toxicity (hemlock) (Kapetanos et al., 2008).

Wild chervil is characterized by the presence of numerous types of biomolecules, such as lignans (Orčić et al., 2021) and other phenolic compounds (Olaru et al., 2016), terpenoids, sterols, carotenoids, anthocyanins, vitamins, etc. (Chupakhina and Maslennikov, 2004; Chen et al., 2014). Lignans are one of the most abundant types of biomolecules present in *Anthriscus sylvestris*, with different biological activities, like cytotoxic, antiproliferative, anti-inflammatory, insecticidal, antioxidant, antiviral (Lin et al., 2004) and cardiovascular activity (Ghisalberti, 1997). Previous studies confirmed their presence in all parts of plant (Orčić et al., 2021; Orčić et al., 2022; Koulman et al., 2003a). The exact content of several lignans was determined in the roots and herbs (Dall'Acqua et al., 2006; Koulman et al., 2003b; Hendrawati et al., 2011; Orčić et al., 2022), but their content in fruits remains unknown, as the current reports deal only with qualitative profile (Ikeda et al., 1998; Janković et al., 2023)

In the present work, we report the content of 14 lignans in the fruits of *A. sylvestris* from Fruška Gora mountain (Serbia) investigated by HPLC-MS method. The obtained results were compared with the content of the same lignans in the roots and herbs of *A. sylvestris* from the same location (Orčić et al., 2022).

2. MATERIALS AND METHODS

2.1. Chemicals

All used standards (Fig. 1): acetylpodophyllotoxin, deoxypodophyllotoxin, podophyllotoxin, podophyllotoxone, picropodophyllotoxone, isopicropodophyllotoxone, guayadequiol, yatein, dimethylmatairesinol, 5'-demethoxypodophyllotoxin, isochaihulactone, nemerosin, kaerophyllin and isokaerophyllin were previously isolated in LAFIB (Laboratory for investigation of natural resources of pharmacologically and biologically active compounds) from *Anthriscus sylvestris* roots (Orčić et al., 2021). Herein used solvents (methanol and formic acid) were obtained from commercial source – Merck (Darmstadt, Germany).

2.2. Plant material

Plant material used for extraction was collected from Fruška Gora mountain (45.15727°N, 19.79375°E, Republic of Serbia), during the senescence phase (7.6.2023), with completely dry herbs and fruits. The fruits were separated from impurities and pulverized. The voucher specimen was confirmed and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium), Faculty of Sciences, University of Novi Sad, under designation 2-0019.

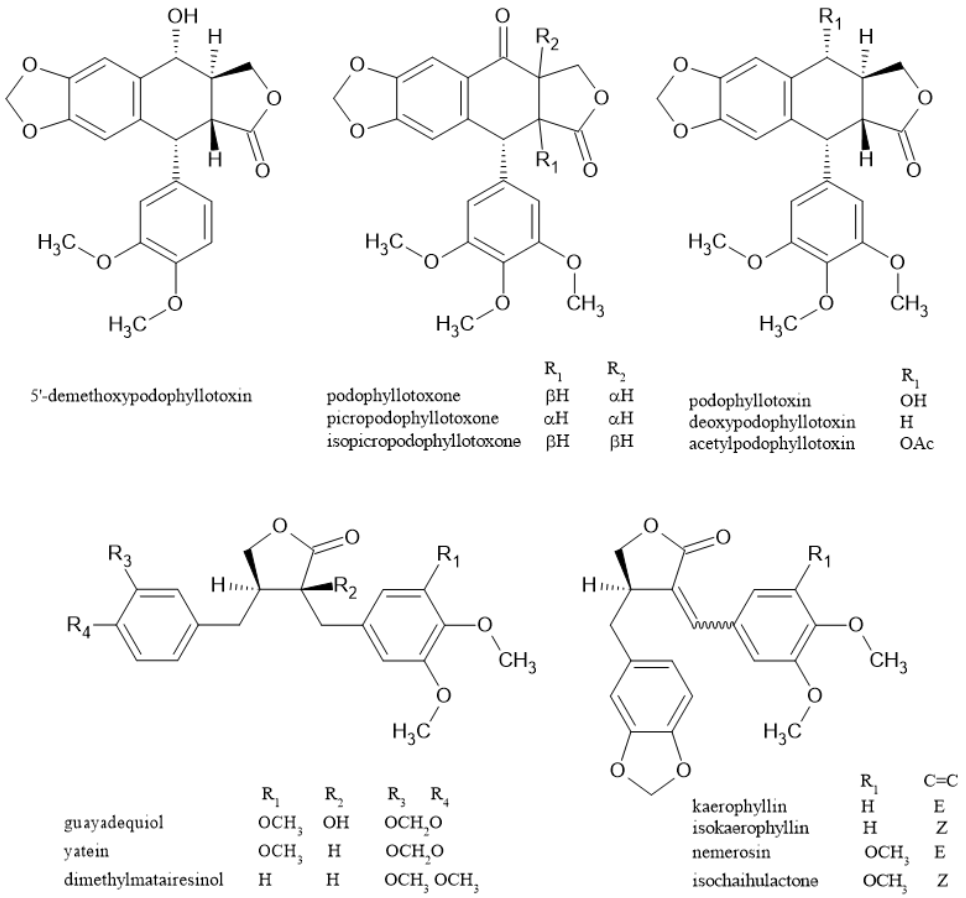

Fig. 1 Structures of the analyzed lignans.

2.3. Extraction

The sample (3.1267 g) was subjected to extraction by maceration with methanol (40 mL per cycle; 4 cycles) with constant shaking. The extract was evaporated at 35 °C under reduced pressure, yielding 88.3 mg of the dried residue. The extract was stored in the dark at 4 °C until the HPLC analysis. The extract sample was dissolved in 1500 μL of cold methanol immediately before the HPLC-MS analysis.

2.4. HPLC-MS analysis

Quantitative analysis was performed using liquid chromatography coupled with mass spectrometric detection on an Agilent Technologies 1200 Series Rapid Resolution liquid chromatograph (consisting of a G1379B vacuum degasser, a G1312B binary pump, a G1367C autosampler, a G1316B thermostated column compartment and a G1315C DAD detector) coupled to a G6410A QqQ MS-MS detector with an electrospray (ESI) ion source. MassHunter Workstation Data Acquisition ver. B.01.02 software (Agilent Technologies) was used for instrument control and data acquisition. The sample (0.2 μL) was injected onto a Zorbax XDB-C18 50 mm × 4.6 mm × 1.8 μm column, thermostated at 50 °C. A gradient of 0.05% HCOOH and methanol (0 min 30% MeOH, 6 min 70%, 9 min 100%, 12 min 100%) was used as the mobile phase, with a flow rate of 1 mL/min. Ion source parameters were: nebulization gas pressure of 50 psi, drying gas temperature of 350 °C and flow rate of 10 L/min, capillary voltage of 4000 V, fragmentor voltage of 100 V, positive mode. Compounds were monitored in MS2Scan mode, with *m/z* range 35–1680. Quantification was performed according to the external standard method (ESTD).

2.5. Calibration curves

A series of mixed standards was used for calibration with concentrations given in Table 1. The identity of the compounds was confirmed by comparing the retention times and spectra with literature data (Orčić et al., 2021; Orčić, 2010). For each compound, a summed peak area for the characteristic abundant ions (Table 1) was used as a response. Calibration curves were generated by OriginPro 9.2.0 software (OriginLab Corporation) (Fig. 2).

Table 1 Chromatographic parameters.

Compound	t_R (min)	Ions *m/z*	Calibration range (μg/mL)
podophyllotoxin	4.5	415, 432, 437, 453	3.125–250
picropodophyllotoxone	4.6	413, 430, 435, 451	0.625–50
deoxypodophyllotoxin	5.5	399, 416, 421, 437, 231	10–800
guayadequiol	5.5	387, 404, 409, 425	6.25–500
dimethylmatairesinol	5.1	387, 404, 409, 425	1.25–100
yatein	5.7	401, 418, 423, 439	6.25–500
nemerosin	5.9	399, 421, 437, 819	6.25–500
isokaerophyllin	6.4	369, 391	0.625–50
podophyllotoxone	4.9	413, 430, 435, 451	12.5–1000
acetylpodophyllotoxin	5.6	474, 479, 495, 397	0.9375–75
5’-demethoxypodophyllotoxin	4.3	402, 407, 423	0.625–50
isopicropodophyllotoxone	4.4	413, 430, 435, 451	0.625–50

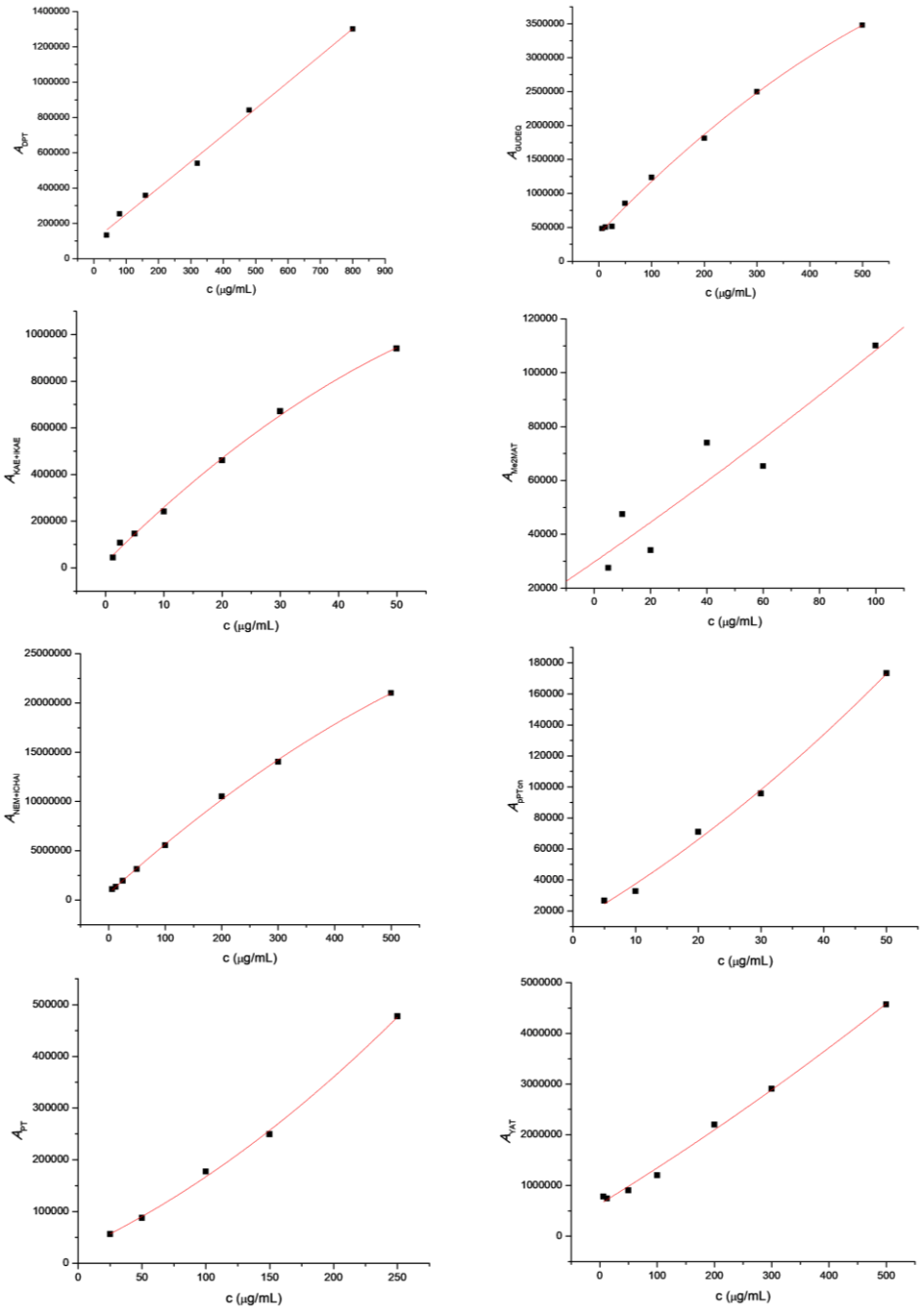

Fig. 2 Calibration curves.

3. RESULTS AND DISCUSSION

The results of quantification are shown in Table 2. Of the investigated 14 lignans, 10 were present above the detection limit. In the previous studies (Orčić et al., 2022; Schmidt et al., 2008), a rapid interconversion of *cis*- and *trans*- isomers of unsaturated dibenzylbutyrolactones was proven, even during the storage in the autosampler. Therefore it was impossible to quantify individual isomers and, consequently, the content of geometric isomer pairs (nemerosin/isochaihulactone; kaerophyllin/isokaerophyllin) is presented as a sum.

Table 2 Content of lignans in the fruit extract of *A. sylvestris* (mg/g).

Compound	Concentration in extract (mg/g)	Concentration in fruits (mg/g)
podophyllotoxin	2.23	0.0628
picropodophyllotoxone	3.99	0.113
deoxypodophyllotoxin	867	24.5
guayadequiol	0.741	0.0209
dimethylmatairesinol	27.7	0.781
yatein	61.0	1.72
nemerosin + isochaihulactone	3.31	0.0935
kaerophyllin + isokaerophyllin	1.84	0.0521
5'-demethoxypodophyllotoxin	< 0.0849*	< 0.00240*
isopicropodophyllotoxone	< 0.0849*	< 0.00240*
podophyllotoxone	< 1.70*	< 0.0480*
acetylpodophyllotoxin	< 0.127*	< 0.00360*

* Below the detection limit

The dominant lignan in the fruit extract of *A. sylvestris* was found to be deoxypodophyllotoxin (86.7% of the mass of dry extract). Other abundant lignans, with content greater than 1% in the dry fruit extract, were yatein (6.10%) and dimethylmatairesinol (2.77%). Comparing the results of the quantitative analysis of fruit extract with the previously determined concentration of lignans in the root and herb extract of *A. sylvestris* from the same location, significantly higher total lignan content (calculated as the total content of all the investigated lignans) in fruit extract was observed (970 mg/g mg/g vs. 4.3–66 mg/g). One possible explanation is the lower content of ballast substances in the fruit extract, in contrast to large amounts of storage oligo- and polysaccharides in the roots, and primary metabolism intermediates in the herbs. Another possible explanation is related to the ecological function of lignans – as compounds with confirmed insecticidal activity (Kozawa et al., 1982), they protect the fruits from herbivorous insects and ensure reproduction. The level of the most potent compound, deoxypodophyllotoxin, was approximately 39 to 389 times higher than in the root and herb extracts, the yatein content was 6.4–179 times higher, and the dimethylmatairesinol content 12–413 times higher. Quantitatively less prominent lignan was picropodophyllotoxone at 3.99 mg/g, still exceeding the values in the roots and herbs. Curiously, its stereoisomer podophyllotoxone, which was the most abundant 7-oxoaryltetralin in herb and root samples, was undetectable in the fruits. This can likely be attributed to photo-induced epimerization at C-8' into the picro isomer, which was already observed during the stability studies (Orčić et al., 2022). The content of unsaturated dibenzylbutyrolactones was

within a range previously observed in herb and root extracts (0.58–10.1 mg/g for nemerosin and isochaihulactone, 0.085–2.0 mg/g for kaerophyllin and isokaerophyllin).

Significant amount of the 8'-epimer of deoxypodophyllotoxin, deoxypicropodophyllotoxin, was also observed. The identity of this compound was previously confirmed by alkaline isomerization of deoxypodophyllotoxin (Orčić, 2010). As this compound was not detected in the roots (Orčić et al., 2021), it is likely that it was produced in the fruits (exposed to the sunlight) by photoisomerization. It was not possible to quantify this compound due to the lack of reference standard.

Based on the results, the fruits of *Anthriscus sylvestris* could be used as an industrial raw material for obtaining deoxypodophyllotoxin. While the amount of available biomass of fruits is much lower than in the case of roots and herbs (Orčić et al., 2021; Orčić et al., 2022), the much simpler chemical profile (including lignans) could make the isolation process simpler and economically more feasible.

Acknowledgement: *The authors would like to thank to the Ministry of Education, Science and Technological Development of the Republic of Serbia for the financial support of this work (Grant No. 451-03-47/2023–01/200125).*

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KVANTITATIVNA ANALIZA LIGNANA IZ PLODA ŠUMSKE KRASULJICE (*ANTHRISCUS SYLVESTRIS* L. (HOFFM.))

Anthriscus sylvestris (L.) Hoffm., u narodu poznata kao šumska krasuljica, velika krasuljica ili velika krbuljica, jeste višegodišnja biljna vrsta koja pripada familiji Apiaceae. S obzirom da je bogata lignanima, šumska krasuljica se koristila u tradicionalnoj medicini za lečenje glavobolje, kao analgetik, antipiretik, diuretik, antitusik, antihipertenziv itd. Kvantitativne studije lignana sprovedene na biljnoj vrsti Anthriscus sylvestis su ograničene na sadržaj nekoliko dominantnih predstavnika lignana u korenu i herbi. Iz tog razloga je HPLC-MS metoda korišćena za kvantifikaciju 14 lignana iz ekstrakta ploda A. sylvestis. Utvrđen je znatno viši sadržaj ukupnih lignana (970 mg/g) u poređenju sa prethodno analiziranim ekstraktima korena i herbe (4,3–66 mg/g) sa istog lokaliteta. Dokazano je da su tri kvantitativno najzastupljenija lignana deoksipodofilotoksin (867 mg/g), jatein (61,0 mg/g) i dimetilmatairezinol (27,7 mg/g). U uzorku je, takođe, primećena značajna količina deoksipikropodofilotoksina, ali on nije kvantifikovan zbog nedostatka referentnog standarda. Zbog ranije potvrđene spontane interkonverzije cis/trans izomera, nemerozin i izohaihulakton, kao i kerofilin i izokerofilin, kvantifikovani su zajedno. Na osnovu dobijenih rezultata, sledi mogućnost upotrebe plodova A. sylvestris kao industrijske sirovine za dobijanje deoksipodofilotoksina. U prilog tome ide i znatno jednostavniji profil lignana ekstrakta ploda šumske krasuljice.

Ključne reči: *Anthriscus sylvestris, Apiaceae, ESI-MS, HPLC, lignan, kvantifikacija.*