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Minireview

Phytochemical and biochemical studies of wild chervil *(Anthriscus sylvestris)*

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Summary. Plants represent important sources of bioactive molecules that can be used directly as medications, or as industrial precursors thereof. Podophyllotoxin, which was first isolated from mayapple (*Podophyllum peltatum* L.), and related natural and semi-synthetic lignans, are important antiviral, anthelmintic and especially antitumor agents, used in traditional and official medicine. Since the exploitation of common sources, such as *Sinopodophyllum hexandrum*, becomes unsustainable, new species are being investigated. One of the most promising is wild chervil (*Anthriscus sylvestris* (L.) Hoffm.), a widely distributed wild-growing Apiaceae species, commonly considered a noxious weed, that is known to be rich in lignans, especially aryltetralins (such as deoxypodophyllotoxin, podophyllotoxin and podophyllotoxone) and dibenzylbutyrolactones (including yatein and nemerosin), but also in various phenylpropanoids and terpenoids. This paper provides an extensive overview of *A. sylvestris* chemical composition investigations conducted thus far, with special focus on recent comprehensive profiling studies based on hyphenated techniques. Additionally, a detailed account of the bioactivity studies of both extracts and isolated compounds, confirming their antioxidant, anti-inflammatory and antiproliferative activity, is given.

Keywords: *Anthriscus sylvestris*, coumarins, essential oil, flavonoids, lignans, phenylpropanoids, polyacetylenes.

INTRODUCTION

Anthriscus sylvestris (L.) Hoffm, also known as cow parsley or wild chervil, is a wild-growing perennial plant from Apiaceae family, tribe Scandiceae Drude, that is widespread in Europe, North America and Asia. In most Western countries, it is considered an invasive weed, except for decorative "raven's wing" variety. However, in Europe and especially Asia (China, Japan, Korea), it has found use in traditional medicine as antipyretic and analgesic, cough remedy, diuretic, hematinic, tonic, digestive, antihypertensive etc. (Kozawa et al. 1982; Milovanovic et al. 1996; Lim et al. 1999; Jeong et al. 2007). Additionally, both roots and aerial parts are used in Japan as a food.

Anthriscus sylvestris is known (Ikeda et al. 1998a, 1998b; Jeong et al. 2007; Hendrawati et al. 2011; Orčić et al. 2021) for numerous secondary biomolecules with pharmacological activity – lignans, phenylpropanoids, coumarins, flavonoids, polyacetylenes and terpenoids. The most notable constituents are dominant lignans – deoxypodophyllotoxin (anthricin), nemerosin and yatein – which are present in sufficient amounts to allow for large-scale isolation. Compounded with significant biomass, including large storage roots, *A. sylvestris* exhibits a potential as an industrial source of lignans to be used either directly or as precursors of semi-synthetic derivatives. This is especially important since podophyllotoxin, the precursor for production of chemotherapeutics etoposide and teniposide, is currently extracted from *Podophyllum peltatum* and *Sinopodophyllum hexandrum*, with the latter becoming increasingly endangered due to over-exploitation (Hendrawa-

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ti et al. 2011; Orčić et al. 2021). Therefore, *A. sylvestris* represents an attractive target of phytochemical and biochemical studies. In this paper, we provide an extensive overview of *A. sylvestris* chemical composition investigations conducted thus far, with special focus on recent comprehensive profiling studies based on hyphenated techniques. Additionally, a detailed account is given regarding the bioactivity studies of both extracts and isolated compounds, confirming their antioxidant, anti-inflammatory and antiproliferative activity.

CHEMICAL COMPOSITION OF *A. SYLVESTRIS* ESSENTIAL OILS

Only a few studies of *A. sylvestris* essential oils composition had ever been published, despite the fact it is a highly aromatic plant, if somewhat pungent, and occasionally used as a food. The seminal studies by Kurihara and coworkers (Kurihara et al. 1978, 1979) were published only in Japanese, and contained only qualitative, but not quantitative data. The root analysis indicated a monoterpenes-dominated essential oil, with *l*-α-fenchyl acetate as the most abundant component, and α-pinene, β-myrcene, *d*-limonene, terpinolene and *p*-cymene as other prominent compounds. The chemical profile of inflorescences and leaves was more complex and could not be fully resolved using contemporary techniques. In addition to terpenoids (*d*-limonene, *p*-cymene, β-myrcene, *d*-sabinyl acetate, β-farnesene and unidentified sesquiterpenes), several non-terpenoid aromatic compounds were detected (benzyl alcohol, *m*- and *p*-cresol, phenethyl alcohol, eugenol), as well as *cis*-3-hexen-1-ol (leaf alcohol) in leaves.

The interest was rekindled in XXI century, when Bos and coworkers (Bos et al. 2002) identified over sixty compounds in *A. sylvestris* leaves' and roots' hydrodistillates. Volatile fraction of fresh leaves was dominated (70%) by monoterpenes, mostly β-phellandrene (38.8%), β-myrcene (16.7%), sabinene (6.2%) and *Z*-β-ocimene (5.4%), while germacrene D (4.2%) and *E,E*-α-farnesene (2.5%) were found to be the main sesquiterpenes (Fig. 1). The composition of root essential oil was qualitatively similar, but with 45.4% of β-phellandrene, 16.9% of *Z*-β-ocimene, 4.6% of α-pinene and 4.2% of germacrene D. The volatile profile of *A. sylvestris* bear no similarity to that of related species – *A. cerefolium*, which was characterized by presence of phenylpropanoids such as estragole and osmorhizole, that were absent in *A. sylvestris* likely due to their rerouting into lignan biosynthesis.

In our studies of Serbian wild chervil whole-plant essential oils (Orčić 2010), in addition to monoterpenes (8.4–20.9% sabinene, 2.3–28.3% limonene, 6.8–11.4% *E*-β-ocimene, 1.8–6.0% α-pinene etc.), a significant amount of *n*-alkanes was found (12.9–27.4% nonane, 0.7–1.1% decane, 3.1-4.4% undecane), likely originating from fatty acids

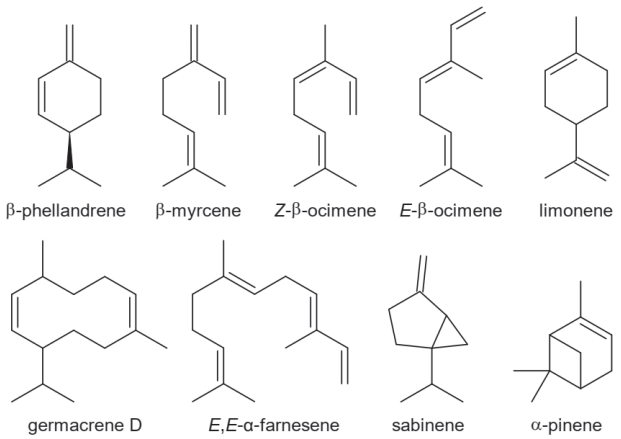

Fig. 1. Structures of major *Anthriscus sylvestris* terpenoids.

degradation, which were previously reported only in traces. The only phenylpropanoid was estragole, amounting up to 1.9%. Headspace studies of the volatiles evolved from the different plant parts indicated high variability in chemical composition with respect to organs, development phase and even plant specimens within a population. The dominant components were usually monoterpenes (especially sabinene, α-pinene, β-myrcene and, in some samples, limonene, β-phellandrene and *Z*- and *E*-β-ocimene) in widely varying ratios, without any discernible trends. Sesquiterpenes (mostly germacrene D and *E,E*-α-farnesene) were abundant only in top parts – leaves (0.0–61.5%), inflorescences (2.0–37.0%) and seeds (2.9–81.1%), while *n*-alkanes were abundant only in vegetative parts, reaching up to 76.9% (vs. up to 11.7% in inflorescences and seeds). Non-terpenoid aliphatic alcohols (hexanol and hexenols) were most prominent in leaves (reaching up to 49.4%).

Several phenylpropanoids (elemicin, myristicin, apiole) were also detected in traces in root samples from Canada (St-Gelais et al. 2015), alongside 23.8–64.7% of β-phellandrene, 8.7–48.4% myrcene, and other monoterpenes in minor amounts. The study by St-Gelais and coworkers confirmed the high variability of *A. sylvestris* volatile profile.

CHEMICAL COMPOSITION OF *A. SYLVESTRIS* EXTRACTS

Lignans

Lignans, 8,8'-linked phenylpropanoid dimers, are the most abundant and the most important secondary biomolecules of *A. sylvestris*. They have been extensively studied, both in academia and industry, due to their biological activity, most notably potent cytotoxicity. Since the seminal work of Kozawa and coworkers (Kozawa et al. 1978), numerous studies confirmed that *A. sylvestris* is a rich source of struc-

Table 1. Lignans detected in *Anthriscus sylvestris*.

Compound	References
aryltetralins	
(–)-deoxypodophyllotoxin (anthricin, DPT)	Kozawa et al. 1978; Kurihara et al. 1978; Van Uden et al. 1997; Ikeda et al. 1998a, 1998b; Lim et al. 1999; Koulman et al. 2001, 2003; Suzuki et al. 2002; Sakakibara et al. 2003; Lee et al. 2004; Dall'Acqua et al. 2006; Jeong et al. 2007; Suh et al. 2009; Yong et al. 2009; Orčić 2010; Quan et al. 2010; Hendrawati et al. 2011, Jung et al. 2013; Orčić et al. 2021
deoxypicropodophyllotoxin (isoanthricin)	Jeong et al. 2007, Kozawa et al. 1978
morelensin	Ikeda et al. 1998b; Lim et al. 1999; Koulman et al. 2003; Orčić et al. 2021
α-peltatin	Hendrawati et al. 2011
β-peltatin	Hendrawati et al. 2011
β-peltatin-a-methylether	Hendrawati et al. 2011
hydroxy- and oxo-aryltetralins	
angeloylpodophyllotoxin	Lim et al. 1999; Koulman et al. 2003; Jeong et al. 2007; Orčić 2010; Hendrawati et al. 2011; Orčić et al. 2021
podophyllotoxin	Koulman et al. 2001; Orčić 2010; Orčić et al. 2021
picropodophyllotoxin	Jeong et al. 2007; Orčić et al. 2021
5'-demethoxypodophyllotoxin (7-hydroxymorelensin)	Orčić 2010; Orčić et al. 2021
podophyllotoxone	Orčić 2010; Hendrawati et al. 2011; Orčić et al. 2021
picropodophyllotoxone (picropodophyllone)	Orčić 2010; Orčić et al. 2021
isopicropodophyllotoxone (isopicropodophyllone)	Orčić et al. 2021; Hendrawati et al. 2011
acetylpodophyllotoxin	Orčić et al. 2021
5'-demethoxypodophyllotoxone	Orčić et al. 2021
5'-demethoxypicropodophyllotoxone (5'-demethoxypicropodophyllone)	Orčić et al. 2021
5'-demethoxyisopicropodophyllotoxone (5'-demethoxyisopicropodophyllone)	Orčić et al. 2021
4-hydroxy-3',4',5-trimethoxy-7-oxo-2,7'-cyclolignano-9',9-lactone ?	Orčić et al. 2021
dibenzylbutyrolactones	
(–)-deoxypodorhizone (yatein)	Kozawa et al. 1978; Ikeda et al. 1998a, 1998b; Koulman et al. 2001, 2003; Suzuki et al. 2002; Sakakibara et al. 2003; Jeong et al. 2007; Orčić 2010; Hendrawati et al. 2011; Orčić et al. 2021
(–)-hinokinin	Ikeda et al. 1998b; Suzuki et al. 2002; Koulman et al. 2003
bursehernin	Lim et al. 1999; Suzuki et al. 2002; Koulman et al. 2003; Sakakibara et al. 2003; Orčić et al. 2021
arctigenin	Koulman et al. 2001, 2003
dimethylmatairesinol (methylarctigenin)	Koulman et al. 2003; Orčić et al. 2021
matairesinol	Suzuki et al. 2002; Sakakibara et al. 2003; Orčić et al. 2021
dimethylthujaplicatin methyl ether (trimethylthujaplicatin)	Koulman et al. 2003; Orčić et al. 2021
pluviatolide	Suzuki et al. 2002; Sakakibara et al. 2003; Orčić et al. 2021
5-*O*-methylthujaplicatin	Sakakibara et al. 2003; Ragamustari et al. 2013
4,5-di-*O*-methylthujaplicatin (thujaplicatin-3,4-dimethylether, hernanol)	Sakakibara et al. 2003; Ragamustari et al. 2013; Orčić et al. 2021

Compound	References
thujaplicatin	Sakakibara et al. 2003
5-methoxyguayaraol	Orčić et al. 2021
hydroxy- and oxo-dibenzylbutyrolactones	
7'-hydroxyyatein	Koulman et al. 2003
8-hydroxy-8'-*epi*-pluviatolide	Orčić et al. 2021
guayadequiol	Orčić et al. 2021
epiwikstromol dimethyl ether (8-hydroxy-8-*epi*-matairesinol)	Orčić et al. 2021
wikstromol dimethyl ether	Orčić et al. 2021
8-hydroxy-8-*epi*-yatein	Orčić et al. 2021
8-hydroxy-trimethoxylignano-9,9'-lactone ?	Orčić et al. 2021
3',8-dihydroxy-3,4,4'-trimethoxylignano-9,9'-lactone ?	Orčić et al. 2021
(-)-podorhizol (7-hydroxyyatein)	Orčić et al. 2021
(+)-podorhizon (7-oxoyatein)	Orčić et al. 2021
unsaturated dibenzylbutyrolactones	
anhydropodorhizol (nemerosin)	Ikeda et al. 1998a; Koulman et al. 2001, 2003; Suzuki et al. 2002; Dall'Acqua et al. 2006; Jeong et al. 2007; Orčić 2010; Hendrawati et al. 2011; Orčić et al. 2021
isosuchilactone	Ikeda et al. 1998a; Orčić et al. 2021
kaerophyllin	Ikeda et al. 1998a; Orčić 2010; Orčić et al. 2021
isokaerophyllin	Orčić 2010; Orčić et al. 2021
jatrophan	Ikeda et al. 1998a
7'-hydroxyanhydropodorhizol	Koulman et al. 2003
sylvestrin	Jeong et al. 2007
3,4,5-trimethoxy-3',4'-dihydroxylign-7-eno-9,9'-lactone	Orčić 2010; Orčić et al. 2021
(*E*)-3'-demethyljatrophan	Orčić et al. 2021
(*Z*)-3'-demethyljatrophan	Orčić et al. 2021
(*E*)-7,8-didehydro-dimethylmatairesinol	Orčić et al. 2021
(*Z*)-7,8-didehydro-dimethylmatairesinol	Orčić et al. 2021
7,8-didehydroguayarol	Orčić et al. 2021
7,8-didehydroisoarctigenin	Orčić et al. 2021
tetrahydrofurofurans	
phylligenin	Koulman et al. 2001
tetrahydrofurans	
lariciresinol	Suzuki et al. 2002
9-acetoxy-7'-oxo-3,3',4,4'-tetramethoxy-7,9'-epoxylignan	Orčić et al. 2021
arylnaphthalenes	
tetradehydropodophyllotoxin	Orčić et al. 2021
dibenzylbutandiols	
secoisolariciresinol	Suzuki et al. 2002; Sakakibara et al. 2003; Orčić et al. 2021

turally diverse lignans (Table 1). The most abundant classes were found to be aryltetralins (with or without oxygenation in position C-7) and saturated and unsaturated dibenzylbutyrolactones, typically bearing guaiacyl (3-methoxy-4-hydroxy), veratryl (3,4-dimethoxy), piperonyl (3,4-methylenedioxy) or syringyl (3,4,5-trimethoxy) substitution (Fig. 2).

The majority of conducted studies focused on structural elucidation and/or bioactivity determination of isolated compounds (Kozawa et al. 1978, 1982; Kurihara et al. 1978; Van Uden et al. 1997; Ikeda et al. 1998a, 1998b; Lim et al. 1999; Jeong et al. 2007; Suh et al. 2009; Yong et al. 2009; Jung et al. 2013), therefore offering only a limited overview of *A. sylvestris* chemical composition. Several more recent studies, relying on GC-MS (Koulman et al. 2001, 2003; Suzuki et al. 2002), HPLC-DAD-MS/MS (Orčić et al. 2021) and HPLC-MS-NMR (Hendrawati et al. 2011) provided better insight into lignan profile and enabled identification of minor components. For example, our systematic screening for lignans (Orčić et al. 2021), based on HPLC-MS/MS-guided chromatographic enrichment and purification, resulted in full or partial identification of 46 lignans, including 19 previously unknown.

Despite the importance of lignans, there had been only a few attempts to quantify these compounds in *A. sylvestris*. The published studies (Koulman et al. 2001, 2003; Dall'Acqua et al. 2006) focused on dominant lignans – deoxypodophyllotoxin (DPT), nemerosin and yatein – and indicated high variability of content with regards to growing conditions and plant age. For example, DPT concentration varied between 0.01 and 4.0 mg/g d.w. in herb, and between 0.08 and 17.3 mg/g in root. It should be noted that all these studies likely severely underestimated lignans' content due to suboptimal extraction conditions and the use of GC-MS, which can result in degradation. Our current study (unpublished results) measured content of 14 lignans by HPLC-DAD, and found significantly higher levels of DPT (2.0–42.8 mg/g in root), yatein (up to 18.5 mg/g), and (*Z*)- and (*E*)-anhydropodorhizols (2.1–24.2 mg/g), as well as significant amounts of podophyllotoxone (up to 20.5 mg/g). Total lignan content was 11.3–106.2 mg/g in root and 2.1–15.1 mg/g in aboveground parts.

Phenylpropanoids

Phenylpropanoids are a class of phenolic compounds originating from phenylalanine, bearing C_6C_3 skeleton with various degree of oxygenation and unsaturation. These compounds, especially hydroxycinnamic acids and their esters with quinic acid – chlorogenic acids – are universally present in plants.

The reports on phenylpropanoid acids are surprisingly scarce. Dall'Acqua et al. (2006) reported the effect-guided isolation of 5-*O*-caffeoylquinic acid ("chlorogenic acid", Fig. 3), which was found to be the most potent antioxidant in aerial parts extract. Our study (Orčić et al. 2021) also indicated the presence of *p*-hydroxyphenethyl ferulate (tyrosol ferulate), that was previously found in related *Chaerophyllum* species. The attempt (Orčić 2010) to screen for chlorogenic acids by HPLC-DAD-MS/MS resulted in detection of three caffeoylquinic acids: 5-CQA, 3-CQA and minor amounts of 1-CQA, and all six dicaffeoylquinic acids, with 1,3-C_2QA and 3,5-C_2QA as the most abundant isomers. A numer of acylated derivatives were also detected, albeit in smaller amounts: five different acetyl-dicaffeoylquinic acids, four malonyl-dicaffeoylquinic acids and two acetyl-malonyl-dicaffeoylquinic acids. Low content of acylated chlorogenic acids differentiated *A. sylvestris* from related species *A. cerefolium*, *Chaerophyllum* spp. and *Scandix pecten-veneris*. Our quantitative study found 5-CQA (0.91–9.00 mg/g d.w. and 0.24–8.4 mg/g in root and herb extracts, respectively), caffeic acid (<0.08 to 0.28 mg/g and <0.04 to 0.056 mg/g) and ferulic acid (<0.08 to 0.89 mg/g and <0.04 to 0.053 mg/g), while sinapic acid and *p*-coumaric acid were not detected (<0.08 mg/g).

In addition to phenylpropanoid acids, *A. sylvestris* contains a number of C_6C_3 compounds with, typically, (OCH_3) (OCH_2O) substitution on benzene ring, and oxygenation in C_3 chain, which is often further acylated with angelic, tiglic and/or sarracinic acid (Figs 4, 5). Untargeted and effect-directed fractionations (targeting cytotoxicity) lead to isolation of anthriscinol methyl ether (Kozawa et al. 1982; Ikeda et al. 1998a; Hendrawati et al. 2011), 1-(3-methoxy-

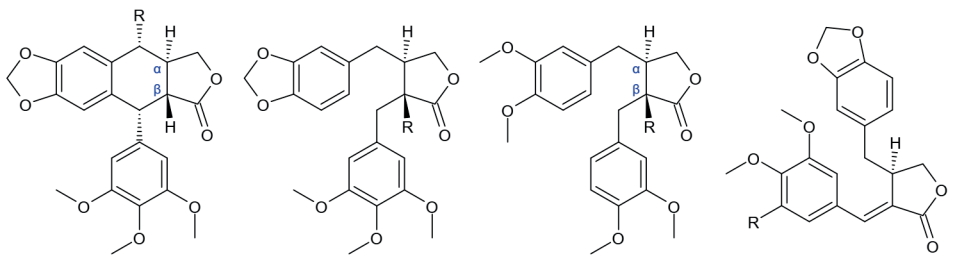

Fig. 2. Structures of major *Anthriscus sylvestris* lignans.

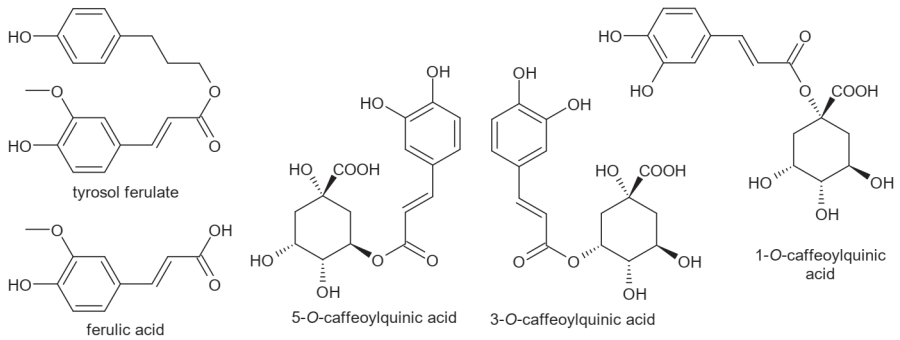

Fig. 3. Structures of some phenylpropanoid acids found in *Anthriscus sylvestris*.

4,5-methylenedioxyphenyl)-1ξ-methoxy-2-propene (1'-methoxymyristicin) (Ikeda et al. 1998a; Dall'Acqua et al. 2006; Hendrawati et al. 2011), 1-(3',4'-dimethoxyphenyl)-1ξ-hydroxy-2-propene (Ikeda et al. 1998b), crocatone (Kozawa et al. 1978), as well as several esters – 3',4'-dimethoxycinnamyl (*Z*)-2-angeloyloxymethyl-2-butenoate and (*Z*)-2-tigloyloxymethyl-2-butenoate (Ikeda et al. 1998b), *O*-[(*Z*)-2-angeloyloxymethyl-2-butenoyl]-3-methoxy-4,5-methylenedioxycinnamyl alcohol (anthriscusin) (Kozawa et al. 1978; Ikeda et al. 1998a; Dall'Acqua et al. 2006; Hendrawati et al. 2011), and 1-(3-methoxy-4,5-methylenedioxyphenyl)-2-angeloyloxy-propan-1-one (Kozawa et al. 1978).

Due to low concentrations, HPLC analysis of raw extracts typically fails to detect the majority of phenylpropanoids, but Hendrawati et al. (2011) managed to register appreciable peaks of anthriscinol methyl ether, anthriscusin and its regioisomer, *O*-[(*Z*)-4-angeloyloxy-2-methyl-2-butenoyl]-3-methoxy-4,5-methylenedioxycinnamyl alcohol. Our HPLC-MS/MS guided fractionation study (Orčić et al. 2021) lead to detection and, in some cases, isolation of phenylpropanoids based on *O*-prenylated coniferol, never before reported in *Anthriscus* spp.

The most volatile phenylpropanoids are usually reported as components of essential oils (Kurihara et al. 1979; Ragamustari et al. 2013; St-Gelais et al. 2015), but ocasionally isolated through fractionation of extracts (Ikeda et al. 1998a). These compounds include elemicin, estragole, myristicin, eugenol and apiole.

Coumarins

Coumarins are lactone derivatives of *o*-hydroxylated phenylpropenoic acids, containing benzopyrone moiety. Over 1300 distinct structures have been discovered in about 30 plant families, with Apiaceae family containing the widest range of structures (Matos et al. 2015). While no studies focused on coumarins in *A. sylvestris* so far, non-targeted fractionation by Jeong and coworkers (Jeong et al. 2007) resulted in isolation of several coumarins from root extract – scopoletin, isoscopoletin and linear furanocoumarin 5-methoxypsoralen (bergapten) (Fig. 6). In our studies (Orčić et al. 2021), only scopoletin was detectable by LC-MS even after extensive fractionation. The only attempt to quantify coumarins in *A. sylvestris* (Orčić 2010) found only minor amounts of scopoletin (<0.06 to 0.21 mg/g d.w. and <0.04 to 0.088 mg/g in root and herb extracts, respectively), while aesculetin was not detectable (<0.08 mg/g in root and herb extracts).

C_6C_1 phenols

In addition to C_6C_3 phenolic acids, plants often contain C_6C_1 acids (hydroxybenzoic acids), aldehydes and alcohols.

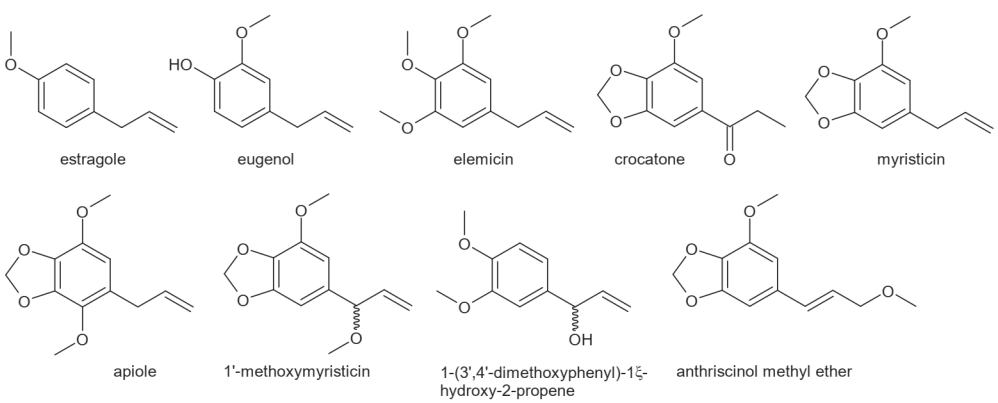

Fig. 4. Structures of simple phenylpropanoids found in *Anthriscus sylvestris*.

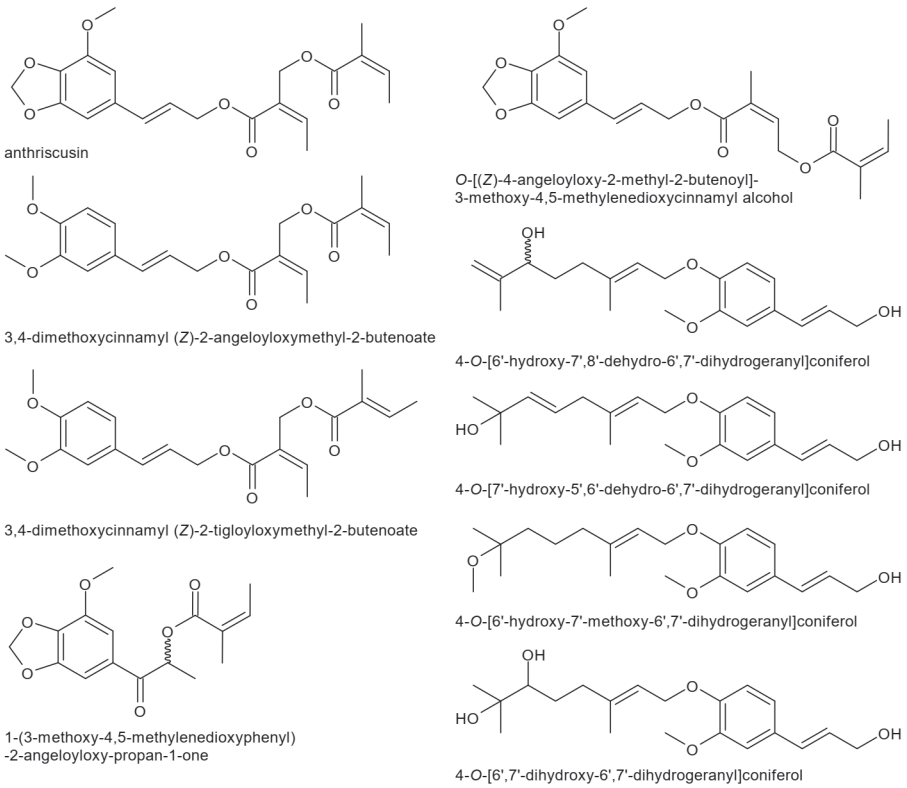

Fig. 5. Structures of conjugated phenylpropanoids found in *Anthriscus sylvestris*.

While they can be sometimes detected as such in plant material, they are mostly present as conjugates in tannins and lignin.

The reports on C_6C_1 compounds in *A. sylvestris* are extremely scarce, and are limited to detection of benzyl alcohol and traces of *p*-cuminaldehyde and myristic aldehyde (Fig. 7) in essential oil (Kurihara et al. 1979; Lajayer et al. 2020). In our quantitative study (Orčić 2010), we detected only *p*-hydroxybenzoic acid (<0.08 mg/g d.w. in root extracts and 0.083–1.38 mg/g in herb extracts), while syringic acid and gallic acid were below quantification limit (<0.08 mg/g).

Flavonoids

Flavonoids are the largest group of plant phenols, with nearly seven thousand known structures reported by 2017 (Flavonoid Database 2017). They are characterized by two benzene rings connected through C_3-chain, usually forming a flavan ring system that is further oxygenated and/or unsaturated. They are known to exhibit a wide range of biological activities, including antioxidant, anti-inflammatory, antibacterial, antimutagenic, antineoplastic, vasoprotective and antithrombotic.

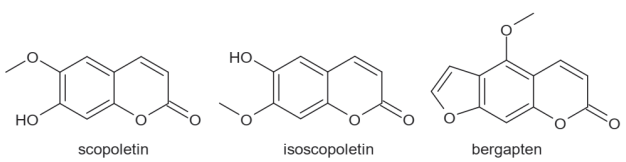

Fig. 6. Structures of coumarins found in *Anthriscus sylvestris*.

The presence of flavonoids in *A. sylvestris* was first confirmed by Kurihara et al. (1978), who reported non-targeted isolation of luteolin and apigenin (Fig. 8) from flowers. Nearly two decades later, fractionation of aqueous-ethanolic extract of aerial parts resulted in isolation of common flavonoids – quercetin, rutin (quercetin-3-*O*-rutinoside) and apigenin (Milovanovic et al. 1996). Apigenin was confirmed to be the most potent component for preventing lard rancidity. Activity-guided fractionation of aerial parts extract by Dall'Acqua and coworkers (Dall'Acqua et al. 2006), directed towards antioxidant components, yielded luteolin-7-*O*-β-D-glucoside (cynaroside) as a component of the most active fraction. Žemlička et al. (2014) also isolated this compound from aerial parts, in a moderate yield (124 mg per 1 kg of raw plant), and confirmed its antimutagenic and antioxidant activity, as well as potent antimicrobial activity towards some of the investigated bacteria.

Abdulmanea et al. (2012) conducted a detailed chromatographic study of flavonoids in a number of Apiaceae species, including *A. sylvestris*, finding rutin (8.0 mg/kg d.w. in leaves), quercetin (3.8 mg/kg) and apigenin (2.9 mg/kg). Surprisingly, the presence of isoflavonoids (Fig. 9) daidzein,

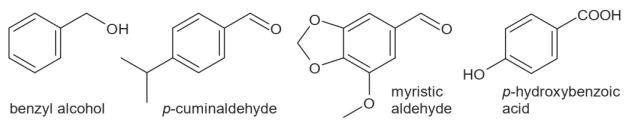

Fig. 7. Structures of C_6C_1 compounds found in *Anthriscus sylvestris*.

daidzin (daidzein-7-*O*-β-D-glucopyranoside, 2.4 mg/kg), sissotrin (biochanin A 7-*O*-β-D-glucopyranoside), formononetin, genistin (genistein-7-*O*-β-D-glucopyranoside, 0.8 mg/kg), 4',6,7-trihydroxyisoflavone (desmethylglycitein, 4.0 mg/kg) and isoformononetin (8.5 mg/kg), traditionally associated with Fabaceae family, was confirmed by HPLC-ELISA and HPLC-MS.

Another attempt on comprehensive study of *A. sylvestris* lignans using HPLC-MS/MS technique was conducted in our laboratory (Orčić 2010), resulting in detection of extensive satellite sets. Based on UV spectra and characteristic *m/z* values, we identified *O*-glycosides of luteolin (hexoside, two malonylhexosides and two acetylhexosides), chrysoeriol (hexoside, malonyl hexoside and acetylhexoside) and apigenin (hexoside and two acetylhexoside). Curiously, previously reported quercetin derivatives were not found in *A. sylvestris* and closely related species, *A. cerefolium*, only in *Chaerophyllum* species. Apiin (apigenin-7-*O*-apiogluycoside), another flavonoid characteristic for Apiaceae family, including *A. cerefolium*, was also not detected. An attempt was made to quantify aglycones – quercetin, luteolin, kaempferol, apigenin – by HPLC-MS/MS, but they were undetectable in root extract (<0.08 mg/g) and only sporadically found in herb extracts (with luteolin reaching up to 0.14 mg/g). Minute amounts of apigenin-7-*O*-glucoside were quantified in herb extracts (<0.04 to 0.10 mg/g).

Polyacetylenes

Polyacetylenes (polyyines) are secondary biomolecules, derived from unsaturated fatty acids, characterized by the presence of unusual triple bonds in aliphatic chain. Their biological and ecological role is poorly known, but it is assumed that they protect plants from fungal infections, in addition to insecticidal and allelopathic effects. While they are suspected to be widely distributed, their presence in plants is rarely investigated due to their highly unstable nature. A number of C_{17} polyacetylenes have been found in various edible Apiaceae species, including carrot (*Daucus carota*), celery (*Apium graveolens*) and parsley (*Petroselinum crispum*), where they represent bitter off-flavour principles, but also in toxic plants such as water hemlock (*Cicuta* spp.) and water dropwort (*Oenanthe* spp.).

Several non-targeted, activity-guided fractionation studies (Ikeda et al. 1998a, 1998b; Lim et al. 1999; Jeong et al. 2007) were conducted with *A. sylvestris* root and aboveground parts, resulting in isolation of falcarindiol (Fig. 10), that was found to be active towards k562, MK-1, HeLa, B16F10 and HL-60 cancer cell lines. Kramer et al. (2011) conducted systematic quantitative study of polyacetylene in several Apiaceae species, including *A. sylvestris* root, which they found to contain falcarindiol (3.6 mg/g dw), as well as falcarindiol-3-acetate (0.21 mg/g) and falcarinol (0.028 mg/g).

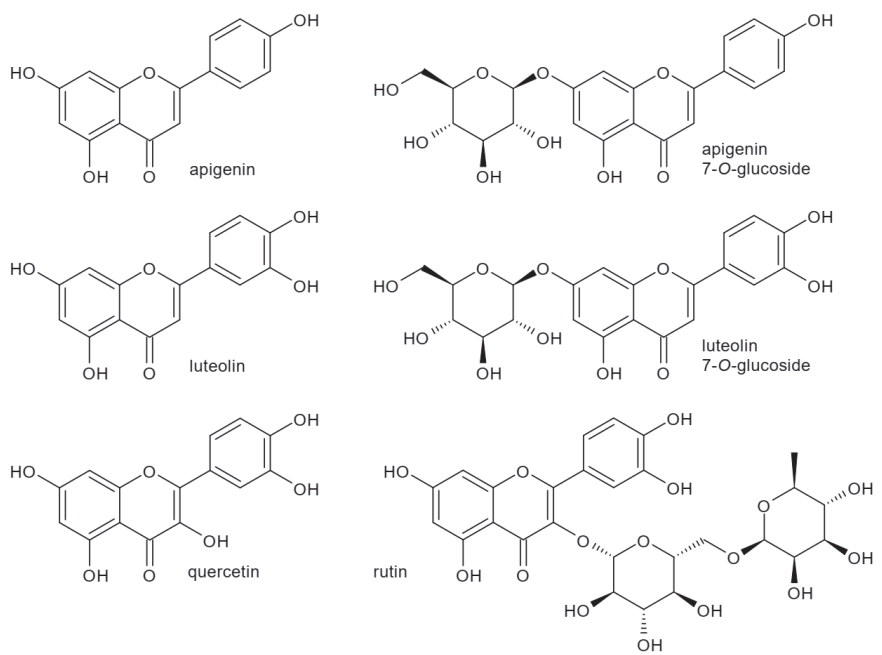

Fig. 8. Structures of main flavonoids found in *Anthriscus sylvestris*.

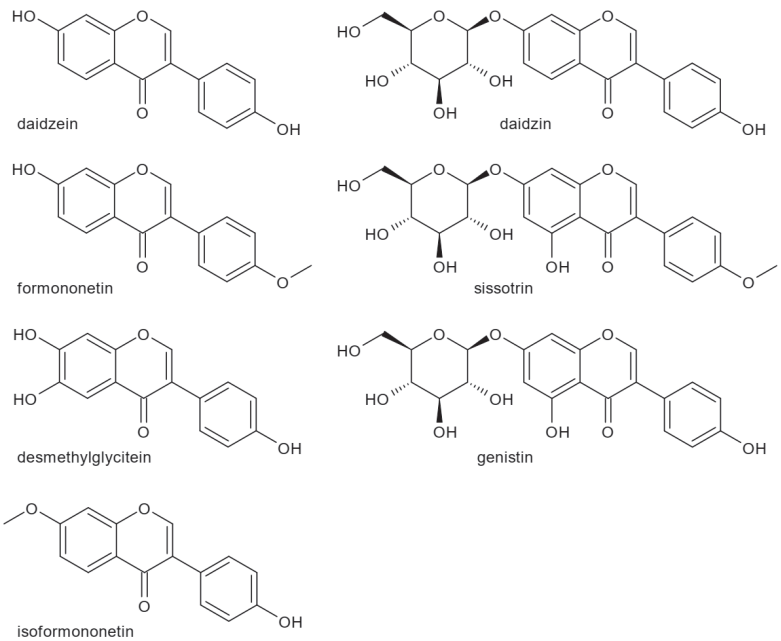

Fig. 9. Structures of isoflavonoids found in *Anthriscus sylvestris*.

BIOLOGICAL ACTIVITY OF *A. SYLVESTRIS* AND ITS CONSTITUENTS

Anti-inflammatory activity

Owing to a high concentration of extremely potent lignans, *A. sylvestris* bioactivity can mostly be traced to this class of compounds. The main uses (Lee et al. 2004; Jeong et al. 2007) of *A. sylvestris* in Korean traditional medicine – as an antitussive, antipyretic, analgesic and diuretic – can be at least partially attributed to anti-inflammatory properties of DPT. Lee et al. (2004) found that DPT causes dual inhibition of COX-2 (cyclooxygenase, prostaglandin-endoperoxide synthase 2) and 5-LOX (arachidonate 5-lipoxygenase), thus inhibiting production of prostaglandin D_2 (PGD_2) in a dose-dependent manner. DPT exhibited excellent selectivity towards COX-2 (inducible isoform) vs. COX-1 (constitutive isoform), with IC_{50} values of 0.01 μmol/L and 12.1 μmol/L in *in vitro* enzyme assay, and 1.89 mol/L vs. 65.3 μmol/L in an assay using murine bone marrow-derived mast cells (BMMC). The mechanism was confirmed to be through direct enzyme inhibition, since DPT did not affect expression of either enzyme, nor did it inhibit phospholipase A_2 ($sPLA_2$), the first enzyme in the arachidonic acid cascade. DPT also inhibited leukotriene C_4 (LTC_4) production by 5-LOX in BMMC, with IC_{50} of 0.37 μmol/L, thus showing promise in treating allergies and asthma.

Our experiments (Orčić 2010), monitoring the induced production of 12-hydroxyicosatetraenoic acid (12-HETE) and 12-hydroxyheptadecatrienoic acid (12-HHT) in human platelets, also confirmed anti-inflammatory activity of *A. sylvestris*. Raw herb and root extracts inhibited both 12-HHT production (IC_{50} of 0.49 and 0.39 mg/mL) and 12-HETE (IC_{50} 0.77 and 0.78 mg/mL), surpassing the other investigated plants from Scandiceae tribe (*A. cerefolium*, *Chaerophyllum temulentum*, *C. hirsutum*, *C. bulbosum*, *Scandix pecten-veneris*). Despite the lower levels of lignans, aerial parts extract exhibited the similar activity to the root extract, thus implying the effects of additional inhibiting compounds, such as flavonoids, coumarins and phenolic acids.

Antiproliferative activity

Study by Nakano and coworkers (Nakano et al. 1998) conducted to screen for plants with cytotoxic activity pointed out to *A. sylvestris* as extremely potent, with IC_{50} for raw herb and root extracts being below 2 μg/mL for all tested tumor cell lines (HeLa, MK-1 and B16F10). The activity surpassing that of other Apiaceae species, compounded with lower polyacetylenes content, implied some other active principles. The follow-up activity-guided fractionation studies (Ikeda et al. 1998a, 1998b) resulted in isolation of several active compounds, with DPT (IC_{50} 0.0005–0.001 μg/mL) and podophyllotoxin (IC_{50} 0.001–0.006 μg/mL) being by far the most potent. The study by Lim et al. (1999) confirmed that DPT is the main cytotoxic constituent of *A. sylvestris*, with IC_{50} towards cancer cell lines 5–100x lower than that of commercial chemotherapeutics etoposide and doxorubicin. An additional compound, angeloylpodophyllotoxin, was also found to be exceptionally potent, indicating the need for further studies. Our screening for active Scandiceae tribe species (Orčić 2010) also confirmed the high potency of *A. sylvestris*

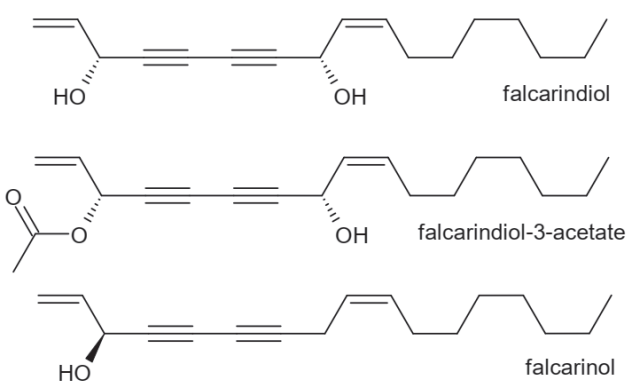

Fig. 10. Structures of polyacetylenes found in *Anthriscus sylvestris*.

raw extracts, but also indicated problems with selectivity – activity of root extract towards healthy cell line MRC-5 was significantly higher than towards cancer cell lines MCF-7, HeLaS3 and MDA-MB-231 (0.77 μg/mL vs. 2.6–19 μg/mL).

Within our comprehensive studies, total of 34 compounds were isolated (Orčić et al. 2021) and tested (unpublished results) for cytotoxicity towards healthy cells and three cancer cell lines (HeLa, MCF7, HT29). High activity, with IC_{50} < 1 μg/mL for at least one cell line, was detected for nine compounds: DPT, podophyllotoxin, morelensin, 7-hydroxymorelensin, acetylpodophyllotoxin, isokaerophyllin, podophyllotoxone, podorhizon, and an unidentified lignan, possibly 7-hydroxy-3,4,4'-trimethoxy-9,9'-epoxylignan. While selectivity of the commonly studied compounds – DPT and podophyllotoxin – was poor, it was significantly better for their 5'-demethoxylated derivatives – morelensin and 7-hydroxymorelensin (with IC_{50} towards healthy cells 3–165x times higher than towards cancer cells), as well as for podorhizon (3–8x). The highest activity was observed for aryltetralins, their 7-hydroxy and 7-acyloxy-derivatives, moderate for unsaturated dibenzylbutyrolactones, 7-oxoaryltetralins and 7-oxo dibenzylbutyrolactones, and the poorest for saturated dibenzylbutyrolactones.

The prediction of lignans activity is complicated by a multitude of possible mechanisms (Gordaliza et al. 2000; Lee and Xiao 2005; Orčić et al. 2021), with the preferential mechanism depending heavily on several structural factors, including the configuration on aryltetralin's position C-7, the presence of hydroxyl on C-4', and the presence of a bulky substituent on C-7. Podophyllotoxin is known to be a spindle poison – it reversibly binds to tubulin, preventing its polymerization into microtubules and formation of mitotic spindle, leading to arrest of the cell cycle in the metaphase, and subsequently to cell death. On the other hand, semi-synthetic podophyllotoxin derivatives etoposide and teniposide covalently bind to DNA-topoisomerase II complex, stabilize the form that catalyzes DNA cleavage, inducing single- and double-strand DNA breaks and fully preventing mithosis by blocking the cell in late S or G_2 phase. In a study by Jeong et al. (2007), DPT, angeloylpodophyllotoxin, deoxypicropodophyllotoxin and picropodophyllotoxin were all demonstrated to cause DNA fragmentation. In the same study, it was found that these compounds, as well as polyacetylene falcarindiol, also induce apoptosis by activating caspase-3. Study by Yong et al. (2009) confirmed that DPT induces cell cycle arrest at G2/M phase through several mechanisms, including inhibition of tubulin assembly and loss of the tubulin network, change in expression of cyclins A and B1, and activation of caspase-3 or -7. Jung et al. (2013) indicated that DPT additionally inhibits Akt/mTOR signaling pathway, blocking the autophagy while promoting apoptosis.

Multiple structurally dependent mechanisms of cytotoxicity, compounded with the presence of a wide range of structurally diverse lignans in *A. sylvestris*, open up the opportunity for synergistic effects of extracts when compared to individual compounds or synthetic chemotherapeutics. Additionally, more studies are needed to gain better insight into structure-activity relationship of these valuable compounds.

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