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CAFFEIC ACID DERIVATIVES AND FLAVONOIDS FROM APIACEAE AND *SALVIA* SPECIES

PhD thesis

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List of Abbreviations

AVP	After the vegetation period
BVC	Beginning of vegetation cycle
CDAs	Caffeoyl-daucic acids
CDHPLAs	Caffeoyl-dihydroxyphenyllactic acids
C4H	Cinnamate-4-hydroxylase
C3H	Coumarate 3-hydroxylase
CID	Collision induced dissociation
4CL	4-coumarate:CoA ligase
CQAs	Caffeoylquinic acids
DHPLA	3,4-dihydroxyphenyllactic acid
DIA	Data-independent acquisition
DiCDA	Dicaffeoyl-daucic acid
DiMDiCQA	Dimalonyl-dicaffeoylquinic acid
DMEM	Dulbecco's Modified Eagle's Medium
DPPH	2,2-diphenyl-1-picrylhydrazyl
DW5	Aqueous treatment, 5 min
DW15	Aqueous treatment, 15 min
DW30	Aqueous treatment, 30 min
DW45	Aqueous treatment, 45 min
DW60	Aqueous treatment, 60 min
100 °C, DW45	Aqueous treatment at 100 °C for 2 min, followed by room-temperature holding for 45 min
100 °C, DW60	Aqueous treatment at 100 °C for 2 min, followed by room-temperature holding for 60 min
ESI	Electrospray ionization
FBS	Fetal bovine serum
FCDAs	Feruloyl-caffeoyl-daucic acids
FMGls	Flavonoid-malonyl-glucosides
FS	Flowering stage
HQT	Hydroxycinnamoyl transferase
MdiCQA	Malonyl dicaffeoylquinic acid
PAL	Phenylalanine ammonia lyase
PBS	Phosphate-buffered saline
PRM	Parallel reaction monitoring

RAS	Rosmarinic acid synthase
TAL	Tyrosine ammonia lyase
UT	Untreated samples

1. INTRODUCTION

1.1. Background

Secondary metabolites are produced by plants primarily as part of their defense mechanisms; these specialized metabolites protect plants from a wide range of pathogens, pests, and predators. These metabolites also possess various therapeutic properties that can be exploited for medical applications (1). A significant part of commercially available therapeutic compounds has been derived from plants. The isolation of morphine from *Papaver somniferum* L. in 1803 marked the beginning of the drug discovery era from plant species (2). Later on, a wide range of well-known therapeutic agents, such as aspirin, paclitaxel, digitoxin, cocaine, pilocarpine, codeine, quinine, artemisinin, and silymarin, were isolated from medicinal plants, thereby contributing significantly to advancements in drug discovery (3, 4). Hence, investigating the phytochemical composition of underexplored plants holds a significant promise for novel drug discovery.

Phenolic compounds are one of the largest groups of secondary metabolites found in plants, including phenolic acids and flavonoids as main subclasses. Phenolic acids are characterized by the presence of a carboxylic acid group (5). Hydroxycinnamic acids (6) are the most common phenolic acids, containing a phenyl ring (C6) linked to a propenoic acid side chain (C3), thus forming a phenylpropanoid (C6-C3) scaffold. The hydroxycinnamic acid derivatives can be differentiated based on the numbers and positions of hydroxyl groups and other substituents attached to the phenyl ring. The most common hydroxycinnamic acids include *p*-coumaric, caffeic, ferulic, and sinapic acids (7). Among these, caffeic acid is considered the most abundant hydroxycinnamic acid in plants (8).

1.2. Chemical and structural features of caffeic acid and flavonoids

Caffeic acid ((2E)-3-(3,4-dihydroxyphenyl)prop-2-enoic acid) was first identified in 1867 (9, 10). The phenyl ring of caffeic acid is substituted with two adjacent hydroxyl groups, thus forming a catechol moiety (Figure 1). The olefinic bond in the propenoic acid side chain predominantly exists in the *trans* (E) configuration (11). Caffeic acid commonly forms ester bonds with alcohols, yielding caffeic acid phenethyl esters; amide bonds with amines, producing caffeic acid amides; and glycosidic bonds with sugars,

generating caffeic acid glycosides, which altogether improve its solubility, stability, and pharmacological activities (11-13).

Flavonoids contain C₆–C₃–C₆ skeleton, which is based on two 6-carbon aromatic rings (A and B) linked by a three-carbon heterocyclic ring (C) (Figure 1) (14). Based on the degree of oxidation, the extent of saturation in the C₃ subunit, and the pattern of substitutions, flavonoids are categorized into several major classes, including flavanones, dihydroflavonols, flavones, isoflavones, flavonols, and anthocyanins (15). Moreover, flavonoids may undergo structural modifications such as glycosylation, methylation, and acylation, which alter their solubility, stability, and biological activities (16).

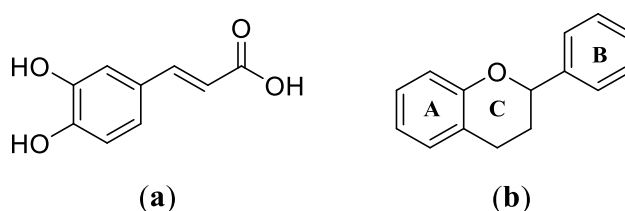


Figure 1. Representation of (a) caffeic acid and (b) flavonoid core structure.
Source: Structures drawn by the author based on the literature (11, 14).

1.3. Biosynthesis of caffeic acid

Caffeic acid is synthesized via the phenylpropanoid pathway from the amino acids phenylalanine or tyrosine. The deamination of L-phenylalanine is catalyzed by phenylalanine ammonia lyase (PAL), resulting in the formation of cinnamic acid, which is hydroxylated to *p*-coumaric acid under the action of cinnamate-4-hydroxylase (C4H). On the other hand, L-tyrosine is transformed into *p*-coumaric acid after the deamination mediated by tyrosine ammonia lyase (TAL) (17). Subsequently, *p*-coumaric acid is directly converted into caffeic acid after undergoing hydroxylation catalyzed by coumarate 3-hydroxylase (C3H). Alternatively, *p*-coumaric acid is transformed to coumaroyl-CoA by 4-coumarate:CoA ligase (4CL), which undergoes hydroxylation to yield caffeoyl-CoA mediated by C3H. Subsequently, the hydrolysis of caffeoyl-CoA by CoA thioesters results in the formation of caffeic acid (Figure 2) (9, 18).

Caffeic acid acts as an activated acyl donor (caffeoyl-CoA), generating diverse secondary metabolites. The hydroxycinnamoyl-CoA:quinic acid hydroxycinnamoyl transferase (HQT) mediates the transfer of the caffeoyl group from caffeoyl-CoA to quinic acid, forming caffeoylquinic acids (19). Caffeoyl-dihydroxyphenyllactic acids, such as rosmarinic acid, are produced by the condensation of caffeic acid with 3,4-

dihydroxyphenyllactic acid catalyzed by rosmarinic acid synthase (RAS) (20). The biosynthetic pathway of caffeoyl daucic acids has not yet been reported. The specific acyltransferase(s) that attach the caffeoyl moiety to daucic acid have not been identified.

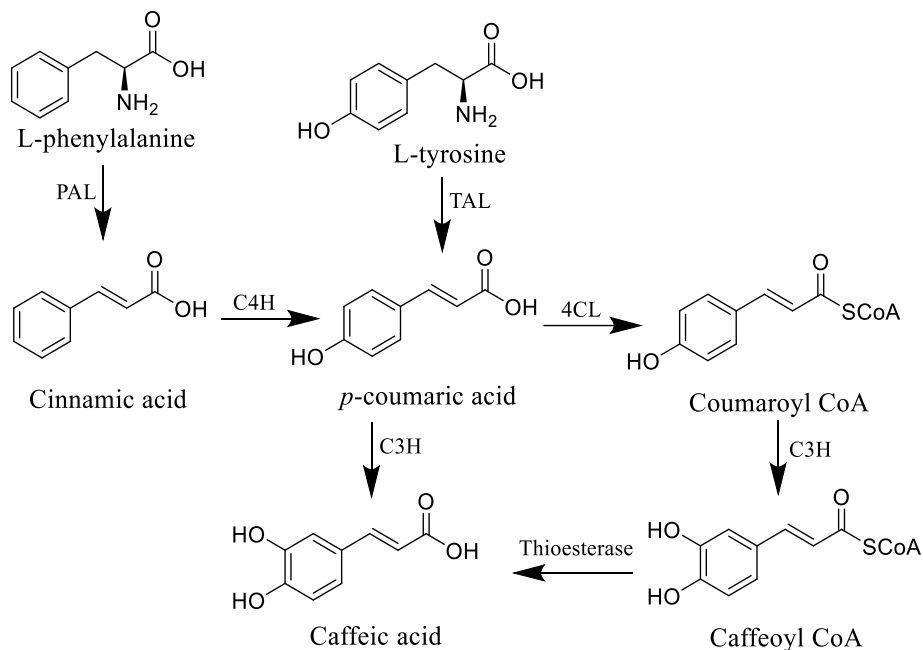


Figure 2. Biosynthesis of caffeic acid from its precursor amino acids.

Abbreviations: PAL; L-phenylalanine ammonia lyase, TAL; tyrosine ammonia lyase, C4H; cinnamate-4-hydroxylase, 4CL; 4-coumarate:CoA ligase, C3H; coumarate 3-hydroxylase.

Source: Illustration prepared by the author based on the literature (9, 17, 18, 19, 20).

1.4. Caffeic acid derivatives and flavonoids studied in the present work

The most common naturally occurring caffeic acid derivatives include caffeoyl ester derivatives, caffeic acid amide derivatives, caffeoyltartaric acid, caffeoyl shikimic acid, caffeoylquinic acid, and caffeoyl glycoside (21). We will discuss only those derivatives that are closely related to our research topic.

1.4.1. Caffeic acid derivatives

1.4.1.1. Caffeoylquinic acids

Caffeoylquinic acids (CQAs) are formed by the esterification of quinic acid with one to four caffeic acid residues (21). Quinic acid has four esterification positions for caffeoyl groups, giving rise to 15 possible substitution patterns (four monoCQAs, six diCQAs, four triCQAs, and one tetraCQA), while assuming a fixed stereochemical configuration of quinic acid (22). Some commonly known CQAs include neochlorogenic

1.4.2. Flavonoid-glucosides

Quercetin and kaempferol belong to the flavonol class, whereas luteolin is a flavone derivative (Figure 3) (15). These flavonoids predominantly occur as *O*-glycosides rather than as free aglycones (33). The glycosylation improves the bioavailability, stability, and therapeutic efficacy of flavonoids (34). The hydroxyl groups of the sugar moiety may be further acylated with malonyl, acetyl, coumaroyl, and feruloyl groups (35).

1.5. Distribution of caffeic acid derivatives and flavonoid-glucosides in plants

Coffea arabica L. (Rubiaceae family) and *Camellia sinensis* L. (Theaceae family) are the richest sources of chlorogenic acid (5-CQA) (36). A wide range of mono and diCQAs have been identified in Apiaceae species, such as *Centella asiatica* L. (37), *Daucus carota* L. (38), and *Carum carvi* L. (39). TriCQAs were found in the Asteraceae species, such as *Duhaldea nervosa* (Wall. ex DC.) Anderb. (40), *Anthemis tinctoria* L. (41), and *Chrysanthemum morifolium* Ramat. (42). TetraCQA is the least occurring CQA, which was reported in another species of the Asteraceae family, i.e., *Pluchea indica* L. (43).

Reports on the presence of CDAs are scarce, and they have been mainly identified in the Apiaceae family. These species include *Oenanthe javanica* (Blume) DC., featuring 4,5-di-*O*-caffeoyl daucic acid (*lyxo* configuration) and 4-*O*-caffeoyl-5-*O*-feruloyl daucic acid (28), and *Ligusticum chuanxiong* Hort., containing methyl ester derivative of 4,5-di-*O*-caffeoyl daucic acid (31). Moreover, 4,5-di-*O*-caffeoyl daucic acid (*xylo* configuration) was reported in *Ipomoea batatas* (L.) Lam. from the Convolvulaceae family (30), thus highlighting stereochemical variations across species.

CDHPLAs are predominantly found in the Lamiaceae family, especially within the Nepetoideae subfamily, where rosmarinic acid serves as a key chemotaxonomic marker compound. Some of these species include *Rosmarinus officinalis* L. (44), *Salvia officinalis* L. (45), *Melissa officinalis* L. (46), and *Thymus vulgaris* L. (47). Salvianolic acids are mainly distributed across *Salvia* species: *Salvia miltiorrhiza* Bge. is considered the most enriched source of salvianolic acids A, B, C, D, E, F, T, and U (48-52). The other species include *Salvia officinalis* L., containing salvianolic acids L, and Y

(53, 54), *Salvia cavaleriei* H.Lév., containing salvianolic acids H, and I (55), *Salvia flava* Forrest ex Diels., containing salvianolic acid J (56), *Salvia officinalis* L., containing salvianolic acid K (57), *Salvia deserta* Schang., containing salvianolic acid M (58), and *Salvia yunnanensis* C.H.Wright., containing salvianolic acid N (59).

The glucoside derivatives of quercetin, kaempferol, and luteolin have been identified across a wide range of plant families, including Polygonaceae, Solanaceae, Moraceae, Ranunculaceae, Leguminosae, Verbenaceae, Lamiaceae, Asteraceae, Scrophulariaceae, and Liliaceae (60).

1.6. Therapeutic significance of caffeic acid derivatives and flavonoid-glucosides

Given the numerous studies on the biological activities of caffeic acid derivatives and flavonoids, the following subchapter focuses only on the main biological effects of the derivatives most closely related to our research topic (Table 1).

Table 1. Anti-tumor and antioxidant activities of the caffeic acid and flavonoid derivatives.

Effects	Compounds	Results	References
Anti-tumor	3,5- <i>O</i> -dicaffeoyl-4- <i>O</i> -malonylquinic acid	The compound showed ~25% and ~80% collagenase inhibition at 50 and 250 µg/ml concentrations, respectively.	(61)
	1,3-dicaffeoyl-5-malonyl- δ -quinide	The compound exhibited non-selective cytotoxicity toward leukemic cells (MOLM-13) and non-cancerous rat kidney epithelial cells with EC ₅₀ between 400 and 600 µM	(62)
	Rosmarinic acid	Rosmarinic acid showed 41.3%, 27.4%, 48.7%, and 50.8% inhibition of T98G, PANC1, HT-29, and MDA-MB 436 cell lines, respectively, at 60 ng concentration.	(63)
	Salvianolic acid B	Savianolic acid B inhibited the growth of JHU-022, JHU-013, MDA-MB-231, and MCF-7 cells with IC ₅₀ of 18, 50, 125, and 120 µM, respectively.	(64, 65)
	Quercetin-3- <i>O</i> -glucoside	The compound showed significant cytotoxicity against the Caco-2 cell line with an IC ₅₀ of 79 µg/mL and moderate cytotoxicity against the HepG2 cell line with an IC ₅₀ of 150 µg/mL.	(66)

Antioxidant	Kaempferol 7- <i>O</i> -glucoside	The compound showed cytotoxic activities towards A-549 and MIA-PaCa cell lines with IC ₅₀ of 35.34 and 48.43 µg/mL, respectively.	(67)
	Luteolin-7- <i>O</i> -glucoside	The compound showed cytotoxic activities towards MCF-7 and U87 with IC ₅₀ of 3.98 and 26.34 µg/mL, respectively.	(68, 69)
	3,5- <i>O</i> -dicaffeoyl-4- <i>O</i> -malonylquinic acid	The compound showed an IC ₅₀ value of 2.29 µg/ml, which is comparable to that of ascorbic acid. i.e., 2.1 µg/ml.	(61)
	3- <i>O</i> -malonyl-5- <i>O</i> -(<i>E</i>)-caffeoylquinic acid	Both compounds showed 1.34 and 1.65 mM TE _s activity, while quercetin dihydrate (standard) showed 5.85 mM	(27)
	4- <i>O</i> -(<i>E</i>)-caffeoyl-5- <i>O</i> -malonylquinic acid	TE _s activity.	
	4,5-dicaffeoyldaucic acid	The compound exhibited 5.56 and 6.44 mM TE _s activity in DPPH and FRAP assays, which was comparable to that of gallic acid, i.e., 4.55 and 5.67 mM TE _s .	(30)
	Rosmarinic acid	Rosmarinic acid showed higher DPPH radical scavenging activity (IC ₅₀ 10.09 µmol/L), than methyl rosmarinates and ethyl rosmarinate (IC ₅₀ 12.5 and 16.5 µmol/L).	(70)
	Salvianolic acid B	Salvianolic acid B showed the inhibition of 18.59 mg/100 ml for ABTS and 13.02 mg/100 ml for DPPH radicals.	(71)
	Quercetin 3- <i>O</i> -(6''- <i>O</i> -malonyl)-β-D-glucoside	The compound exhibited ~90% DPPH radical scavenging activity at 1000 µM concentration.	(72)
	kaempferol-3- <i>O</i> -glucoside	The compound showed better DPPH inhibition (IC ₅₀ 1.25 µg/ml) than ascorbic acid (IC ₅₀ 3.08 µg/ml).	(73)
	Luteolin 7- <i>O</i> -β-glucoside	The compound showed DPPH inhibition with an IC ₅₀ of 21.2 µM.	(74)

Abbreviations: TE_s; Trolox equivalents, FRAP; ferric reducing antioxidant power, ABTS; 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), DPPH; 2,2-diphenyl-1-picrylhydrazyl.

1.7. Description of the studied plant species

1.7.1. Apiaceae species

The Apiaceae family includes 434 genera and nearly 3780 species, which are mainly distributed in northern temperate regions (75).

1.7.1.1. *Anthriscus cerefolium* (L.) Hoffm.

A. cerefolium (French parsley, chervil) is used as a seasoning herb for stews, soups, and sauces (76) (Figure 4). In folk medicine, *A. cerefolium* is used for its anti-

inflammatory, hypotensive, and diuretic effects (77, 78). Several caffeoylquinic acids (CQAs), specifically malonyl-1,4-*O*-dicaffeoylquinic acid, malonyl-1,5-*O*-dicaffeoylquinic acid, and malonyl-4,5-*O*-dicaffeoylquinic acid, were tentatively characterized in *A. cerefolium* aerial parts via HPLC combined with HR-MS/MS (79). However, their exact structural confirmations have not been performed. Flavonoids (luteolin 6-*C*-glucoside, quercetin 3-*O*-glucoside, kaempferol 3-*O*-hexoside, apigenin 7-*O*-glucoside) have also been identified in *A. cerefolium* (62, 80).

1.7.1.2. *Anthriscus sylvestris* (L.) Hoffm.

A. sylvestris (wild chervil, cow parsley) is used as a spice in salad dressings and infused with cheese as a flavoring agent (Figure 4). The roots of *A. sylvestris* are used as food, while the stems are pickled (81, 82). It is traditionally used as a tonic, anti-tussive, antipyretic, analgesic, and diuretic (83). The roots of *A. sylvestris* are known as the main source of lignans, including deoxypodophyllotoxin, podophyllotoxin, podophyllotoxone, nemerosin, and yatein (84).

1.7.1.3. *Chaerophyllum bulbosum* (L.)

Tubers of *C. bulbosum* (tuberous-rooted chervil) are used as a vegetable in raw or cooked form (Figure 4) (85). Inhibitory activity of *C. bulbosum* extracts was investigated against various enzymes such as acetylcholinesterase, urease, tyrosinase, α -amylase, and α -glucosidase (86). Flavonoid glycosides, including quercetin-3-*O*- β -D-glucopyranoside, apigenin-7-*O*- β -D-glucopyranoside, and luteolin-7-*O*- β -D-glucopyranoside were isolated from aerial parts of *C. bulbosum* (86).

1.7.1.4. *Myrrhis odorata* (L.) Scop.

M. odorata (sweet cicely) is used as a sweetener in desserts and to flavor liqueurs (Figure 4) (87, 88). The seeds are sprinkled over fruit salads, while the leaves are added to omelets and soups (88). *M. odorata* possesses antimalarial, antianemic, antiseptic, diuretic, expectorant, and tonic effects (88, 89). No comprehensive investigation of the non-volatile constituents has been reported for this species.

1.7.1.5. *Anthriscus caucalis* (M.) Bieb., and *Anthriscus nitida* (Wahlenb.) Hazsl.

The culinary uses of *A. caucalis* (burr parsley) and *A. nitida* (glossy cow parsley) have not been observed (Figure 4). Moreover, the ethnobotanical literature on these species is also limited, and the non-volatile phytochemicals are still underexplored.

1.7.2. *Salvia* species

Salvia is the largest genus in the family Lamiaceae, comprising approximately 1,000 species distributed across temperate, subtropical, and tropical regions worldwide. *Salvia* species are well known for their medicinal, culinary, and ornamental importance (90).

1.7.2.1. *Salvia officinalis* (L.)

S. officinalis (sage) leaf is included in the European Pharmacopeia (Ph. Eur.), possessing strong antioxidant effects, which improve brain function, prevent depression, dementia, diabetes, and obesity (Figure 4) (91). *S. officinalis* is considered one of the main sources of rosmarinic acid, while salvianolic acid B and salvianolic acid K have also been identified (92).

1.7.2.2. Other *Salvia* species

In addition to *S. officinalis*, four *Salvia* species common in Europe and Central Asia including *S. sclarea*, *S. nemorosa*, *S. pratensis*, and *S. verticillata*, have been less extensively studied for their specialized metabolites (Figure 4). However, their aerial parts are also used in traditional medicine (93). These species possess well-developed root systems that, in addition to their aerial parts, may represent novel sources of bioactive compounds.

1.7.2.2.1. *Salvia nemorosa* (L.)

S. nemorosa (woodland sage) is traditionally used as an antiseptic, antidiarrheal, anti-flatulent, diuretic, and stomach tonic (94) (Figure 4). Various metabolites, including caffeic acid, luteolin-7-*O*- β -D-glucuronide, apigenin-7-*O*- β -D-glucuronide, and rosmarinic acid, have been identified (95).

1.7.2.2.2. *Salvia pratensis* (L.)

S. pratensis (meadow sage) tea is used to cure abdominal pain, skin diseases, and ulcers (Figure 4). Various phytochemicals, such as rosmarinic acid, apigenin-7-glycoside, and luteolin-7-glycoside, have been identified in *S. pratensis* extracts (96); however, the isolation of bioactive compounds has not yet been reported.

1.7.2.2.3. *Salvia sclarea* (L.)

S. sclarea (clary sage) tea is used as an antidiarrheal and a tranquilizer in folk medicine (Figure 4) (97). Rosmarinic acid, salvianolic acid K, and flavonoid glycosides

(apigenin 7-*O*-glucuronide, luteolin 7-*O*-glucuronide, and luteolin 4'-*O*-glucuronide) have been isolated from *S. sclarea* (98).

1.7.2.2.4. *Salvia verticillata* (L.)

S. verticillata (lilac sage) is used as a flavouring agent in cheese preparation and a preservative for meat products (Figure 4). *S. verticillata* tea is used as an expectorant, disinfectant for the oral cavity, and cataplasm for wound healing (99). Rosmarinic acid, salvianolic acid B, salvianolic acid C, and salvianolic acid K have been identified in aerial parts of *S. verticillata* extracts, while the root part is still not investigated (100).

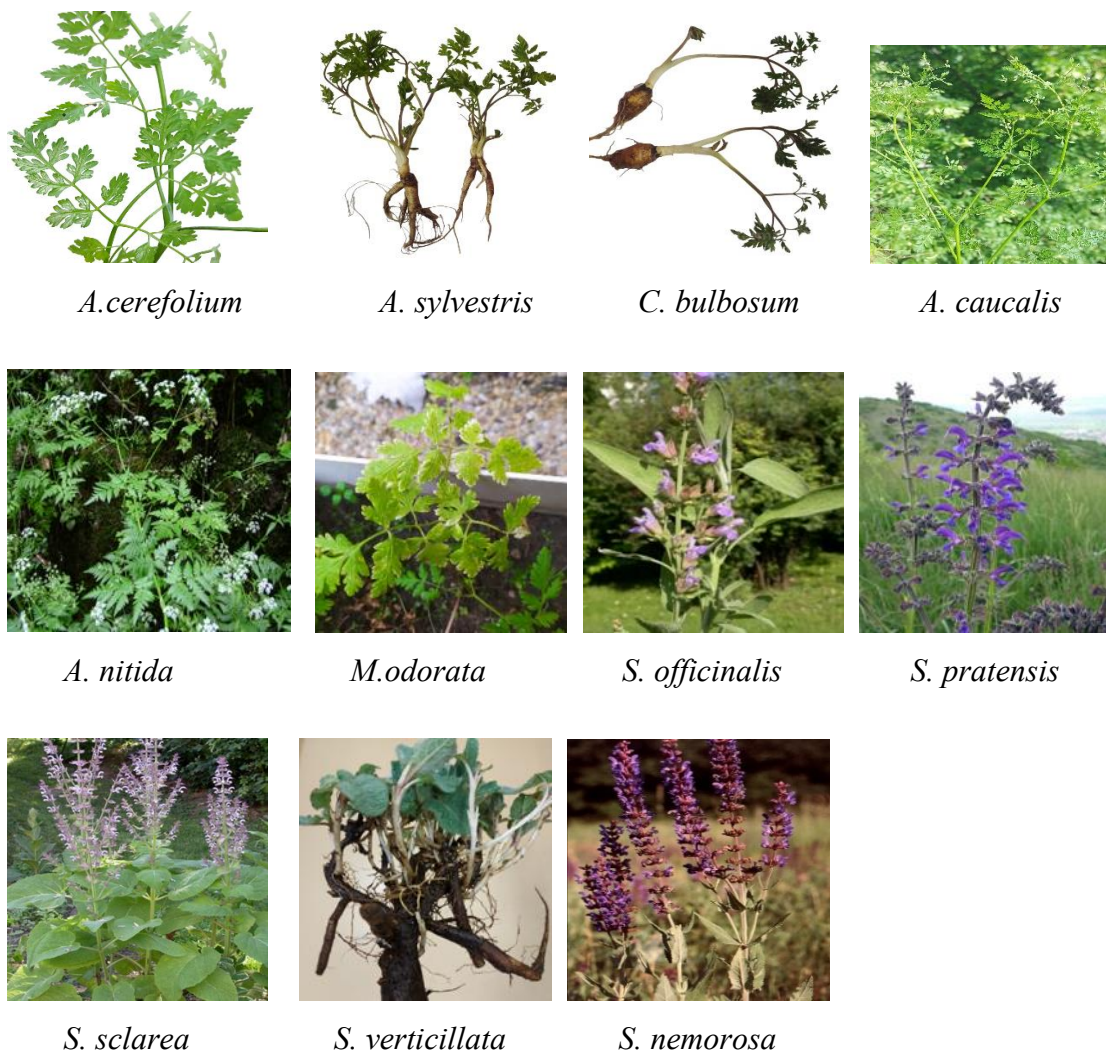


Figure 4. Illustrative photographs of selected plant species analyzed in the present study.
Source: Original photographs captured by the author.

2. OBJECTIVES

The primary aim of this study was the phytochemical characterization of selected, poorly investigated Apiaceae and *Salvia* species, followed by the isolation and structural elucidation of novel bioactive compounds, as well as the evaluation of their bioactivities.

To achieve this, the following objectives were defined for this study:

- 1) Identification of compounds in crude extracts prepared from different organs of selected Apiaceae and *Salvia* species through HPLC-UV-Orbitrap-HR-MS/MS analysis.
- 2) Quantifying the compounds in the organs of selected species collected in different phases of the vegetation cycle by an HPLC-UV-based external calibration method.
- 3) Identifying optimal tissues with relatively high levels of identified compounds, enabling their isolation using a one-step preparative HPLC method.
- 4) Investigating the impact of various treatment/extraction conditions on the metabolite composition of *Salvia* species, considering the consumption of these species in the form of teas and infusions.
- 5) Optimizing the treatment/extraction conditions, enabling the isolation of conversion products.
- 6) Detailed structural characterization of all the isolated compounds through NMR spectroscopy, with particular focus on the differentiation of regioisomers and stereoisomers.
- 7) Analyze the MS/MS fragment ion profiles of compounds to identify diagnostic ions suitable for distinguishing isomeric compounds.
- 8) Evaluate the in vitro antioxidant and cytostatic activities of the isolated compounds to demonstrate their potential for drug development.

3. MATERIAL AND METHODS

3.1. Materials and reagents

Formic acid, acetonitrile, distilled water, methanol-*d*₄, and acetonitrile-*d*₆ were acquired from Reanal (Hungary), while DMSO-*d*₆ and D₂O were sourced from VWR Chemicals (Belgium). DPPH (2,2-diphenyl-1-picrylhydrazyl), rutin, chlorogenic acid, kaempferol, quercetin, and trolox were purchased from Sigma-Aldrich (Steinheim, Germany). PBS (phosphate-buffered saline), DMEM (Dulbecco's Modified Eagle's Medium), trypan blue, and L-glutamine were purchased from Lonza (Basel, Switzerland). Nonessential amino acids, penicillin, streptomycin, and trypsin were sourced from Gibco (Thermo Fisher Scientific, Waltham, MA), while FBS (fetal bovine serum) was obtained from EuroClone (Pero, Italy).

3.2. Plant material

Myrrhis odorata L., *Salvia sclarea* L., and *S. officinalis* L. were cultivated in home gardens from plants purchased from commercial horticultural nurseries and were likewise collected during the flowering stage and autumn between 2022 and 2025. Commercial filter tea of *Salvia officinalis* L. (Sample 2) was obtained from a local pharmacy and complied with pharmacopeial quality requirements. Other plant samples were collected from various locations in Hungary during different phases of the vegetation cycle between 2022 and 2025. Plant parts (inflorescence, leaf, and root) were separated, washed, lyophilized, and ground to a fine powder. The voucher specimens of the plant samples were deposited in the Department of Plant Anatomy, Eötvös Loránd University, Budapest, Hungary.

3.3. Preparation of extracts

3.3.1. Preparation of untreated extracts

Untreated extracts were prepared from both Apiaceae and *Salvia* species for metabolite identification and isolation.

3.3.1.1. For HPLC-HR-Orbitrap-MS analysis

The extraction of each powdered plant part (5-20 mg) was performed with 3 mL of methanol (Apiaceae tissues) or 80% (v/v) methanol (*Salvia* tissues) at 60 °C for 30 minutes in 5 mL screw-capped vials. Thereafter, the extracts were centrifuged at 5,000

rpm, and diluted with methanol or 80% (v/v) methanol prior to HPLC-UV-HR-MS analysis.

3.3.1.2. For the isolation of compounds

Two consecutive extractions of pulverized plant tissues (2-10 g) were carried out with 10-60 mL of methanol (Apiaceae tissues) or 80% (v/v) methanol (*Salvia* tissues) at 60 °C for 30 minutes each in screw-capped vials. Subsequently, the extracts were centrifuged at 5,000 rpm, while supernatants were combined and dried using a standard laboratory rotary vacuum evaporator. The dried extracts were dissolved in 5-10 mL of methanol, followed by centrifugation. The centrifuged supernatant was filtered using Minisart RC 15 syringe filters (0.2 µm pore diameter, Sartorius AG, Goettingen, Germany), and the filtrates were used for preparative HPLC separation of compounds.

3.3.2. Sample treatments for investigating the effects of endogenous enzymes on metabolite composition

These treatments were performed on the pulverized tissues of selected *Salvia* species.

3.3.2.1. For HPLC-HR-Orbitrap-MS analysis

Treatment 1. Pulverized plant tissues (20 mg) were incubated with 600 µL distilled water for 5, 15, 30, 45, and 60 min.

Treatment 2. Pulverized plant tissues (20 mg) were suspended in 600 µL hot distilled water and incubated at 100 °C for 2 min, then kept at room temperature for an additional 45 or 60 min.

Following both treatments, 2.4 mL of absolute methanol was added to achieve a final methanol concentration of 80% (v/v) in each sample. Subsequently, samples were heated at 60 °C for 30 min, centrifuged at 5,000 rpm, and diluted with 80% (v/v) prior to analysis. The Treatment 1 samples were coded as DW5, DW15, DW30, DW45, and DW60 whereas the Treatment 2 samples were coded as 100 °C, DW45 and 100 °C, DW60.

3.3.2.2. For isolation of conversion products

Conversion products (**18**, **19**, **21**) were isolated from aqueous-treated extracts. Pulverized plant tissues (10 g) were incubated with 40 mL of distilled water for 30 min (**18**, **21**) or 5 min (**19**). The resultant conversion products were extracted in two consecutive steps with 50 mL of methanol at 60 °C for 30 min each, following the same

subsequent extraction process as mentioned above for the compound isolation under section 3.3.1.2.

3.4. Analysis of compounds by HPLC-HR-Orbitrap-MS

A Dionex Ultimate 3000 HPLC system (3000RS diode array detector (DAD), TCC-3000RS column thermostat, HPG-3400RS pump, SRD-3400 solvent rack degasser, WPS-3000TRS autosampler) connected to an Orbitrap Q Exactive Focus Mass Spectrometer equipped with electrospray ionization (ESI) (Thermo Fisher Scientific, Waltham, MA, USA) was used.

Analysis of Apiaceae species; Gemini NX-C18 column (150 × 3 mm; 3 μm) (Phenomenex, Torrance, CA, USA). Eluents: eluent A, 0.1% v/v formic acid, eluent B, acetonitrile:0.1% v/v formic acid (80:20, v/v), while the analysis was repeated with eluent A, 0.3% v/v formic acid, eluent B, acetonitrile:0.3% v/v formic acid (80:20, v/v). Gradient program 1: 0.0 min, 20% B; 15.0 min, 90% B (linear gradient); 18.0 min, 90% B (isocratic). Gradient program 2 was employed for the improved separation of compounds **5** and **6** from *A. sylvestris* extract: 0.0 min, 15% B; 15.0 min, 50% B (linear gradient); 16.0 min, 90% B (linear gradient); 19.0 min, 90% B (isocratic). Flow rate: 0.4 mL/min; column temperature: 25 °C; injected volume: 1.0–5.0 μL.

Analysis of Salvia species; Kinetex C18 column (75 × 3 mm; 2.6 μm) (Phenomenex, USA). Eluents: eluent A, 0.1% v/v formic acid, eluent B, acetonitrile:0.1% v/v formic acid (80:20, v/v). Gradient Program 3: 0.0 min, 10% B; 10.0 min, 50% B (linear gradient); 12.0 min, 90% B (linear gradient); 13.5 min, 90% B (isocratic). Flow rate: 0.3 mL/min; column temperature: 25 °C; injected volume: 2.0 μL. Ionization: ESI in positive and negative ionization mode (switching mode).

Fragmentation of compounds **1–10**: data-independent acquisition (DIA) with isolation widths of 100–300 *m/z*, 295–500 *m/z*, 495–800 *m/z*, and 795–1100 *m/z*; parallel reaction monitoring (PRM) method with an isolation width of 0.4 *m/z*, and collision-induced dissociation (CID) energies ranging from 10 eV to 45 eV.

Fragmentations of compounds **11–13**: DIA with isolation widths of 50–300 *m/z*, 50–500 *m/z*, 53–800 *m/z*, and 73–1100 *m/z*; PRM method with an isolation width of 0.4 *m/z*, and CID energies ranging from 10 eV to 45 eV.

Fragmentations of compounds **14–17**: DIA was recorded with an isolation widths of 100–300 m/z , 295–500 m/z , 495–800 m/z , and 795–1100 m/z ; PRM method with an isolation width of 0.4 m/z , and collision energies ranging from 10 eV to 45 eV.

All the compounds were analysed with Resolution: full MS, 70,000; MS/MS, 35,000. Full MS scans range: 100–1500 m/z . UV scan range: 230–600 nm.

3.5. Quantification of compounds

The quantification was carried out by employing an external standard method through HPLC-DAD technique. Linear regression analysis was performed for the isolated compounds in the following concentration ranges: 0.2670–100.0 $\mu\text{g/mL}$ (**1**), 0.4120–100.0 $\mu\text{g/mL}$ (**2**, **3**, **8**), 0.240–300.0 $\mu\text{g/mL}$ (**4**, **9**), 0.1953–50.0 $\mu\text{g/mL}$ (**10**), 0.20–50.0 $\mu\text{g/mL}$ (**11**), 0.2500–100.0 $\mu\text{g/mL}$ (**14**), 0.2500–200 $\mu\text{g/mL}$ (**15**), 0.20–400.0 $\mu\text{g/mL}$ (**16**), 0.40–200.0 $\mu\text{g/mL}$ (**17**), 0.20–100.0 $\mu\text{g/mL}$ (**18**), 0.16–100.0 $\mu\text{g/mL}$ (**19**), and 0.20–100.0 $\mu\text{g/mL}$ (**21**).

All compounds exhibited r^2 values greater than 0.999. The quantities of compounds **5**, **6**, and **7** were calculated using the calibration curve of compound **4**. Moreover, compounds **12** and **13** were quantified using the calibration equation of compound **11**, while compound **20** was quantified using the calibration equation of compound **19**. These calibration standards were structurally related to the corresponding test compounds and exhibited quite similar UV spectra. Consequently, the concentrations reported for compounds **5**, **6**, **7**, **12**, **13**, and **20** should be considered semiquantitative rather than absolute quantitative values.

3.6. Isolation of compounds by preparative HPLC with UV detection

A Dionex UltiMat 3000 HPLC System (VWD-3400RS photometer, HPG-3200BX pump, WPS-3000TSL autosampler, VF-F11-A-01 Fraction Collector F) (Thermo Fisher Scientific, Waltham, MA, USA) was used. Detection: 330, 336, and 348 nm. Initially, the extracts were purified by using RediSep Rf Gold C18 reversed column (Teledyne Isco, Lincoln, USA). Subsequent isolation was performed by using a Gemini NX-C18 column (250 $\times$ 10 mm; 5 μm) (Phenomenex, Torrance, CA, USA). Eluents: eluent A, 0.1% v/v formic acid, eluent B, acetonitrile. Flow rate: 5 mL/min.

Gradient program 1 for compounds **1–13**: 0.0 min, 15% B; 4.0 min, 15.0% B (isocratic); 32 min, 25% B (linear gradient); 42.0 min, 90% B (linear gradient). Gradient

program 2 for compounds **14–17**: 0.0 min, 15% B; 5.0 min, 15.0% B (isocratic); 20.0 min, 30% B (linear gradient); 25.0 min, 35% B (linear gradient); 26.0 min, 80% B (linear gradient); 30.0 min, 80% B (isocratic). Gradient program 3 for compounds **18, 19, and 21**: 0.0 min, 15% B; 5.0 min, 15.0% B (isocratic); 20.0 min, 30% B (linear gradient); 35.0 min, 45% B (linear gradient); 37.0 min, 80% B (linear gradient); 40.0 min, 80% B (isocratic).

3.7. Measurement of optical activity

The specific absorbance, λ_{max} values, and optical rotations were measured in MeOH at 25 °C through a Jasco V-550 UV/Vis spectrophotometer and a Jasco-P-200 polarimeter (JASCO International Co., Ltd., Tokyo, Japan), respectively.

3.8. Structural characterization of isolated compounds through NMR

NMR spectra of isolated compounds were recorded by using a Bruker Avance III HD 500 (500/150 MHz) spectrometer at 295 K, while the HMBC spectra of compounds **6** and **13** were recorded at 328 K and 313 K, respectively. The NMR spectra of compound **12** were recorded with a Bruker Avance III HD 700 (700/176 MHz) spectrometer at 313 K. The spectra of different compounds were recorded in various solvents, including compounds **1–10** in DMSO- d_6 , compounds **15, 17, 19** in DMSO- d_6 +D₂O, compounds **11–14, 16** in methanol- d_4 , and compounds **18, 21** in acetone- d_6 . The spectra were acquired through Bruker TopSpin 3.5 software using standard pulse sequences provided in their software library. Spectra were processed and analyzed by MestreNova NMR processor. ¹H and ¹³C chemical shifts (δ) are given in ppm, while coupling constants (J) are measured in Hz. The assignments were based on 1D ¹H NMR, ¹³C NMR and 2D ¹H–¹H COSY, ¹H–¹H ROESY, 2D ¹H–¹H NOESY, ¹H–¹³C HSQC, and ¹H–¹³C HMBC experiments.

3.9. In vitro antioxidant activity test

A previously published protocol was followed for the execution of DPPH radical scavenging assay (101). Serially diluted test compounds were incubated with 2.5 ml DPPH solution for 6 min. The absorbance was measured by a HITACHI U-2000 spectrophotometer (Hitachi Ltd., Tokyo, Japan) at 515 nm. The triplicate analysis was performed, and results are reported as IC₅₀ values (μM) $\pm$ SEM.

3.10. In vitro cytostatic activity test

In vitro, the cytostatic effect of the compounds was evaluated on numerous cell lines using Alamar Blue end-point viability assay. Cells were cultivated in DMEM medium consisting of 10% FBS, 2 mM L-glutamine, 50 IU/mL penicillin/50 µg/mL streptomycin antibiotic cocktail, 1 mM sodium pyruvate, and 1% non-essential amino acid mixture at 37 °C in a humidified atmosphere with 5% CO₂. After reaching confluency, the cells were seeded into 96-well tissue culture plates at an initial count of 5000 cells/well. Following 24 hours, cells were treated with serially diluted compounds and incubated overnight. Afterwards, cells were rinsed with a serum-free medium and cultured for another 72 hours. Subsequently, 22.5 µL Alamar Blue (resazurin sodium salt, Merck) solution (0.15 mg/mL in PBS) was added into each well, and after 4 hrs of incubation, the fluorescence was recorded at $\lambda_{\text{Ex}} = 530/30$ and $\lambda_{\text{EM}} = 610/10$ nm using a Synergy H4 multi-mode microplate reader (BioTek). The assay was performed in quadruplicate, and results are represented as IC₅₀ values (µM)±SEM.

3.11. Structural characterization of isolated novel compounds (4–9, 12, 17, 19) and previously misidentified compound (13)

1,5-dicaffeoyl-3-malonylquinic acid (**4**). light yellow amorphous solid; $[\alpha]^{25}_{\text{D}} = -110.2$ (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 335 (4.49); for ¹H NMR and ¹³C NMR data, see Table 6; HRESIMS (negative) *m/z* 601.1198 [M - H]⁻ (calcd for 601.1199, C₂₈H₂₅O₁₅).

3,5-dicaffeoyl-1-malonylquinic acid (**5**). light yellow amorphous solid; $[\alpha]^{25}_{\text{D}} = -147.5$ (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 335 (4.49); for ¹H NMR and ¹³C NMR data, see Table 6; HRESIMS (negative) *m/z* 601.1205 [M - H]⁻ (calcd for 601.1199, C₂₈H₂₅O₁₅).

3,5-dicaffeoyl-4-malonyl-*epi*-quinic acid (**6**). light yellow amorphous solid; $[\alpha]^{25}_{\text{D}} = -141.5$ (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 335 (4.49); for ¹H NMR and ¹³C NMR data, see Table 6; HRESIMS (negative) *m/z* 601.1207 [M - H]⁻ (calcd for 601.1199, C₂₈H₂₅O₁₅).

1,5-dicaffeoyl-4-malonylquinic acid (**7**). light yellow amorphous solid; $[\alpha]^{25}_{\text{D}} = -114.3$ (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 335 (4.51); for ¹H NMR and ¹³C NMR

data, see Table 6; HRESIMS (negative) m/z 601.1201 $[M - H]^-$ (calcd for 601.1199, $C_{28}H_{25}O_{15}$).

Luteolin-7-*O*-(2'',6''-di-*O*-malonyl)- β -D-glucoside (**8**). yellow amorphous solid; $[\alpha]^{25}_D = -83.4$ (c 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 348 (4.25); for ^1H NMR and ^{13}C NMR data, see Table 11; HRESIMS (negative) m/z 619.0943 $[M - H]^-$ (calcd for 619.0941, $C_{27}H_{23}O_{17}$).

1,5-dicaffeoyl-3,4-dimalonylquinic acid (**9**). light yellow amorphous solid; $[\alpha]^{25}_D = -135.3$ (c 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 335 (4.48); for ^1H NMR and ^{13}C NMR data, see Table 7; HRESIMS (negative) m/z 687.1205 $[M - H]^-$ (calcd for 687.1203, $C_{31}H_{27}O_{18}$).

4-caffeoyl, 5-feruloyl daucic acid (**12**). light yellow amorphous solid; $[\alpha]^{20}_D = +36$ (c 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 327 (4.33), 218 (3.89); for ^1H NMR and ^{13}C NMR data, see Table 8; HRESIMS (negative) m/z 541.0992 $[M - H]^-$ (calcd for 541.0988, $C_{26}H_{21}O_{13}$).

4-feruloyl, 5-caffeoyl daucic acid (**13**). light yellow amorphous solid; $[\alpha]^{20}_D = +35$ (c 1.0, MeOH); UV (MeOH) λ_{max} (log ϵ) 330.5 (4.51), 219.5 (4.01); for ^1H NMR and ^{13}C NMR data, see Table 8; HRESIMS (negative) m/z 541.0994 $[M - H]^-$ (calcd for 541.0988, $C_{26}H_{21}O_{13}$).

Salvianolic acid O (**17**). light yellow amorphous solid; $[\alpha]^{25}_D = 56.9$ (c 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 320.5 (4.19), 288 (4.12); for ^1H NMR and ^{13}C NMR data, see Table 9; HRESIMS (negative) m/z 555.1144 $[M - H]^-$ (calcd for 555.1144, $C_{27}H_{23}O_{13}$).

Salvianolic acid P (**19**). light yellow amorphous solid; $[\alpha]^{25}_D = -37.2$ (c 0.2, MeOH); UV (MeOH) λ_{max} (log ϵ) 331 (4.32), 300 (4.31), 249.5 (3.41); for ^1H NMR and ^{13}C NMR data, see Table 10; HRESIMS (negative) m/z 509.1090 $[M - H]^-$ (calcd for 509.1089, $C_{26}H_{21}O_{11}$).

4. RESULTS

4.1. HPLC-UV-Orbitrap-HR-MS analysis of extracts

4.1.1. Identification of metabolites in Apiaceae species

The HPLC-UV analysis of crude extracts from selected Apiaceae species showed thirteen peaks corresponding to the compounds of interest (Figure 5). Compounds with comparable retention times in the chromatograms of analyzed extracts showed identical HR-MS profiles, thus suggesting structural identity.

Compounds **1–3** and **8** showed a UV absorption maximum in the range of 340–360 nm, characteristic of flavonoids. Based on molecular formulas, compound **1** ($C_{24}H_{22}O_{15}$) is the malonyl-glucoside of quercetin (102), while compounds **2** and **3** (both $C_{24}H_{22}O_{14}$) are malonyl-glucosides of either kaempferol or luteolin (103, 104). Comparing the molecular formula of compound **8** ($C_{27}H_{24}O_{17}$) with that of compounds **2** and **3**, the presence of an additional malonyl subunit is depicted. Hence, compound **8** is identified as the dimalonyl-glucoside derivative of either kaempferol or luteolin. All these flavonoid-malonyl-glucosides (FMGLs) were detected in *C. bulbosum* extracts.

Compounds **4–7** exhibited a UV absorption maximum in the range of 280–330 nm, characteristic of caffeic acid-containing compounds. Moreover, these compounds possess the same molecular formula $C_{28}H_{26}O_{15}$, which aligns with malonyl dicaffeoylquinic acid (MDiCQA) isomers (105, 106). Compounds **9** and **10** exhibited UV absorption characteristics similar to those of compounds **4–7**, but their molecular formula, $C_{31}H_{28}O_{18}$, suggests a more complex structure. Comparison of this molecular formula with those of compounds **4–7** indicated the presence of an additional malonyl unit in compounds **9** and **10**, thereby supporting their identification as dimalonyl-dicaffeoylquinic acid (DiMDiCQA) isomers. Compound **4** was present in *A. cerefolium*, *C. bulbosum*, and *A. sylvestris*. Compounds **5** and **6** appeared as an overlapped peak in *A. sylvestris* (at 7.56 min); hence, they were further separated by an improved HPLC method using gradient program 2. Compounds **7** and **9** were present in *A. cerefolium* and *C. bulbosum*, while compound **10** was detected in *A. caucalis*.

The UV spectra of compounds **11–13** also showed the characteristics of caffeic acid with a UV absorption band at 280–330 nm. The molecular formula of compound **11** ($C_{25}H_{20}O_{13}$) suggested its identification as dicaffeoyldaucic acid (DiCDA) (28). The molecular formula of compounds **12** and **13** ($C_{26}H_{22}O_{13}$) indicated the presence of an

additional methylene subunit, thus supporting their identification as feruloyl-caffeoyl-*daucic acid* (FCDAs) isomers (28). Compounds **11–13** were present in *M. odorata*, while compound **11** was also detected in *A. nitida*.

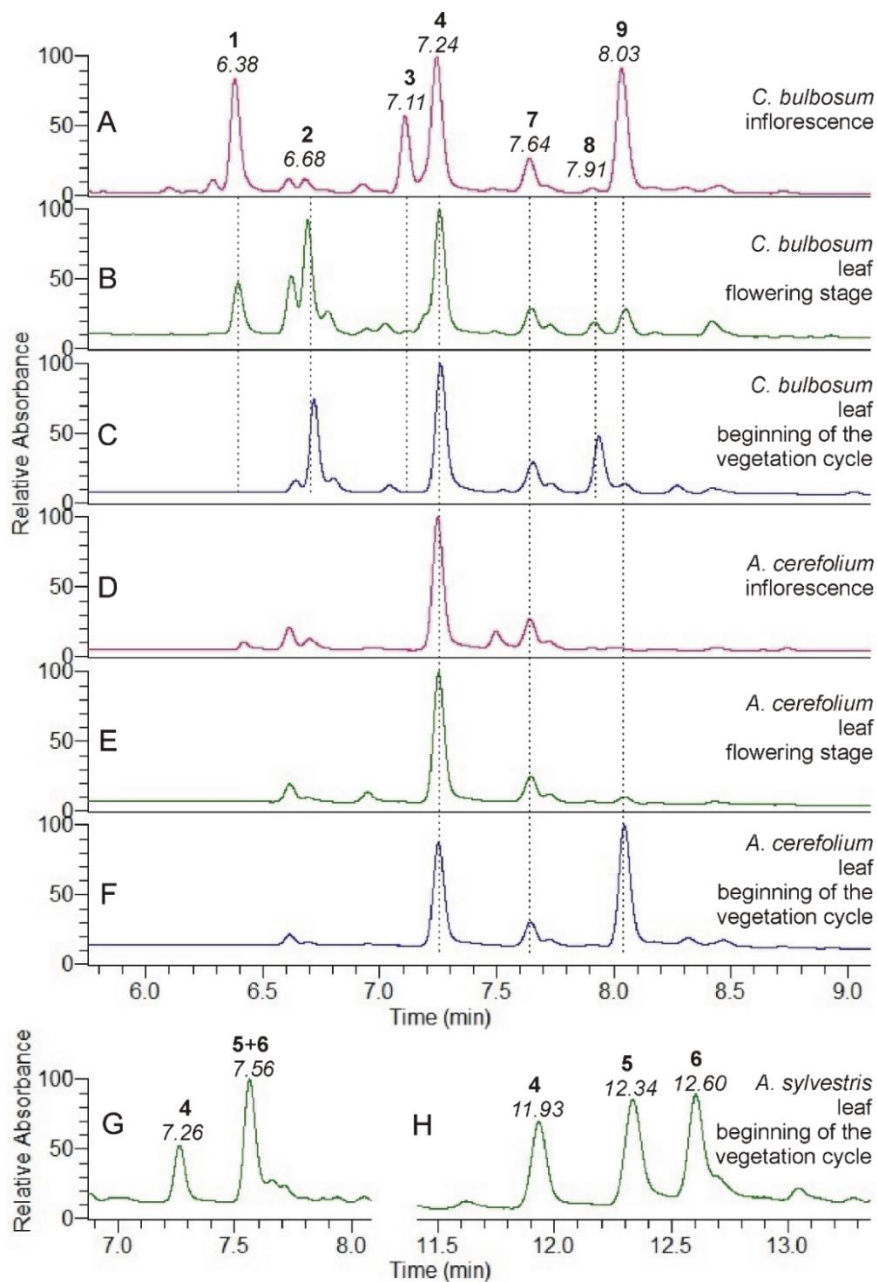


Figure 5a. HPLC-UV ($\lambda = 230\text{--}600$ nm, total scan) analysis of the *C. bulbosum* (A–C) and *A. cerefolium* (D–F) extracts using gradient program 1, while *A. sylvestris* (G, H) extract using both gradient program 1 (G) and gradient program 2 (H). Peak numbers (in bold) correspond to the numbering of the identified compounds, with retention times indicated in italics.

Source: (A–F) Author's own work; (G–H) reproduced with permission from Ausbüttel T (107).

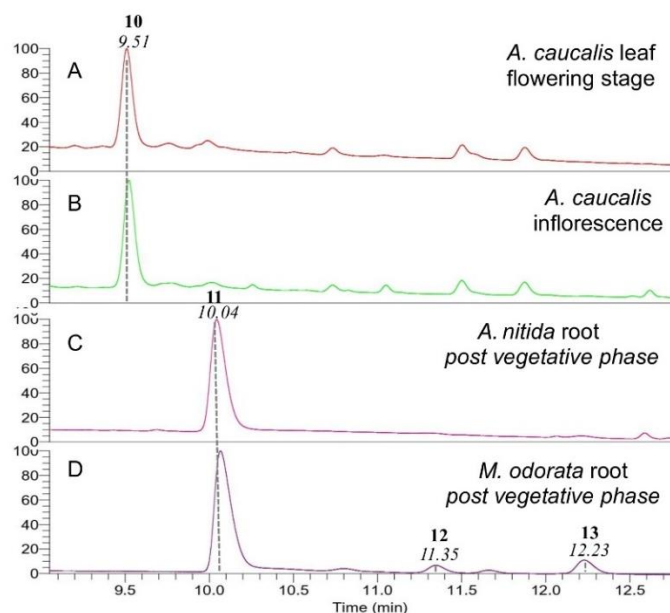


Figure 5b. HPLC-UV ($\lambda = 230\text{--}600$ nm, total scan) analysis of *A. caucalis* (A, B), *A. nitida* (C), and *M. odorata* (D) extracts. Peak numbers (in bold) correspond to the numbering of the identified compounds, with retention times indicated in italics.

Source: Author's own work.

During the HR-MS analysis of caffeoylquinic acid (CQAs) (**4–7**, **9**, **10**) and caffeoyl daucic acid (CDAs) (**11–13**) derivatives, additional fragments were observed alongside parent ion peaks when using a commonly used eluent composition (0.1% formic acid). In the case of MdiCQAs (**4–7**), along with the predominant deprotonated ion $[M-H]^-$ at m/z 601.12, additional ions at m/z 597.10 and m/z 599.09 were also detected. In the case of DiMdiCQAs (**9**, **10**), ions at m/z 685.10 and m/z 683.09 were more intense than the deprotonated ion $[M-H]^-$ at m/z 687.12. Similarly, the HR-MS spectrum of DiCDA (**11**) exhibited ions at m/z 523.05 and m/z 525.06, with higher intensity than the deprotonated ion $[M-H]^-$ at m/z 527.08. Both FCDA (**12**, **13**) also showed ions at m/z 539.08 and 537.08, corresponding to mass losses of 2 and 4 Da, respectively, relative to their deprotonated ions at m/z 541.09. The ions with 2 and 4 Da less than the deprotonated ones were also detected in positive ionization mode, demonstrating that their formation is independent of the polarity of the ionization mode.

Earlier HPLC-MS studies on catechol-containing molecules have revealed that oxidation in the ion source promotes the loss of two hydrogen atoms from the ortho-hydroxy groups of the catechol moiety, leading to the formation of 2 Da lighter ions than the non-oxidized species (108). Currently, our identified compounds contain one or two

catechol residues. Based on these literature findings and the presence of two catechol units in our compounds, we suggest that one or both catechol units undergo in-source oxidation during MS analysis. This process produces singly and doubly oxidized ions at m/z values 2 and 4 Da lower than those of their deprotonated counterparts, respectively. We hypothesized that the pH of the HPLC eluents played a role in the formation of oxidized analogs. Therefore, HPLC-MS analysis was repeated using a higher formic acid concentration (0.3%), which suppressed the formation of oxidized ions, resulting in better spectral clarity, with deprotonated ions being predominant.

4.1.2. Identification of metabolites in *Salvia* species

4.1.2.1. Identification of metabolites in methanolic extracts

The HPLC-UV analysis of crude extracts from selected *Salvia* species showed four major peaks, indicating the presence of main metabolites (Figure 6).

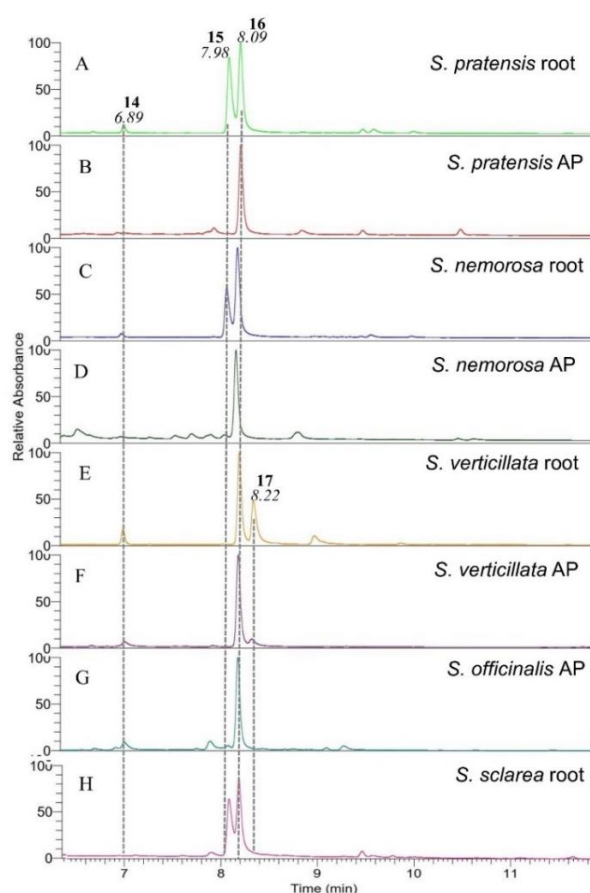


Figure 6. HPLC-UV ($\lambda = 230\text{--}600$ nm, total scan) analysis of *S. pratensis* (A, B), *S. nemorosa* (C, D), *S. verticillata* (E, F), *S. officinalis* (G), and *S. sclarea* (H). Peak numbers (in bold) correspond to the numbering of the identified compounds, with retention times indicated in italics.

Source: Author's own work.

Compound **16** exhibited two absorption bands at 265–300 nm (λ_{max} 290 nm), 310–360 nm (λ_{max} 328nm), and a molecular formula of $\text{C}_{18}\text{H}_{16}\text{O}_8$, consistent with rosmarinic acid (109). Rosmarinic acid was identified in all selected *Salvia* species. Based on the molecular formula of compound **14** ($\text{C}_{24}\text{H}_{26}\text{O}_{13}$), the additional $\text{C}_6\text{H}_{10}\text{O}_5$ subunit relative to rosmarinic acid indicates the presence of a glucosyl unit. This glycosylation induced a hypsochromic effect, with λ_{max} values at 285 nm and 318 nm (110). Rosmarinic acid glucoside was detected in *S. verticillata*, *S. pratensis*, *S. nemorosa*, and *S. officinalis*. The UV spectra of compounds **15** and **17** showed two absorption bands at 280–290 nm and 320–330 nm, and both compounds possess the same molecular formula $\text{C}_{27}\text{H}_{24}\text{O}_{13}$, suggesting the presence of salvianolic acid K (111) and its isomer. Compound **15** was present in *S. pratensis*, *S. sclarea*, and *S. nemorosa*, while compound **17** was detected in *S. verticillata*.

4.1.2.2. Identification of conversion products in aqueous-pretreated extracts

The powdered plant tissues were subjected to aqueous pretreatment for different time periods, such as 5, 15, 30, and 45 min, followed by methanolic extraction (Figure 7).

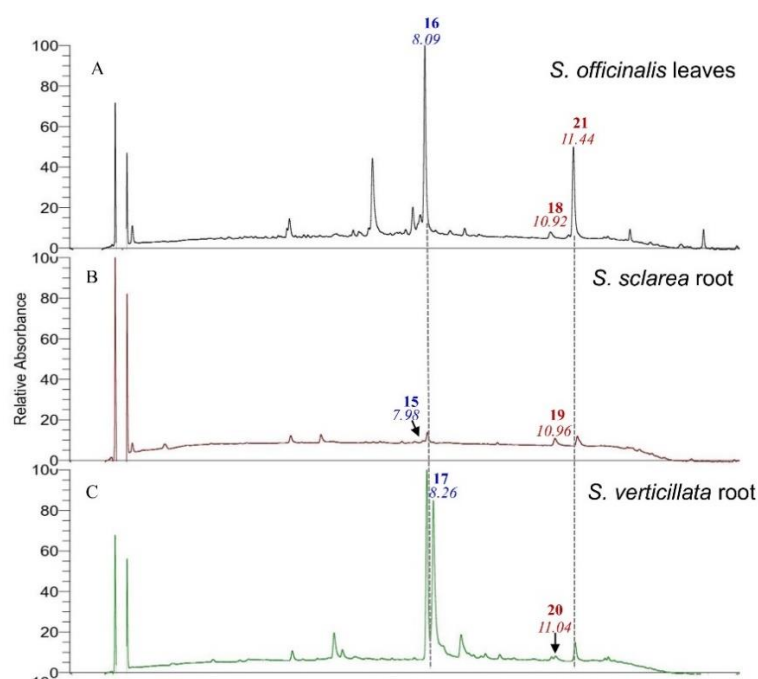
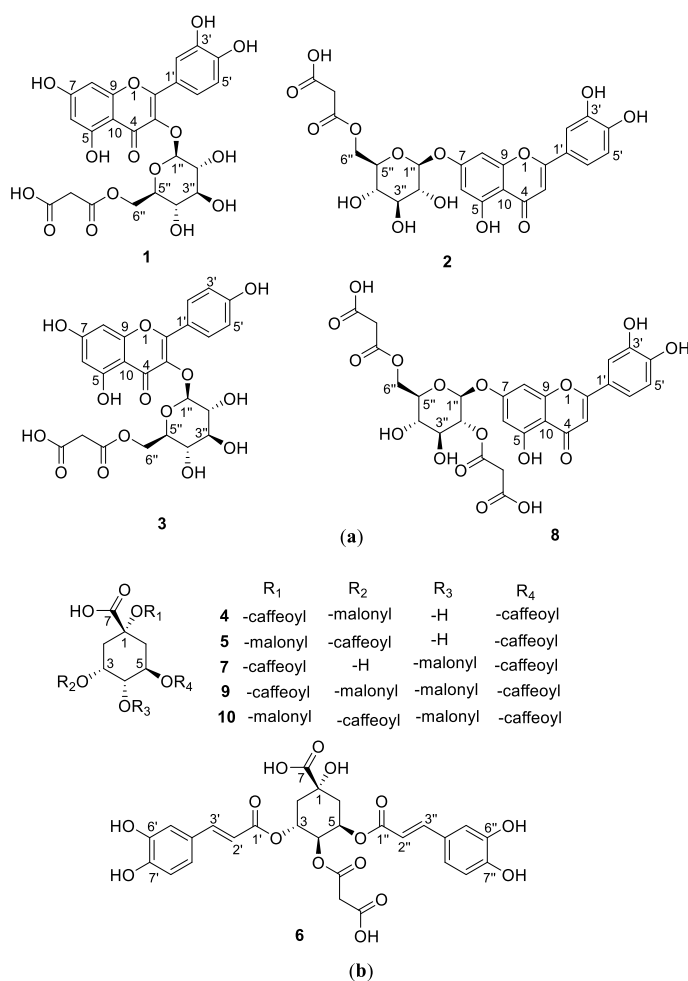


Figure 7. HPLC-UV ($\lambda = 230\text{--}600$ nm, total scan) analysis of aqueous-treated *S. officinalis* (A), *S. sclarea* (B), and *S. verticillata* (C) extracts. Blue peak numbers (in bold) correspond to the parent compound, and red peak numbers (in bold) refer to the conversion products, while the retention times are indicated in italics.

Source: Author's own work.

During these aqueous pretreatments, the pulverized tissues were suspended in distilled water and incubated at room temperature for the periods specified above. Upon aqueous treatment, all identified metabolites (**14–17**) decomposed. Parallel to the decomposition of these compounds, amounts of their corresponding conversion products increased. The conversion products (**18–21**) are formed by the theoretical loss of a formic acid moiety through oxidative decarboxylation involving the concomitant removal of C-8' carboxyl group and C-7' hydrogen from parent compounds (as detailed in section 4.3.3). Rosmarinic acid (**16**) was transformed into two isomeric products with molecular formula $C_{17}H_{14}O_6$, supporting their identification as nepetoidin A (**18**) and nepetoidin B (**21**), respectively (112). The transformation of rosmarinic acid glucoside (**14**) may involve loss of the glucose moiety, yielding rosmarinic acid. The conversion products (**19**, **20**), generated from the isomeric compounds **15** and **17**, showed a similar molecular formula $C_{26}H_{22}O_{11}$, which were named as salvianolic acid P (**19**) and salvianolic acid Q (**20**), representing the novel compounds (Figure 8).



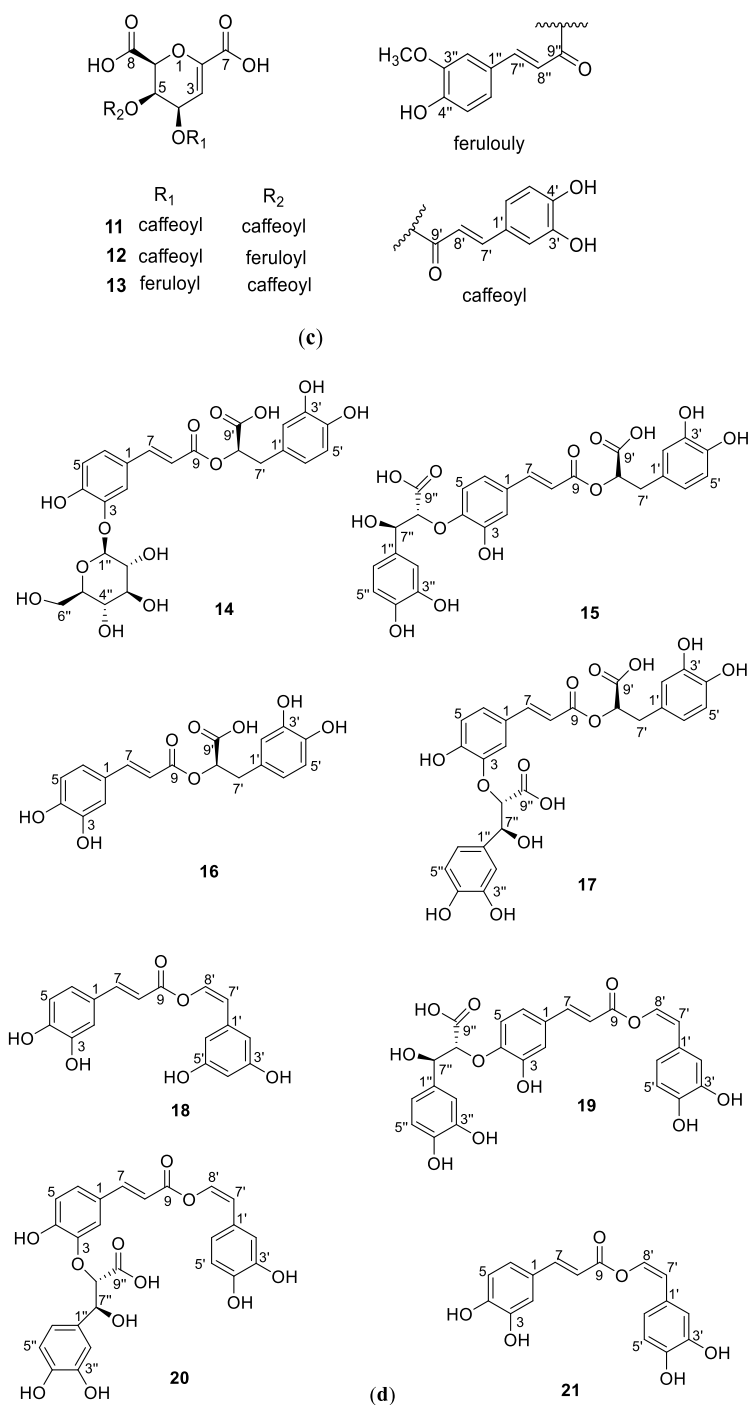


Figure 8. Chemical structures of identified compounds (a) flavonoid malonyl-glucosides (1–3, 8), (b) malonyl dicaffeoylquinic acids (4–7, 9, 10), (c) malonyl caffeoyl-daucic acids (11–13), (d) caffeoyl-dihydroxyphenyllactic acids (14–17), and conversion products (18–21).

Source: Author's own work.

4.2. Mass fragmentation study of metabolites by HPLC-HR-MS/MS

The MS fragmentation profiles of identified compounds were analyzed in negative ionization mode at different CID energies (Table 2–5).

4.2.1. MS fragmentation study of malonyl dicaffeoylquinic acids (4–7, 9, 10)

Fragment ion profiles of MDiCQAs 4–7, generated from their deprotonated ions at m/z 601, showed identical fragment ions, thus confirming their isomeric structures consisting of identical structural units (Table 2).

Table 2. Relative abundance of characteristic ions in the Tandem Mass Spectra of isomeric malonyl dicaffeoylquinic acids (4–7).

No.	CID (eV)	Relative abundances (%) of characteristic ions (CIs)													
	CIs→	[M-H] ⁻	[M-H-CO ₂] ⁻	[M-H-MA] ⁻	[M-H-MA-H ₂ O] ⁻	[M-H-CA] ⁻	[M-H-CA-CO ₂] ⁻	[M-H-CA-CO ₂ -H ₂ O] ⁻	[M-H-CA-MA] ⁻	[M-H-CA-MA-H ₂ O] ⁻	[M-H-2×CA-CO ₂] ⁻	[QA-H] ⁻	[CA-A-H] ⁻	[QA-H-H ₂ O] ⁻	[CA-A-H-H ₂ O] ⁻
4	m/z→	601.12	557.13	515.12	497.11	439.09	395.10	377.09	353.09	335.08	233.07	191.06	179.03	173.04	161.02
	15	13.9	9.5	6.6	0.13	15.5	28.4	2.3	1.4	0.38	14.7	0.41	054	0.11	0.45
	25	-	0.30	0.74	-	0.98	11.1	1.0	3.9	1.2	65.6	1.4	1.9	0.70	1.6
	30	-	-	-	-	-	2.4	0.59	2.9	1.2	68.0	3.5	2.7	1.6	2.2
	35	-	-	-	-	-	0.41	0.28	1.3	1.1	60.9	4.9	3.3	3.3	3.0
	45	-	-	-	-	-	-	-	0.13	0.44	30.4	7.7	4.4	8.0	4.4
7	15	14.5	6.8	3.9	-	8.7	35.5	2.5	0.87	0.17	18.8	0.18	0.80	0.29	0.47
	25	0.10	-	0.51	-	0.59	10.0	0.79	2.2	0.43	69.0	0.92	2.3	2.8	1.5
	30	-	-	-	-	0.13	2.5	0.40	1.8	0.58	68.0	1.9	3.4	6.8	2.0
	35	-	-	-	-	-	0.44	0.23	0.77	0.55	59.1	3.5	4.1	15.2	2.3
	45	-	-	-	-	-	-	-	-	0.13	28.0	4.3	3.9	38.7	2.8
	5	15	2.0	20.2	9.7	2.5	0.23	46.4	0.48	5.1	1.4	0.086	0.061	2.8	0.11
25		-	0.26	0.56	1.2	-	35.2	1.1	12.8	11.1	1.4	0.85	18.5	0.61	1.1
30		-	-	-	0.33	-	11.7	0.62	9.0	12.4	12.1	2.3	28.9	2.1	2.1
35		-	-	-	-	-	1.8	0.23	3.4	5.6	5.4	2.8	24.6	2.3	1.6
45		-	-	-	-	-	-	0.12	0.93	3.4	1.5	6.9	42.6	4.3	5.4
6		15	2.8	4.1	5.8	-	12.1	58.7	0.57	7.5	0.29	5.2	0.19	0.67	0.33
	25	-	-	0.46	-	0.71	32.3	0.10	15.2	1.2	36.4	2.1	2.8	3.3	0.71
	30	-	-	-	-	-	11.2	-	11.2	1.2	48.5	5.5	5.8	9.7	1.1
	35	-	-	-	-	-	2.7	-	6.5	1.1	40.9	10.0	10.1	18.7	1.9
	45	-	-	-	-	-	-	-	0.77	0.52	14.5	13.6	12.7	39.0	2.9

Abbreviations: CID; collision-induced dissociation, CIs; characteristic ions, CA; caffeoyl group, MA; malonyl group, CA.A; caffeic acid, QA; quinic acid.

Note: The values in bold provide the main basis for isomer differentiation.

These compounds formed fragment ions at m/z 515, m/z 439, and m/z 353, corresponding to the deprotonated dicaffeoylquinic acid, malonyl-caffeoylquinic acid, and caffeoylquinic acid subunits, respectively.

The MS/MS spectra of DiMDiCQAs **9** and **10** generated from their identical deprotonated ions at m/z 687 showed a fragment ion at m/z 601 formed by the loss of a malonyl group (Table 3). All MDiCQAs (**4–7**) and DiMDiCQAs (**9, 10**) showed further fragment ions at m/z 557, m/z 439, m/z 395, m/z 377, and m/z 233. In MDiCQAs (**4–7**), these fragment ions were formed by the loss of CO₂, a caffeoyl group, a caffeoyl group + CO₂, a caffeoyl group + CO₂ + H₂O, and two caffeoyl groups + CO₂, respectively. In DiMDiCQAs (**9, 10**), there was an initial loss of a malonyl group, followed by the subsequent elimination of similar moieties as described for MDiCQAs (**4–7**). Furthermore, all MDiCQAs and DiMDiCQAs showed fragment ions at m/z 191 and m/z 179 corresponding to deprotonated quinic acid and caffeic acid units, respectively.

Table 3. Relative abundance of characteristic ions in the Tandem Mass Spectra of isomeric dimalonyl-dicaffeoylquinic acids (**9, 10**).

No.	CID (eV)	Relative abundances (%) of characteristic ions (CIs)																	
		CIs→																	
		[M-H] ⁻	[M-H-CO ₂] ⁻	[M-H-MA] ⁻	[M-H-MA-CO ₂] ⁻	[M-H-CA-CO ₂] ⁻	[M-H-CA-MA] ⁻	[M-H-CA-2×CO ₂] ⁻	[M-H-CA-MA-CO ₂] ⁻	[M-H-CA-MA-CO ₂ -H ₂ O] ⁻	[M-H-2×CA-2×CO ₂] ⁻	[M-H-2×CA-MA-CO ₂] ⁻	[M-H-2×CA-MA-CO ₂ -H ₂ O] ⁻	[QA-H] ⁻	[CA-A-H] ⁻	[QA-H-H ₂ O] ⁻	[CA-A-H-H ₂ O] ⁻		
		<i>m/z</i> →	687.12	643.13	601.12	557.13	481.10	439.09	437.11	395.10	377.09	275.08	233.07	215.06	191.06	179.03	173.04	161.02	
9	15		18.3	3.7	9.5	4.4	6.1	0.80	19.7	2.7	-	21.1	0.35	-	-	0.25	-	0.48	
	30		-	-	-	-	0.22	0.26	0.84	4.8	0.33	67.3	13.3	1.2	-	2.5	0.19	1.4	
	45		-	-	-	-	-	-	-	-	-	35.8	16.8	9.4	0.32	4.3	3.9	2.2	
10	15		2.6	6.7	5.7	3.6	3.6	0.86	45.4	3.3	1.4	0.5	-	-	-	0.87	-	0.17	
			6																
	30		-	-	-	-	-	0.30	23.4	11.7	15.8	2.0	4.1	1.1	0.1	20.9	0.38	2.6	
			0																
	45		-	-	-	-	-	-	0.27	1.3	2.1	1.0	9.3	1.3	1.1	36.1	4.0	7.2	

Abbreviations: CID; collision-induced dissociation, CIs; characteristic ions, CA; caffeoyl group, MA; malonyl group, CA.A; caffeic acid, QA; quinic acid.

Note: The values in bold provide the main basis for isomer differentiation.

4.2.2. MS fragmentation study of caffeoyl daucic acids (**11–13**)

The MS/MS spectra of the isomeric compounds **12** and **13**, obtained from their deprotonated ions at m/z 541, exhibited fragments at m/z 379 and m/z 365, corresponding to the removal of caffeoyl and feruloyl groups, respectively (Table 4).

Table 4. Relative abundance of characteristic ions in the Tandem Mass Spectra of isomeric feruloyl-caffeoyl daucic acids (**12**, **13**).

No. ^a	CID ^b (eV)	Relative abundances (%) of characteristic ions (CIs)										
CI→		[M-H] ⁻	[M-H-CA] ⁻	[M-H-FE] ⁻	[DA-H] ⁻	[FE-A-H] ⁻	[DA-H-H ₂ O]	[CA-A-H] ⁻	[FE-A-H-CO ₂] ⁻	[DA-H-H ₂ O-CO ₂] ⁻	[CA-A-H-CO ₂] ⁻	
<i>m/z</i> →		541.09	379.06	365.05	203.01	193.04	185.00	179.03	149.05	141.01	135.04	
12	10	14.9	46.2	16.5	1.9	3.9	1.4	1.8	-	1.07	-	
	15	2.5	52.3	14.7	9.3	10.1	5.8	3.3	-	3.0	-	
	30	-	2.2	-	19.6	27.5	8.2	4.6	0.78	12.1	1.2	
	45	-	-	-	9.2	18.4	0.73	3.0	2.3	5.4	3.9	
13	10	6.3	83	-	-	0.07	4.3	0.22	-	0.73	-	
	15	0.51	68.7	-	0.46	0.51	15.5	-	-	1.5	-	
	30	-	3.2	-	1.9	3.0	29.6	0.49	0.22	6.7	-	
	45	-	-	-	0.70	1.9	2.7	0.63	0.06	2.9	1.2	

Abbreviations: CID; collision-induced dissociation, CIs; characteristic ions, CA; caffeoyl group, MA; malonyl group, CA.A; caffeic acid, QA; quinic acid, FE; feruloyl group, FE.A; ferulic acid.

Note: The values in bold provide the main basis for isomer differentiation.

Further fragments at *m/z* 203, *m/z* 193, and *m/z* 179 correspond to deprotonated daucic acid, ferulic acid, and caffeic acid units, respectively. Moreover, the fragments at *m/z* 185 and *m/z* 141 refer to the loss of H₂O, followed by CO₂ from daucic acid, respectively. Fragment ions at *m/z* 149 and *m/z* 135 were generated by the removal of CO₂ from ferulic acid and from caffeic acid, respectively. Compound **11** (*m/z* 527) generated a fragment ion at *m/z* 365 by the elimination of a caffeoyl group. Other fragment ions at *m/z* 203 and *m/z* 179, *m/z* 185, and *m/z* 141 and *m/z* 135 correspond to the same rationale as described for compounds **12** and **13**.

4.2.3. MS fragmentation study of caffeoyl-dihydroxyphenyllactic acids (**14–17**) and conversion products (**18–21**)

The MS/MS spectra of nepetoidins A (**18**) and B (**21**), obtained from the precursor ion at *m/z* 313, displayed identical fragmentation patterns. The characteristic product ions included a dehydrated caffeoyl moiety at *m/z* 161, a deprotonated hydroxyvinyl catechol moiety at *m/z* 151, and its dehydrated fragment at *m/z* 133. The four structurally related trimeric phenylpropanoids, salvianolic acids K (**15**), O (**17**), P (**19**), and Q (**20**), exhibited highly similar MS/MS fragmentation pathways. The initial fragmentation step involved the sequential loss of H₂O and CO₂ from the common 3-(3,4-dihydroxyphenyl)-2,3-dihydroxypropanoic acid moiety, generating product ions containing a vinylcatechol unit.

These product ions were detected at m/z 493 for salvianolic acids K and O (Table 5) and at m/z 447.11 for salvianolic acids P and Q. Subsequent cleavage of the vinylcatechol unit produced the characteristic ions at m/z 359 and m/z 313, respectively. The MS/MS spectra of rosmarinic acid (**16**) and its glucoside derivative (**14**), generated from their deprotonated ions at m/z 359 and m/z 521, respectively, showed fragment ions at m/z 197 and m/z 179 corresponding to the dihydroxyphenyllactic acid and caffeic acid, respectively.

Table 5. Relative abundance of characteristic ions in the Tandem Mass Spectra of isomeric salvianolic acids (**15**, **17**).

No.	CID (eV)	Relative abundances (%)c of characteristic ions (CIs)											
CIs→		[M-H] ⁻	[M-H-H ₂ O] ⁻	[M-H-H ₂ O-CO ₂] ⁻	[M-H-CO ₂ -3,4-DHB] ⁻	[RA-H] ⁻	[NP-H] ⁻	[NP-H-H ₂ O] ⁻	[3,4-DPA-H] ⁻	[CA-A-H] ⁻	[CA-A-H-H ₂ O] ⁻	[CA-A-H-CO ₂] ⁻	
m/z→		555.11	537.10	493.11	401.087	359.07	313.07	295.06	197.04	179.03	161.02	135.04	
15	10	37.3	4.1	75.9	13.7	100	0.9	0.8	1.2	1.9	4.2	2.4	
	12	12.7	2.8	41.6	10.1	100	1.5	1.1	1.4	2.7	5.1	2.8	
	15	2.5	1.8	17.6	8	100	1.8	2.4	2.5	4.4	9.3	5.0	
	30	-	-	-	-	13.2	0.6	9.3	32.5	17.7	100	55.1	
	45	-	-	-	-	-	-	1	20.1	23.4	92.4	100	
17	10	28.1	0.2	100	1.9	12.9	1.5	1.4	0.3	0.7	1.2	0.6	
	12	13.6	0.3	100	2.3	23.2	2.7	2.7	0.4	1.5	2	0.9	
	15	4.4	-	100	3.4	58.7	7.3	10.3	1.6	5.5	6.6	2.5	
	30	-	-	-	-	18.9	3.6	65.2	33	26.4	100	83.6	
	45	-	-	-	-	-	-	4.9	14.5	22.2	57.3	100	

Abbreviations: CID; collision-induced dissociation, CIs; characteristic ions, 3,4-DHB; 3,4-dihydroxy benzene, RA; rosmarinic acid, NP; nepetoidin A/B, 3,4-DPA; 3,4-dihydroxyphenyllactic acid, CA.A; caffeic acid.

Note: The values in bold provide the main basis for isomer differentiation.

4.2.4. MS fragmentation study of flavonoid-malonyl-glucosides (1–3, 8)

FMGLs **1** (m/z 549), **2**, **3** (both at m/z 533), and **8** (m/z 619) exhibited fragment ions at m/z 463, m/z 447, and m/z 533, respectively, which were generated after the loss of one malonyl group from each deprotonated flavonoid. Moreover, compound **1** showed a fragment ion at m/z 301 corresponding to quercetin, while compounds **2**, **3**, and **8** exhibited the ion at m/z 285, suggesting the presence of either luteolin or kaempferol as an aglycone.

4.3. Structural characterization of isolated compounds through NMR

The structural elucidation of isolated compounds (**1–18**, **19**, **21**) was based on 1D (^1H , ^{13}C) and 2D (^1H – ^1H COSY, ^1H – ^1H ROESY, ^1H – ^1H NOESY, ^1H – ^{13}C HSQC, and ^1H – ^{13}C HMBC) NMR experiments (Tables 6–11, Figure 8).

4.3.1. Structural characterization of malonyl dicaffeoylquinic acids (**4–7**, **9**, **10**)

The ^1H NMR spectrum of compound **4** showed six aromatic proton signals δ_{H} 7.07 (1H, d, $J = 2.0$ Hz, H-5'), 7.06 (1H, d, $J = 2.0$ Hz, H-5''), 7.03 (1H, dd, $J = 8.2, 2.0$ Hz, H-9'), 7.01 (1H, dd, $J = 8.2, 2.0$ Hz, H-9''), 6.78 (1H, dd, $J = 8.2$ Hz, H-8''), 6.77 (1H, d, $J = 8.2$ Hz, H-8') which appeared as two ABX systems (Table 6). Moreover, four trans-oriented protons δ_{H} 7.50 (1H, d, $J = 15.9$ Hz, H-3''), 7.49 (1H, d, $J = 15.9$ Hz, H-3'), 6.28 (1H, d, $J = 15.9$ Hz, H-2''), 6.23 (1H, d, $J = 15.9$ Hz, H-2') formed two vinylene groups. HMBC correlations H-3'/C-1', H-3'/C-4', H-3'/C-5', H-3'/C-9' and H-3''/C-1'', H-3''/C-4'', H-3''/C-5'', H-3''/C-9'' confirmed these subunits form two caffeoyl groups. A pair of sp^3 methylene protons δ_{H} 3.29 (1H, d, $J = 15.9$ Hz) and 3.19 (1H, m (ov)) was correlated with two carbonyl carbons at δ_{C} 166.6 (RCOOR') and 167.8 (RCOOH), thus forming a malonyl moiety. Four sp^3 methylene protons δ_{H} 2.44 (1H, m (ov), H-6), 2.41 (2H, m (ov), H-2), 1.95 (1H, dd, $J = 13.1, 9.9$ Hz, H-6) and three oxymethine protons δ_{H} 5.32 (1H, q, $J = 4.1$ Hz, H-3), 5.19 (1H, td, $J = 9.1, 4.1$ Hz, H-5), 3.87 (1H, dd, $J = 8.8, 3.4$ Hz, H-4) were considered a part of 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid. The protons were coupled as $\text{CH}_2\text{-CH-CH-CH-CH}_2$, while HMBC H-6/COOH correlation indicated that the carboxylic group is attached at position-1 of the cyclohexane ring.

The H-4 was considered a diagnostic proton to determine the relative configuration of 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid ring, which showed a large trans-diaxial coupling constant $^3J_{4/5} = 8.8$ Hz, indicating that H-4 holds an axial position. This finding is consistent with earlier studies demonstrating that dicaffeoylquinic acids adopt a chair-like conformation in DMSO- d_6 with the equilibrium slightly shifted towards the conformer holding H-4 and H-5 in axial positions (113–115). Consequently, δ_{H} 5.32 (q, $J = 4.1$ Hz) and 5.19 (td, $J = 9.1, 4.1$ Hz) were attributed to H-3 (equatorial) and H-5 (axial), respectively. The ^1H NMR spectrum of compound **4** in D_2O exhibited a higher *trans*-diaxial coupling constant $^3J_{4/5} = 10.1$ Hz, thus indicating that equilibrium is markedly shifted towards the conformer holding H-4 and H-5 in axial positions (Figure 9), as

previously described (115, 116). These findings indicate that 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid represents a quinic acid moiety.

Table 6. ^1H NMR and ^{13}C data (δ in ppm, J in Hz) of the malonyl-dicaffeoylquinic acids (4–7) recorded in DMSO- d_6 at 500/125 MHz.

Position	4		5		6		7	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	-	78.7	-	78.8**	-	72.3	-	78.9
2	2.41, m (ov)	31.7	2.42, m (ov)	31.6	2.21, m 2.00, m	35.1	2.39, m (ov) 2.31, m	33.7***
3	5.32, q (4.1)	71.7	5.34, q (3.8)	70.7	5.32, br s	67.3	4.27, q (3.4)	64.8***
4	3.87, dd (8.8, 3.4)	68.7	3.87, dd (8.3, 2.5)	68.7	5.15, dd (7.3, 3.3)	70.3	4.93, dd (9.1, 3.4)	74.4***
5	5.19, td (9.1, 4.1)	69.8	5.22, td (8.6, 3.9)	69.8	5.39, dt (7.2, 3.7)	67.8	5.46, td (9.3, 4.3)	66.3***
6	2.44, m (ov) 1.95, dd (13.1, 9.9)	35.6	2.38, m (ov) 1.95, br t (11.1)	35.6	2.28, dd (13.2, 3.2) 1.95, dd (13.5, 7.6)	35.1	2.40, m (ov) 2.02, dd (13.0, 10.5)	35.9***
7	-	172.0	-	171.6	-	175.2	-	n.d.
	1-caffeoyl		3-caffeoyl		3-caffeoyl		1-caffeoyl	
1'	-	165.2	-	166.1	-	165.3	-	165.2
2'	6.28, d (15.9)	113.8	6.29, d (15.9)	114.3	6.15, d (15.8)	113.5	6.22, d (15.8)	114.2
3'	7.49, d (15.9)	146.0	7.47, d (15.9)	145.3/ 145.2	7.45, d (15.8)	145.64	7.47, d (15.8)	145.62/ 145.58
4'	-	125.4	-	125.6/ 125.5	-	125.47/ 125.53	-	125.4
5'	7.07, d (2.0)	115.1	7.06, d (2.0)/ 7.05, d (2.0)	115.0/ 114.9	7.06, d (1.9)/ 7.04, d (1.9)	114.9	7.05, m	115.0/ 114.9
6'	-	145.6	-	145.55/ 145.58	-	145.55	-	145.62/ 145.58
7'	-	148.6	-	148.4/ 148.3	-	148.4/ 148.5	-	148.50/ 148.48
8'	6.77, d (8.2)	115.8	6.77, d (8.1)/ 6.76, d (8.1)	115.8	6.77, d (8.1)/ 6.76, d (8.1)	115.74/ 115.77	6.78, d (8.0)/ 6.76, d (8.0)	115.8
9'	7.03, dd (8.2, 2.0)	121.4	7.02, d (2.0)/ 7.00, d (2.0)	121.4/ 121.3	7.00, dd (8.1, 1.9)	121.5	7.00, m (ov)	121.2
	5-caffeoyl		5-caffeoyl		5-caffeoyl		5-caffeoyl	
1''	-	166.0	-	165.8	-	165.9	-	165.8
2''	6.23, d (15.9)	113.9	6.22, d (15.9)	114.0	6.20, d (15.8)	114.0	6.18, d (15.8)	113.6
3''	7.50, d (15.9)	145.4	7.49, d (15.9)	145.3/ 145.2	7.49, d (15.8)	145.4	7.46, d (15.8)	145.62/ 145.58
4''	-	125.5	-	125.6/ 125.5	-	125.47/ 125.53	-	125.4
5''	7.06, d (2.0)	114.9	7.06, d (2.0)/ 7.05, d (2.0)	115.0/ 114.9	7.06, d (1.9)/ 7.04, d (1.9)	114.9	7.05, m	115.0/ 114.9
6''	-	145.6	-	145.55/ 145.58	-	145.55	-	145.62/ 145.58
7''	-	148.5	-	148.4/ 148.3	-	148.4/ 148.5	-	148.50/ 148.48
8''	6.78, dd (8.2)	115.8	6.77, d (8.1)/ 6.76, d (8.1)	115.8	6.77, d (8.1)/ 6.76, d (8.1)	115.74/ 115.77	6.78, d (8.0)/ 6.76, d (8.0)	115.8
9''	7.01, dd (8.2, 2.0)	121.4	7.02, d (2.09)/ 7.00, d (2.0)	121.4/ 121.3	7.00, dd (8.1, 1.9)	121.3	7.00, m (ov)	121.2
	3-malonyl		1-malonyl		4-malonyl		4-malonyl	
CH ₂	3.29, d (15.9) 3.19, m (ov)*	41.8	3.35, d (15.9)	41.9	3.36, s	41.6	3.31, d (15.7) 3.25, d (15.7)	42.6
RCOOR'	-	166.6	-	166.2	-	166.2	-	167.1
RCOOH	-	167.8	-	167.3	-	167.7	-	168.2

Note: *the signal overlaps with the signal of residual methanol; **signal deduced from the HMBC spectrum; *** chemical shifts deduced from HSQC and HMBC spectra; n.d., not detected.

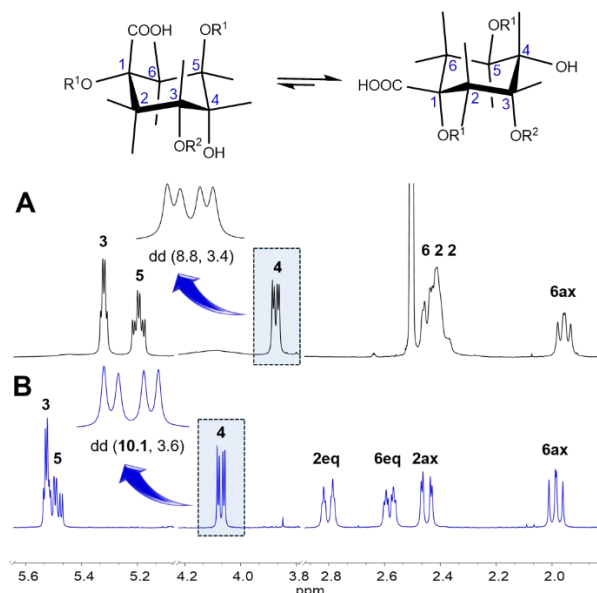


Figure 9. Comparison of spin-spin coupling constants for diagnostic proton (H-4) of compound **4** in DMSO- d_6 (A) and D $_2$ O (B), highlighting the solvent-driven conformational preferences of quinic acid (R_1 = caffeoyl, R_2 = malonyl).

Source: Author's own work.

The substitution positions of quinic acid core were confirmed based on the HMBC correlations. The H-3 was coupled with carbonyl carbon δ_C 166.6 (RCOOR'), indicating the substitution of malonyl group at position 3. Moreover, H-5 showed a correlation with a carbonyl carbon δ_C 166.0, confirming the attachment of caffeoyl group at position 5. The other caffeoyl unit was assigned at position 1 due to the absence of its carbonyl carbon δ_C 165.2 correlation with any quinic acid proton. Thus, compound **4** was identified as 1,5-dicaffeoyl-3-malonylquinic acid (Figure 8).

The NMR spectra of compound **7** displayed the resonances corresponding to a quinic acid moiety, one malonyl, and two caffeoyl groups. Contrary to compound **4**, in this case, H-3 was upfield-shifted, while downfield-shifted H-4 was coupled with carbonyl carbon δ_C 167.1 of malonyl subunit. Caffeoyl groups were substituted at the same positions as noticed in compound **4**, thus leading to identification of compound **7** as 1,5-dicaffeoyl-4-malonylquinic acid (Figure 8).

The NMR analysis of compound **5** indicated the presence of two caffeoyl, one malonyl, and a quinic acid unit. The shift patterns of oxymethine protons were quite similar to those noticed in the case of compound **4**, i.e., H-4 shifted upfield while H-3 and H-5 shifted downfield. In this case, H-3 and H-5 were correlated with carbonyl carbons δ_C 166.1 and 165.8 of caffeoyl groups, respectively. The malonyl group was

assigned at position 1 based on the absence of carbonyl carbon δ_C 166.2 correlation with any oxymethine proton. Thus, compound **5** was identified as 3,5-dicaffeoyl-1-malonylquinic acid (Figure 8).

Compound **6** also possessed two caffeoyl and one malonyl group; however, signals corresponding to the 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid showed variations in multiplicity patterns and spin-spin coupling constants compared to compound **4**, indicating a different relative configuration of this core structure. The diagnostic H-4 showed a relatively lower coupling constant value ^3J (7.3 Hz), suggesting the presence of an *epi*-quinic moiety (117-119), which adopts a chair-like conformation in DMSO- d_6 (115) (Figure 10). Based on the coupling constants calculated for both conformers of 3,5-dicaffeoyl-*epi*-quinic acid (115), compound **6** appears to adopt two major chair conformers in approximately 1:1 ratio. This equilibrium supports the multiplicity patterns observed for H-3 and H-5 protons, δ_H 5.32 (1H, br s) and 5.39 (1H, dt, $J = 7.2, 3.7$ Hz), respectively. The ^1H NMR spectrum of compound **6** in D $_2$ O exhibited a markedly increased $^3J_{3/4}$ value (8.9 Hz) and altered multiplicity patterns for H-3 (axial) and H-5 (equatorial) protons, δ_H 5.66 (1H, m (ov)) and 5.69 (1H, q, $J = 3.7$ Hz). This indicates the equilibrium shift towards the conformer having both H-3 and H-4 in axial positions. Afterwards, the substituent positions of *epi*-quinic acid were assigned based on HMBC correlations.

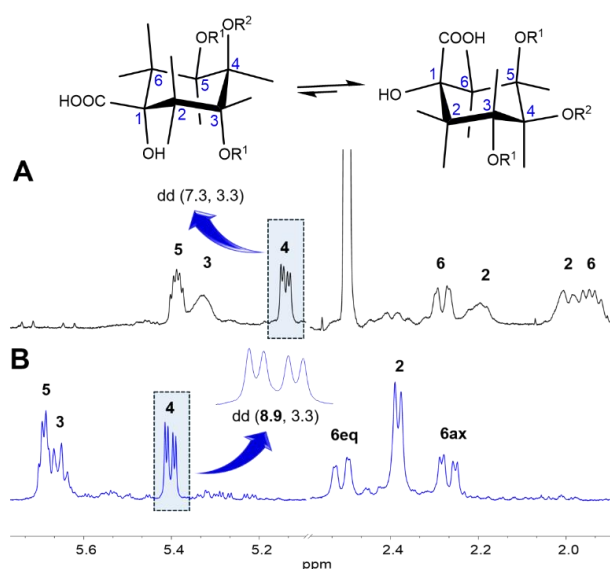


Figure 10. Comparison of spin-spin coupling constants for diagnostic proton (H-4) of compound **6** in DMSO- d_6 (A) and D $_2$ O (B), highlighting the solvent-driven conformational preferences of *epi*-quinic acid (R_1 = caffeoyl, R_2 = malonyl).

Source: Author's own work.

The H-3 and H-5 protons were coupled with carbonyl carbons δ_C 165.3 and δ_C 165.9 of caffeoyl groups. Moreover, H-4 was correlated with carbonyl carbon δ_C 166.2 of malonyl subunit. Thus, compound **6** was identified as 3,5-dicaffeoyl-4-malonyl-*epi*-quinic acid (Figure 8). The NMR spectra of compounds **9** and **10** showed similar structural components to compound **4**, along with an additional malonyl subunit (Table 7). Based on HMBC correlations, compounds **9** and **10** were identified as 1,5-dicaffeoyl-3,4-dimalonylquinic acid and 3,5-dicaffeoyl-1,4-dimalonylquinic acid, respectively (Figure 8). All the currently identified CQAs represent novel natural products.

Table 7. ^1H NMR and ^{13}C data (δ in ppm, J in Hz) of the dimalonyl-dicaffeoylquinic acids (**9**, **10**) recorded in DMSO- d_6 at 500/125 MHz.

Position	9		10	
	δ_H	δ_C	δ_H	δ_C
1	-	78.2		n.d
2	2.53, m (ov)	31.4	2.54, m (ov)	31.7
3	5.47, q (3.8)	68.5	5.53, q (4.0)	67.6
4	5.25, dd (9.6, 3.8)	71.2	5.25, dd (9.5, 3.6)	71.6
5	5.41, td (9.8, 4.3)	66.5	5.42, td (4.2, 9.6)	66.4
6	2.52, m (ov) 2.12, dd (13.3, 10.8)	35.8	2.47, m (ov) 2.13, d (12.2)	*35.8
7	-	171.5	-	171.00
8	-	-	-	-
1-caffeoyl			3-caffeoyl	
1'	-	165.2		165.90
2'	6.32, d (15.8)	113.6	6.32, d (15.8)	113.56
3'	7.51, d (15.8)	146.2	7.48, d (15.8)	145.73
4'	-	125.43/125.36		125.46
5'	7.09, d (1.9)	115.2	7.08, d (2.0)	115.25
6'	-	145.6		145.49
7'	-	148.7/148.6		148.43
8'	6.78, d (8.1)/ 6.76, d (8.1)	115.81/115.76	6.77, d (8.1)	115.71
9'	7.05, m (ov)	121.5	7.06, dd (8.2, 1.9)	121.33
OCH₃	-	-	-	-
5-caffeoyl			5-caffeoyl	
1''	-	165.7		165.47
2''	6.20, d (15.8)	113.3	6.18, d (15.9)	113.36
3''	7.47, d (15.8)	145.9	7.45, d (15.8)	145.69
4''	-	125.43/125.36		125.41
5''	7.04, d (1.9)	115.0	7.04, d (2.1)	114.9
6''	-	145.6		145.52
7''	-	148.7/148.6		148.48
8''	6.78, d (8.1)/ 6.76, d (8.1)	115.81/115.76	6.76, d (8.1)	115.71
9''	7.00, dd (8.1, 1.9)	121.6	6.99, dd (8.2, 2.1)	121.45
OCH₃	-	-	-	-
3-malonyl			1-malonyl	
CH₂	3.28, m (ov) 3.15, m (ov)	41.6	3.26, m (ov) 3.40, m (ov)	41.3
RCOOR'	-	166.4		166.19
RCOOH	-	167.6		167.39
4-malonyl			4-malonyl	
CH₂	3.28, s	41.5	3.31, m (ov)	41.3
RCOOR''	-	166.3		166.04
RCOOH	-	167.7		167.16

* Signals identified from HSQC and HMBC spectra.

4.3.2. Structural characterization of caffeoyldaucic acids (11–13)

The NMR spectra of compound **11** confirmed the presence of two caffeoyl groups; however, it possessed a different core structure (Table 8). This structural unit was based on four protons, i.e., δ_{H} 5.98–5.99 (3H, br s (ov), H-3–H-5), and 5.10 (1H, br s, H-6), which were attached to oxygenated sp^3 methine carbons δ_{C} 66.4 (C-4), 65.2 (C-5), 76.4 (C-6), and a sp^2 methine carbon δ_{C} 108.7 (C-3). These protons showed HMBC correlations with hydroxyl carbonyl carbons δ_{C} 164.8 (C-7), 170.4 (C-8), and a non-protonated carbon δ_{C} 146.21 (C-2). These resonance values and coupling patterns support the presence of daucic acid moiety (28). Moreover, H-4 and H-5 protons were coupled with carbonyl carbons δ_{C} 167.77 and 167.84 of caffeoyl groups, respectively. Hence, compound **11** was identified as 4,5-di-*O*-(*E*)-caffeoyldaucic acid, which is an already known compound (Figure 8) (28).

Table 8. ^1H and ^{13}C NMR data (δ in ppm, J in Hz) of daucic acid derivatives (**11–13**). Compounds **11** and **13** were recorded in Methanol- d_4 at 500/125 MHz, while compound **12** was recorded in Methanol- d_4 at 700/176 MHz, 313 K.

Position	11		12		13	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	-	-	-	-	-	-
2	-	146.21	-	n.d	-	*146.5
3	5.98, br s (ov)	108.7	5.81, br s	*105.4	5.97, m (ov)	*108.2
4	5.99, br s (ov)	66.4	5.98, br s	*67.21	5.99, m (ov)	66.4
5	5.99, br s (ov)	65.2	6.10, br s	*65.03	6.01, m (ov)	65.2
6	5.10, br s	76.4	4.90, br s	78.13	5.09, br s	*76.7
7	-	164.8	-	n.d	-	n.d
8	-	170.4	-	n.d	-	n.d
	4-caffeoyl		4-caffeoyl		4-feruloyl	
1'	-	127.50	-	*127.4	-	127.44
2'	6.99, d (2.1)	114.84	6.97, d (2.1)	*114.77	7.01, d (1.9)	111.9
3'	-	146.81	-	*146.6	-	149.28
4'	-	149.78	-	*149.2	-	150.7
5'	6.69, d (8.2)	116.41	6.66, d (8.2)	*116.19	6.72, d (8.1)	116.45
6'	6.81, dd (8.2, 2.1)	123.52	6.77, dd (8.2, 2.1)	*123.15	6.97, dd (8.2, 1.9)	124.0
7'	7.49, d (15.9)	147.8	7.46, d (15.9)	*147.40	7.51, d (15.9)	147.7
8'	6.16, d (15.9)	114.14	6.13, d (15.9)	*114.24	6.25, d (15.9)	114.58
9'	-	167.77	-	**167.40	-	167.61
OCH₃	-	-	-	-	3.71, s	56.2
	5-caffeoyl		5-feruloyl		5-caffeoyl	
1''	-	127.55	-	*127.4	-	127.55
2''	7.03, d (2.1)	115.2	7.10, br s	*111.8	7.03, d (2.0)	115.2
3''	-	146.81	-	*149.2	-	146.9
4''	-	149.87	-	*150.6	-	149.92
5''	6.76, d (8.2)	116.51	6.80, d (8.1)	*116.43	6.75, d (8.2)	116.52
6''	6.91, dd (8.2, 2.1)	123.43	7.03, br d (8.1)	*123.88	6.90, dd (8.2, 2.0)	123.4
7''	7.53, d (15.8)	148.3	7.55, d (15.8)	*147.51	7.53, d (15.9)	148.4
8''	6.31, d (15.8)	114.01	6.34, d (15.8)	*114.74	6.32, d (15.8)	114.11
9''	-	167.84	-	**167.45	-	167.89
OCH₃	-	-	3.86, br s	*56.3	-	-

*Signals identified from HSQC and HMBC spectra, **Shift values deduced from band-selective HMBC spectrum, n.d; not detected.

The NMR spectra of compound **12** indicated the presence of an additional methyl group, i.e., δ_{H} 3.86 (3H, s), $^*\delta_{\text{C}}$ 56.3. This methyl group exhibited a long-range coupling with $^*\delta_{\text{C}}$ 149.2, which is a superimposed resonance for C-3" and C-4'. In COSY spectrum, the coupling of methyl protons with δ_{H} 7.10 (1H, br s, H-2") suggested that the adjacent C-3" hydroxyl of caffeic acid is methylated, thereby forming a feruloyl moiety. This feruloyl moiety was further differentiated based on the long-range couplings of β -vinylic proton δ_{H} 7.55 (1H, d, J = 15.8 Hz, H-7") with aromatic carbon $^*\delta_{\text{C}}$ 111.8 (C-2") and carbonyl carbon $^*\delta_{\text{C}}$ 167.45 (C-9"). Subsequently, H-4 proton showed long-range coupling with carbonyl carbon $^*\delta_{\text{C}}$ 167.40 (C-9') of caffeoyl group, while H-5 proton was correlated with $^*\delta_{\text{C}}$ 167.45 (C-9") of feruloyl group. Thus, compound **12** was identified as 4-*O*-(*E*)-caffeoyl-5-*O*-(*E*)-feruloyldaucic acid (Figure 8).

Compound **13** also contained a methyl group, i.e., δ_{H} 3.71 (3H, s), δ_{C} 56.2, which was correlated with δ_{C} 149.28 (C-3'), suggesting its substitution at this position. In this case, H-4 was coupled with carbonyl carbon δ_{C} 167.61 (C-9') of feruloyl, while H-5 was correlated with carbonyl carbon δ_{C} 167.89 (C-9") of caffeoyl subunit. Thus, compound **13** was identified as 4-*O*-(*E*)-feruloyl-5-*O*-(*E*)-caffeoyldaucic acid (Figure 8). One of these feruloyl derivatives is already reported (28), while the other one represents a novel compound.

4.3.3. Structural characterization of caffeoyl-dihydroxyphenyllactic acids (**14–17**) and conversion products (**18, 19, 21**)

The ^1H NMR spectrum of compound **17** showed nine proton resonances δ_{H} 7.03 (1H, br s, H-2), 6.66 (1H, m (ov), H-2'), 6.88 (1H, d, J = 2 Hz, H-2"), 6.8 (1H, d, J = 8.1 Hz, H-5), 6.64 (1H, d, J = 8.0 Hz, H-5'), 6.67 (1H, m (ov), H-5"), 7.15 (1H, dd, J = 8.4, 2.0 Hz, H-6), 6.53 (1H, dd, J = 8.1, 2.1 Hz, H-6'), and 6.73 (1H, dd, J = 8.2, 2.0 Hz, H-6") in form of three ABX systems (Table 9). Two vinylic protons δ_{H} 7.43 (1H, d, J = 15.8 Hz, H-7) and 6.26 (1H, d, J = 16 Hz, H-8) were found. A pair of methylene protons δ_{H} 2.90 (1H, dd, J = 14.4, 8.5 Hz, H-7') and 2.98 (1H, dd, J = 14.4, 4.4 Hz, H-7") was coupled with an oxymethine proton δ_{H} 5.03 (1H, dd, J = 8.4, 4.5 Hz, H-8'), forming a $\text{CH}_2\text{-CH(OH)}$ subunit. Furthermore, two adjacent oxymethine protons δ_{H} 4.83 (1H, d, J = 5.8 Hz, H-7") and 4.65 (1H, br s, H-8") were noticed forming CH(OH)-CH(OH) unit.

Table 9. ^1H and ^{13}C NMR data (δ in ppm, J in Hz) of caffeoyl-dihydroxyphenyllactic acids (**14–17**). Spectra were recorded in Methanol- d_4 (**14**, **16**) and DMSO- d_6 + D_2O (**15**, **17**) at 500/125 MHz.

Positio n	14		15		16		17	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1		127.9		128.7		127.6		125.52
2	7.54, d (2.0)	118.05	7.09, d (2.1)	115.37	7.04, d (2.1)	115.2	7.03, br s	**116.33
3		147.1		*148.13		146.81		**151.1
4		151.1		148.9		149.7		146.5
5	6.87, d (8.3)	117.41	6.62, m (ov)	115.73	6.78, d (8.2)	116.49	6.80, d (8.1)	116.65
6	7.19, dd (8.4, 2.0)	126.2	7.01, dd (8.5, 2.2)	121.2	6.95, dd (8.2, 2.1)	123.1	7.15, dd (8.4, 2.0)	125.25
7	7.59, d (15.9)	146.9	7.45, d (15.9)	145.73	7.55, d (15.9)	*147.6	7.43, d (15.8)	145.95
8	6.38, d (15.9)	115.6	6.34, d (16.0)	115.58	6.27, d (15.9)	114.4	6.26, d (16.0)	114.1
9		168.4		166.2		168.4		166.3
1'		129.6		127.6		129.3		127.6
2'	6.76, d (2.1)	117.65	6.67, m (ov)	116.9	6.75, d (2.1)	117.5	6.66, m (ov)	116.89
3'		146.07		145.04		146.16		145.05
4'		145.2		144.13		145.3		144.13
5'	6.69, d (8.0)	116.3	6.64, m (ov)	115.73	6.70, d (8.1)	116.28	6.64, d (8.0)	115.74
6'	6.62, dd (8.1, 2.1)	121.8	6.52, dd (8.1, 2.1)	120.5	6.61, dd (8.1, 2.1)	121.7	6.53, dd (8.1, 2.1)	120.5
7'	2.99, dd (14.3, 8.6) 3.09, dd (14.3, 4.1)	38.07	2.89, dd (14.4, 8.5) 2.97, dd (14.4, 4.3)	36.3	3.00, dd (14.3, 8.4) 3.10, dd (14.3, 4.3)	37.9	2.90, dd (14.4, 8.5) 2.98, dd (14.4, 4.4)	36.3
8'	5.16, dd (8.6, 4.0)	*75.26	5.02, dd (8.3, 4.3)	73.2	5.18, dd (8.4, 4.3)	74.7	5.03, dd (8.4, 4.5)	73.26
9'		n.d		171.12		173.6		**171.16
1''	4.82, m (ov)	104.2		132.4				132.3
2''	3.51, m (ov)	74.92/77.61/ 78.52	6.87, d (2.0)	115.01			6.88, d (2.0)	115.10
3''	3.51, m (ov)	74.92/77.61/ 78.52		144.71/ 144.72				144.69
4''	3.39, m (ov)	71.52		144.71/ 144.72				144.71
5''	3.51, m (ov)	74.92/77.61/ 78.52	6.66, m (ov)	115.16			6.67, m (ov)	115.10
6''	3.72, m 3.97, dd (12.1, 2.2)	62.54	6.72, dd (8.2, 1.9)	118.7			6.73, dd (8.2, 2.0)	118.7
7''			4.81, d (6.0)	73.09			4.83, d (5.8)	73.13
8''			4.49, br s	*83.2			4.65, br s	**83.2
9''				*171.46				**171.49

*Signals deduced from HSQC and HMBC spectrum; **Signals deduced from HSQC and HMBC spectra recorded at elevated temperature (328 K), n.d; not detected

The ^{13}C NMR resonances further confirmed these conclusions and showed three carbonyl carbons δ_{C} (166.3, 171.16, *171.49). The ^1H – ^{13}C HMBC spectrum revealed that one ABX system was correlated with $\text{CH}_2\text{-CH(OR)-COOH}$, corresponding to a 3,4-dihydroxyphenyllactic acid (DHPLA) subunit. The next ABX system was coupled with CH=CH-COOR , forming a caffeic acid moiety. The third ABX system was correlated with $\text{CH(OH)-CH(OR)-COOH}$, yielding a (3,4-dihydroxyphenyl)glyceric acid residue. Afterwards, the connecting positions of these three subunits were confirmed. The α -oxymethine proton δ_{H} 5.03 (1H, dd, J = 8.4, 4.5 Hz, H-8') of DHPLA was correlated with carbonyl carbon δ_{C} 166.3 (C-9) of caffeic acid, thus confirming the esterification between these two subunits. Moreover, α -glyceric acid proton δ_{H} 4.65 (1H, br s, H-8'') was coupled with H-2 of caffeic acid δ_{H} 7.03 (1H, br s) in ^1H – ^1H NOESY spectrum. It indicates that the vicinal hydroxyl (C-3) of caffeic acid is attached to the (3,4-dihydroxyphenyl)glyceric

acid moiety. Compound **17** represents a novel natural product (Figure 8), which was named as salvianolic acid O. In case of isomeric compound **15**, α -glyceric acid proton δ_{H} 4.49 (1H, br s, H-8'') was coupled with H-5 of caffeic acid δ_{H} 6.62 (1H, m (ov), H-5), suggesting that C-4 hydroxyl of caffeic acid is connected with (3,4-dihydroxyphenyl)glyceric acid moiety. Compound **15** was confirmed as salvianolic acid K (120) (Figure 8). Compounds **14** and **16** were verified as rosmarinic acid-3-*O*- β -D-glucoside (121), rosmarinic acid (122) (Figure 8). The NMR assignments of conversion product **19** are in close agreement with its parent salvianolic acid K (**15**), apart from positions 7' and 8', which contained vinylic protons δ_{H} 5.67 (1 H, d, $J = 7.2$ Hz), δ_{H} 7.15 (1H, d, $J = 7.1$ Hz) (Table 10). Conversion product **19** is a novel derivative (Figure 8) and was named as salvianolic acid P. The isomeric conversion products of rosmarinic acid (**18**, **21**) also possessed olefinic protons at positions 7' and 8'. The conversion product **18**, possessing *m*-positioned hydroxyls in ring B, was elucidated as nepetoidin A (*Z*, *E*) (112), while the conversion product **21** containing *o*-positioned hydroxyls was identified as nepetoidin B (*Z*, *E*) (123) (Figure 8).

Table 10. ^1H and ^{13}C NMR data (δ in ppm, J in Hz) of conversion products **19** (DMSO- d_6 +D $_2$ O), **20**, and **21** (Acetone- d_6) at 500/125 MHz.

Position	19		18	21
	δ_{H}	δ_{C}	δ_{H}	δ_{H}
1		129.9		
2	7.14, m (ov)	116.41	7.24, d (2.0)	7.26, d (2)
3		150.55/150.61		
4		150.61/150.55		
5	6.48, d (8.4)	119.5	6.91, d (8.2)	6.91, d (8.2)
6	7.01, dd (8.4, 2.1)	121.9	7.14, dd (8.2, 2.1)	7.14, dd (8.2, 2.1)
7	7.62, d (15.8)	147.6	7.71, d (15.9)	7.74, d (15.9)
8	6.56, d (15.9)	115.41	6.39, d (16.1)	6.51, d (15.9)
9		164.5		
1'		126.3		
2'	7.22, d (2.1)	116.94	6.80, s	7.38, d (2.0)
3'		145.4		
4'		145.4	6.95, s	
5'	6.73, d (8.1)	116.37		6.82, d (8.2)
6'	6.87, m (ov)	122.2	6.80, s	7.02, dd (8.2, 2.1)
7'	5.67, d (7.2)	113.1	6.40, d (12.6)	5.68, d (7.2)
8'	7.15, d (7.1)	132.6	7.84, d (12.8)	7.27, d (7.2)
1''		133.2		
2''	6.85, d (2.0)	115.60		
3''		144.97		
4''		144.93		
5''	6.67, d (8.1)	115.60		
6''	6.70, dd (8.4, 2.0)	119.5		
7''	4.78, d (6.3)	73.7		
8''	4.21, Br (s)	*86.8		
9''		173.9		

*Signal deduced from HSQC spectrum.

4.3.4. Structural characterization of flavonoid-malonyl-glucosides (1–3, 8)

FMGLs **1**, **2**, and **3** were identified as quercetin-3-*O*-(6"-*O*-malonyl)- β -D-glucoside (124, 125), luteolin-7-*O*-(6"-*O*-malonyl)- β -D-glucoside (126), and kaempferol-3-*O*-(6"-*O*-malonyl)- β -D-glucoside (127, 128) (Figure 8, Table 11), based on the comparison of their NMR data reported in earlier studies.

Table 11. ^1H and ^{13}C NMR data (δ in ppm, J in Hz) of the flavonoid-malonyl-glucosides **1–3, 8** recorded in DMSO- d_6 at 500/125 MHz.

Position	1		2		3		8	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}	δ_{H}	δ_{C}
2	-	156.7	-	164.8	-	156.7	-	164.8
3	-	133.2	6.75, s	103.1	-	133.1	6.76, s	103.1
4	-	177.3	-	181.9	-	177.4	-	181.9
5	-	161.2	-	161.0	-	161.2	-	161.1
6	6.20, d (2.0)	98.7	6.42, d (1.9)	99.8	6.21, d (2.0)	98.8	6.38, d (2.0)	99.8
7	-	164.2	-	162.8	-	164.3	-	162.1
8	6.40, d (2.0)	93.6	6.77, d (2.0)	94.5	6.43, d (2.0)	93.7	6.72, d (2.0)	94.9
8a	-	156.4	-	157.1	-	156.4	-	156.9
4a	-	103.9	-	105.5	-	103.9	-	105.8
1'	-	121.0	-	121.3	-	120.7	-	121.3
2'	7.51, d (2.1)	116.2	7.53, br s	113.5	7.98, d (8.9)	130.8	7.51, brs	113.5
3'	-	144.9	-	146.2	6.88, d (8.9)	115.1	-	146.1
4'	-	148.6	-	150.0	-	160.1	-	150.1
5'	6.83, d (8.4)	115.1	6.87, d (8.4)	115.9	6.88, d (8.9)	115.1	6.87, d (8.4)	115.9
6'	7.49, dd (8.4, 2.2)	121.5	7.41, dd (8.4, 2.1)	118.9	7.98, d (8.9)	130.8	7.42, dd (8.4, 2.2)	119.0
1''	5.37, d (7.4)	101.1	5.07, d (7.3)	100.0	5.34, d (7.5)	101.3	5.38, d (8.1)	97.3
2''	3.24, m (ov)	73.9	3.29, m (ov)	73.1	3.20, m (ov)	74.0	4.84, dd (9.3, 8.4)	73.8
3''	3.24, m (ov)	76.2	3.32, m (ov)	76.1	3.22, m (ov)	76.1	3.58, t (9.2)	73.3
4''	3.18, m	69.5	3.17, m	69.7	3.14, m (ov)	69.5	3.31, m (ov)	69.7
5''	3.31, m (ov)	73.94	3.73, m	74.2	3.31, m (ov)	73.9	3.87, m	74.1
6''	4.19, dd (11.8, 1.8) 3.99, dd (11.9, 5.7)	63.5	4.46, d (11.2) 4.04, dd (11.9, 7.5)	64.0	4.16, dd (11.8, 1.7) 4.00, dd (11.9, 5.7)	63.4	4.46, d (11.0) 4.12, dd (11.9, 7.1)	63.8
2''-malonyl								
CH₂	-	-	-	-	-	-	3.38, m (ov)	41.8
RCOOR'	-	-	-	-	-	-	-	166.2
RCOOH	-	-	-	-	-	-	-	167.8
6''-malonyl								
CH₂	3.07, s	41.5	3.31, m (ov)	42.6	3.07, s	41.6	3.35, m (ov)	42.3
RCOOR''	-	166.8	-	167.5	-	166.8	-	167.3
RCOOH	-	167.9	-	168.0	-	167.9	-	168.0

Note: Ov, signals in the overlapped regions of the spectra and the multiplicities could not be recognized.

The NMR spectra of FMGLs **2** and **8** are quite identical, referring to the presence of luteolin-7-*O*- β -D-glucoside subunit. However, **8** is esterified with two malonyl units (δ_{H} 3.38; δ_{C} 167.8, 166.2, 41.8 and δ_{H} 3.35; δ_{C} 168.0, 167.3, 42.3). The HMBC correlations of glucoside protons, i.e., H-2'' and H-6'' with carbonyl carbons δ_{C} 166.2 and 167.3 of malonyls, respectively, confirmed their substitution positions. Compound **8** was identified as luteolin-7-*O*-(2'',6''-di-*O*-malonyl)- β -D-glucoside (Figure 8), which represents a novel flavonoid.

4.4. Quantification of metabolites in selected Apiaceae species

In addition to collecting inflorescences, the leaves and roots of *A. cerefolium*, *C. bulbosum*, and *A. sylvestris* were collected at the beginning of the vegetation cycle in early spring (BVC) and during the flowering stage (FS). The leaves of *A. caucalis* were collected during the FS along with its inflorescences. The roots and leaves of *M. odorata* and *A. nitida* were collected during their post-vegetative phase in autumn (PVP). The composition of FMGIs (1–3, 8), CQAs (4–7, 9, 10), and CDAs (11–13), was determined in these tissue samples (Table 12).

Table 12. Quantification of flavonoid-malonyl glucosides (1–3, 8), malonyl dicaffeoylquinic acids (4–7, 9, 10), and caffeoyl daucic acids (11–13) in different organs of selected Apiaceae species.

Plant	Tissue	Collection time ^a	Sample code ^b	Amounts of compounds in the dried tissues (mg/g)												
				1	2	3	8	4	7	9	5	6	10	11	12	13
<i>A. cerefolium</i>	Leaf	BVC	AC-L-BVC	-	-	-	-	3.36	1.14	2.84	-	-	-	-	-	-
		FS	AC-L-FS	-	-	-	-	10.64	2.00	1.34	-	-	-	-	-	-
	Inflrsc	FS	AC-I-FS-A	-	-	-	-	28.60	4.62	1.01	-	-	-	-	-	-
			AC-I-FS-B	-	-	-	-	28.53	4.64	1.00	-	-	-	-	-	-
	Root	BVC	AC-R-BVC	-	-	-	-	0.42	0.46	0.22	-	-	-	-	-	-
		FS	AC-R-FS	-	-	-	-	0.30	0.27	0.09	-	-	-	-	-	-
<i>C. bulbosum</i>	Leaf	BVC	CB-L-BVC	-	10.64	-	2.42	8.64	1.76	1.18	-	-	-	-	-	-
		FS	CB-L-FS	2.73	5.63	-	0.76	4.62	0.99	1.59	-	-	-	-	-	-
	Inflrsc	FS	CB-I-FS-A	25.61	1.92	9.98	0.79	20.22	4.57	30.21	-	-	-	-	-	-
			CB-I-FS-B	25.87	1.84	9.66	0.82	20.29	4.56	30.97	-	-	-	-	-	-
	Root	BVC	CB-R-BVC	-	-	-	-	1.34	0.49	0.22	-	-	-	-	-	-
		FS	CB-R-FS	-	-	-	-	0.82	0.36	0.12	-	-	-	-	-	-
<i>A. sylvestris</i>	Leaf	BVC	AS-L-BVC	-	-	-	-	1.79	-	-	2.08	1.91	-	-	-	-
		FS	AS-L-FS	-	-	-	-	1.45	-	-	0.12	0.23	-	-	-	-
	Inflrsc	FS	AS-I-FS	-	-	-	-	2.93	-	-	0.62	0.57	-	-	-	-
	Root	BVC	AS-R-BVC	-	-	-	-	1.32	-	-	0.28	0.15	-	-	-	-
		FS	AS-R-FS	-	-	-	-	3.52	-	-	0.22	0.10	-	-	-	-
	Root	FS	AS-R-FS	-	-	-	-	3.52	-	-	0.22	0.10	-	-	-	-
<i>A. caucalis</i>	Leaf	FS	AC-L-FS	-	-	-	-	-	-	-	-	-	2.05	-	-	-
	Inflrsc	FS	AC-I-FS	-	-	-	-	-	-	-	-	-	3.24	-	-	-
<i>A. nitida</i>	Leaf	PVP	AN-L-PVP	-	-	-	-	-	-	-	-	-	-	-	-	-
	Root	PVP	AN-R-PVP	-	-	-	-	-	-	-	-	-	-	4.39	-	-
<i>M. odorata</i>	Leaf	PVP	MO-L-PVP	-	-	-	-	-	-	-	-	-	-	-	-	-
	Root	PVP	MO-R-PVP	-	-	-	-	-	-	-	-	-	-	9.42	0.75	1.36

^a Plants were collected at the BVC (January to April), FS (May to June), PVP (September to December).

^b Sample codes refer to plant species (AC; *A. cerefolium*, CB; *C. bulbosum*, AS; *A. sylvestris*, AC; *A. caucalis*, AN; *A. nitida*, MO; *M. odorata*), analyzed tissues (L; leaf, I; Inflorescence, R; root), collection time (BVC; beginning of the vegetation cycle. FS; flowering stage, PVP; post vegetative phase).

Note: Values in bold represent the main compound concentration and serve as the basis for selecting the corresponding plant tissue for isolation. Results of *A. sylvestris* are reproduced with permission from Ausbüttel T (107). The italic letters (A, B) in the sample names indicate the independent extraction parallels.

FMGLs **1**, **2**, **3**, and **8** were detected in *C. bulbosum*. The malonyl-glucosides of quercetin (**1**) and kaempferol (**3**) were mainly accumulated in inflorescences of *C. bulbosum* with 25.74 mg/g and 9.82 mg/g concentrations, respectively. On the other hand, the malonyl and dimalonyl-glucoside of luteolin (**2**, **8**) were mainly present in leaves collected at the BVC with 10.6 mg/g and 2.42 mg/g amounts, respectively.

MDiCQAs (**4**, **7**) and DiMDiCQA (**9**) were present in all tissues of *A. cerefolium* and *C. bulbosum*. MDiCQAs (**5**, **6**) were detected in all *A. sylvestris* samples, accompanied by compound **4**. The inflorescences of *A. cerefolium* and *C. bulbosum* were found to be the most concentrated source of compounds **4** and **7**, with 28.56 mg/g and 4.63 mg/g in *A. cerefolium*, while 20.25 mg/g and 4.56 mg/g in *C. bulbosum*. The inflorescences of *C. bulbosum* also possessed the highest concentration of compound **9**, i.e., 30.59 mg/g. The highest levels of compounds **5** and **6** were noticed in leaves of *A. sylvestris* samples collected at the BVC, i.e., 2.08 and 1.91 mg/g, respectively.

DiMDiCQA (**10**) was accumulated in the inflorescences of *A. caucalis* up to 3.24 mg/g. The highest amounts of DiCDA (**11**) were detected in the roots of *M. odorata* collected at the PVP, attaining up to 9.42 mg/g, while the same sample was the only source of FCDA (**12**, **13**) with 0.75 mg/g and 1.36 mg/g concentrations, respectively.

4.5. Quantification of compounds in variously prepared extracts of *Salvia* species

Extracts from pulverized *Salvia* tissues were prepared via three alternative procedures before HPLC-HR-MS-UV analysis. Method 1 used an initial room-temperature incubation of pulverized tissues suspended in distilled water for 5, 15, 30, 45, and 60 min (abbreviated as DW5–DW60 in sample abbreviations). Method 2 incorporated a thermal pretreatment, in which the aqueous suspension was incubated at 100 °C for 2 min and then left at room temperature for 45 or 60 min (abbreviated as 100°C-DW45 or 100°C-DW60 in sample abbreviations). Following these aqueous incubation steps, absolute methanol was added to achieve an 80% (v/v) final concentration, and the samples were heated at 60 °C to extract the compounds. In method 3, untreated tissue extracts were generated by directly extracting the pulverized samples with 80% MeOH at 60 °C, omitting any pre-water treatment (abbreviated as UT in sample abbreviations) (Table 13–15).

4.5.1. Enzymatic conversion of rosmarinic acid into nepetoidins A and B

The untreated *S. officinalis* leaf contained rosmarinic acid (**16**) up to 27.4 mg/g (sample 1; Table 13). Rosmarinic acid was rapidly decomposed during room-temperature aqueous incubation of pulverized tissues. Samples incubated in water at room temperature for 5, 15, 30, 45, and 60 min showed a noticeable reduction in rosmarinic acid content with increasing incubation time: 1.97 mg/g and 0.15 mg/g rosmarinic acid content were detected after 15 and 60 min of aqueous incubation, respectively. On the other hand, thermal pretreatment at 100 °C for 2 min, followed by a 60-min aqueous incubation at room temperature, resulted in a rosmarinic acid content comparable to that observed in untreated samples (sample 100°C-DW60, 29.3 mg/g). While untreated leaves contained negligible amounts of nepetoidin A (**18**) and B (**21**) (sample 1: 0.03 and 0.05 mg/g; sample 2: 0.11 mg/g and 0.08 mg/g, respectively), a room temperature water incubation elevated these values to 0.61 and 1.59 mg/ in sample 1 (5 min incubation), as well as 0.90 and 3.93 mg/g in sample 2 (30 min incubation). Longer incubation periods caused a steady decline in the levels of both compounds (Table 13).

Table13. Quantification of rosmarinic acid (**16**) and its conversion products (**18**, **21**) in aerial tissues of *S. officinalis*.

Species	Sample No	Treatment conditions	Sample name	Compounds, mg/g		
				RA (16)	NEP A (18)	NEP B (21)
<i>S. officinalis</i>	1	UT	SO/1/UT-A	27.1	0.03	0.04
		UT	SO/1/UT-B	27.6	0.03	0.05
		DW5	SO/1/DW5	8.83	0.61	1.59
		DW15	SO/1/DW15-A	2.07	0.51	0.97
		DW15	SO/1/DW15-B	1.86	0.55	0.98
		DW30	SO/1/DW30	0.36	0.33	0.39
		DW45	SO/1/DW45	0.18	0.27	0.17
		DW60	SO/1/DW60	0.15	0.23	0.13
		100 °C, DW60	SO/1/100°C-DW60	29.3	0.14	0.26
	2	UT	SO/2/UT-A	13.9	0.11	0.08
		UT	SO/2/UT-B	15.9	0.11	0.09
		DW5	SO/2/DW5	8.42	0.61	3.25
		DW15	SO/2/DW15-A	4.66	0.66	3.94
		DW15	SO/2/DW15-B	4.55	0.62	3.86
		DW30	SO/2/DW30	2.05	0.90	3.93
		DW45	SO/2/DW45	0.75	0.84	3.21

Note: Values in bold represent the main concentration of compounds and serve as the basis for selecting a plant tissue for isolation.

The italic letters (*A*, *B*) in the sample names indicate the independent extraction parallels.

Abbreviations: RA; rosmarinic acid, NEP A; nepetoidin A, NEP B; nepetoidin B.

4.5.2. Enzymatic Conversion of salvianolic acid K into salvianolic acid P

Salvianolic acid K (**15**) was determined in the root samples of *S. pratensis*, *S. nemorosa*, and *S. sclarea*, ranging from 40.0–43.2, 14.3–17.5, and 5.19–6.37 mg/g, respectively (Table 14A–C). Upon room-temperature aqueous treatment of root tissues, salvianolic acid K converted into salvianolic acid P (**19**). The highest amount of salvianolic acid P was determined after 5 min incubation, with concentrations of 0.55 mg/g in *S. sclarea* (sample 2), 0.45 mg/g in *S. nemorosa* (sample 3), and 0.42 mg/g in *S. pratensis* (sample 1). Prolonged aqueous incubations resulted in a decrease in the levels of salvianolic acid P. In the case of heat-treated extracts (100°C, DW45), salvianolic acid K remained intact with levels comparable to those noticed in untreated (UT) samples.

Table 14A. Quantification of salvianolic acid K (**15**) and its conversion products (**19**) along with other identified metabolites in aerial tissues and roots of *S. pratensis*.

Species	Organ	Sample No	Treatment conditions	Sample name	Compounds, mg/g					
					RA GI (14)	RA (16)	NEPA (18)	NEP B (21)	SAA K (15)	SAA P (19)
<i>S. pratensis</i>	roots	1	UT	SP/R/1/UT-A	3.07	25.1	0.07	0.06	41.6	0.05
				SP/R/1/UT-B	3.14	24.7	0.06	0.06	40.4	0.05
				SP/R/1/UT-C	3.01	22.6	0.06	0.06	38.2	0.04
				SP/R/1/UT-D	3.16	23.5	0.06	0.06	39.8	0.05
		100 °C, DW45	SP/R/1/100°C-DW45-A	2.99	20.1	0.20	0.14	38.7	0.16	
			SP/R/1/100°C-DW45-B	2.68	19.4	0.19	0.14	36.3	0.16	
		DW5	SP/R/1/DW5	0.99	2.46	0.51	0.40	10.5	0.42	
		DW15	SP/R/1/DW15	0.33	0.63	0.49	0.39	2.94	0.41	
		DW30	SP/R/1/DW30	0.03	0.12	0.10	0.10	0.07	0.08	
		DW45	SP/R/1/DW45	-	0.05	tr	0.05	tr	tr	
		2	UT	SP/R/2/UT	2.41	16.2	0.07	0.05	43.2	tr
		3	UT	SP/R/3/UT-A	2.02	16.4	0.07	0.07	41.9	0.05
				SP/R/3/UT-B	1.96	16.3	0.06	0.07	40.7	0.05
	aerial parts	1	NT	SP/AP/1/UT-A	0.13	22.2	tr	tr	tr	-
				SP/AP/1/UT-B	0.13	21.2	tr	tr	tr	-
DW5			SP/AP/1/DW5	tr	5.82	0.22	0.06	tr	-	
DW15			SP/AP/1/DW15	-	0.37	0.14	tr	-	-	
DW30			SP/AP/1/DW30	-	0.10	0.07	tr	-	-	
DW45			SP/AP/1/DW45	-	0.05	tr	tr	-	-	
2		UT	SP/AP/2/UT	-	23.4	tr	tr	tr	-	
3			SP/AP/3/UT	tr	18.6	tr	tr	tr	-	

Note: Values in bold represent the main concentration of compounds and serve as the basis for selecting a plant tissue for isolation.

The italic letters (*A*, *B*, *C*, and *D*) in the sample names indicate the independent extraction parallels

Abbreviations: RA Gl; rosmarinic acid glucoside, RA; rosmarinic acid, NEP A; nepetoidin A, NEP B; nepetoidin B, SAA K; salvianolic acid K, SAA P; salvianolic acid P; tr, trace amount.

Table14B. Quantification of salvianolic acid K (15) and its conversion products (19) along with other identified metabolites in aerial tissues and roots of *S. nemorosa*.

Species	Organ	Sample No	Treatment conditions	Sample name	Compounds, mg/g						
					RA GI (14)	RA (16)	NEPA (18)	NEP B (21)	SAA K (15)	SAA P (19)	
<i>S. nemorosa</i>	roots	1	UT	SN/R/1/UT	2.89	10.8	tr	0.05	14.3	0.04	
		2		SN/R/2/UT-A	1.12	18.1	0.07	tr	17.7	0.05	
				SN/R/2/UT-B	1.13	18.3	0.07	tr	17.3	0.05	
		3		SN/R/3/UT-A	1.28	14.5	0.06	0.05	14.9	0.04	
					SN/R/3/UT-B	1.30	15.3	0.06	0.05	15.0	0.04
				DW5	SN/R/3/DW5	0.43	2.60	0.54	0.61	5.41	0.45
				DW15	SN/R/3/DW15	0.06	0.59	0.34	0.35	0.78	0.28
				DW30	SN/R/3/DW30	tr	0.29	0.13	0.16	0.09	0.11
				DW45	SN/R/3/DW45	tr	0.18	0.07	0.09	0.05	0.06
	aerial parts	1	UT	SN/AP/1/UT	0.27	19.3	tr	tr	tr	-	
		2		SN/AP/2/UT-A	0.46	20.8	tr	tr	tr	tr	
					SN/AP/2/UT-B	0.46	21.8	tr	tr	tr	tr
					DW5	SN/AP/2/DW5	0.35	4.42	0.23	1.24	tr
				DW15	SN/AP/2/DW15	tr	0.23	0.09	0.35	-	tr
				DW30	SN/AP/2/DW30	-	0.18	tr	0.21	-	tr
				DW45	SN/AP/2/DW45	-	0.27	tr	tr	-	tr

The footnote annotations are the same as those described for Table 14A.

Table14C. Quantification of salvianolic acid K (15) and its conversion products (19) along with other identified metabolites in aerial tissues and roots of *S. sclarea*.

Species	Organ	Sample No	Treatment conditions	Sample name	Compounds, mg/g					
					RA GI (14)	RA (16)	NEP A (18)	NEP B (21)	SAA K (15)	SAA P (19)
<i>S. sclarea</i>	roots	1	UT	SS/R/1/UT-A	0.05	3.33	tr	tr	5.29	tr
				SS/R/1/UT-B	0.05	3.08	tr	tr	5.09	tr
		2		SS/R/2/UT-A	0.08	3.92	0.06	tr	5.68	0.04
				SS/R/2/UT-B	0.08	4.37	0.06	tr	7.05	0.05
			DW5	SS/R/2/DW5	-	0.57	0.22	0.45	0.10	0.55
			DW15	SS/R/2/DW15	-	0.11	0.14	0.36	tr	0.35
			DW30	SS/R/2/DW30	-	0.06	0.09	0.22	-	0.21
			DW45	SS/R/2/DW45	-	tr	tr	0.10	-	0.08
	aerial parts	1	UT	SS/AP/1/UT-A	tr	20.0	tr	tr	2.60	tr
				SS/AP/1/UT-B	tr	20.1	tr	tr	2.50	tr
		2		SS/AP/2/UT-A	tr	17.2	tr	tr	2.23	tr
				SS/AP/2/UT-B	tr	16.9	tr	tr	2.22	tr
			DW5	SS/AP/2/DW5	tr	3.46	0.29	0.86	0.32	0.12
			DW15	SS/AP/2/DW15	-	2.29	0.24	0.69	0.20	0.10
			DW30	SS/AP/2/DW30	-	1.40	0.18	0.53	0.05	0.07
			DW45	SS/AP/2/DW45	-	0.42	0.12	0.32	tr	0.05

The footnote annotations are the same as those described for Table 14A.

4.5.3. Enzymatic Conversion of salvianolic acid O into salvianolic acid Q

Salvianolic acid O (17) accumulated in the roots of *S. verticillata* at concentrations ranging from 20.7–78.6 mg/g (Table 15).

Table 15. Quantification of salvianolic acid O (17) and its conversion products (20) along with other identified metabolites in aerial tissues and roots of *S. verticillata*.

Species	Organ	Sample No	Treatment conditions	Sample name	Compounds, mg/g						
					RA G I (14)	RA (16)	NEP A (18)	NEP B (21)	SAA O (17)	SAA Q (20)	
<i>S. verticillata</i>	roots	1	UT	SV/R/1/UT-A	0.48	42.2	0.07	0.04	48.2	tr	
				SV/R/1/UT-B	0.49	42.3	0.07	0.04	50.4	tr	
		2		SV/R/2/UT-A	0.93	51.2	0.08	0.05	31.3	tr	
				SV/R/2/UT-B	0.90	49.4	0.08	0.06	29.3	tr	
				SV/R/2/UT-C	0.94	50.3	0.07	0.06	30.3	tr	
		3		SV/R/3/UT-A	8.26	39.4	tr	0.06	46.7	tr	
				SV/R/3/UT-B	8.18	38.6	tr	0.06	45.8	tr	
		4		SV/R/4/UT	4.51	27.8	tr	0.05	20.7	tr	
		5		SV/R/5/UT	0.31	27.7	tr	0.05	28.5	tr	
				DW5	SV/R/5/DW5	0.11	5.08	0.13	0.41	10.1	0.07
				DW15	SV/R/5/DW15	tr	0.46	0.09	0.20	1.07	0.04
				DW30	SV/R/5/DW30	tr	0.42	0.08	0.19	0.96	0.03
				DW45	SV/R/5/DW45	tr	0.27	0.06	0.18	0.42	tr
		6	UT	SV/R/6/UT-A	tr	46.6	0.04	0.08	60.5	tr	
				SV/R/6/UT-B	tr	42.6	0.04	0.08	55.0	tr	
		7		SV/R/7/UT-A	0.06	33.1	tr	0.05	59.0	tr	
				SV/R/7/UT-B	0.06	31.6	tr	0.05	55.3	tr	
		8		SV/R/8/UT-A	0.09	57.1	tr	0.07	80.1	tr	
				SV/R/8/UT-B	0.08	53.8	tr	0.07	77.0	tr	
				DW5	SV/R/8/DW5	0.04	14.2	0.24	1.35	33.3	0.42
				DW15	SV/R/8/DW15	-	1.92	0.19	0.67	6.77	0.29
				DW30	SV/R/8/DW30	-	1.11	0.11	0.43	1.87	0.19
				DW45	SV/R/8/DW45	-	0.19	0.05	0.12	0.25	tr
		9	UT	SV/R/9/UT-A	3.09	45.8	tr	tr	25.2	tr	
				SV/R/9/UT-B	3.17	47.2	tr	tr	24.4	tr	
			DW45	SV/R/9/DW45	0.05	1.52	tr	0.09	0.20	tr	
			100 °C, DW45	SV/R/9/100°C-DW45-A	2.58	38.4	0.05	0.20	22.7	tr	
				SV/R/9/100°C-DW45-B	2.74	37.7	0.04	0.19	21.4	tr	
	aerial parts	2	UT	SV/AP/2/UT	tr	17.8	tr	0.03	0.52	-	
		3		SV/AP/3/UT-A	-	59.4	tr	tr	1.34	-	
				SV/AP/3/UT-B	-	58.8	tr	tr	1.33	-	
		4		SV/AP/4/UT	-	35.4	tr	0.03	4.09	-	
		5		SV/AP/5/UT	0.31	13.0	tr	tr	0.27	tr	
				DW5	SV/AP/5/DW5	0.11	4.38	0.13	1.59	0.15	tr
				DW15	SV/AP/5/DW15	tr	0.42	0.12	1.32	0.10	tr
				DW30	SV/AP/5/DW30	tr	0.62	0.09	1.10	0.09	tr
				DW45	SV/AP/5/DW45	tr	0.51	0.08	0.99	0.07	tr

The footnote annotations are the same as those described for Table 14A. The additional abbreviations: SAA O; salvianolic acid O, SAA Q; salvianolic acid Q.

During aqueous incubations of root samples at room temperature, salvianolic acid O converted to salvianolic acid Q (20). The maximum concentration of salvianolic acid Q was observed after a 5 min incubation, reaching up to 0.42 mg/g (sample 8). Prolonged aqueous treatments resulted in a decrease in salvianolic acid Q content. Similar to rosmarinic acid and salvianolic acid K, salvianolic acid O also remained intact during

room-temperature aqueous incubations when the samples were subjected to a prior thermal treatment at 100 °C for 2 min.

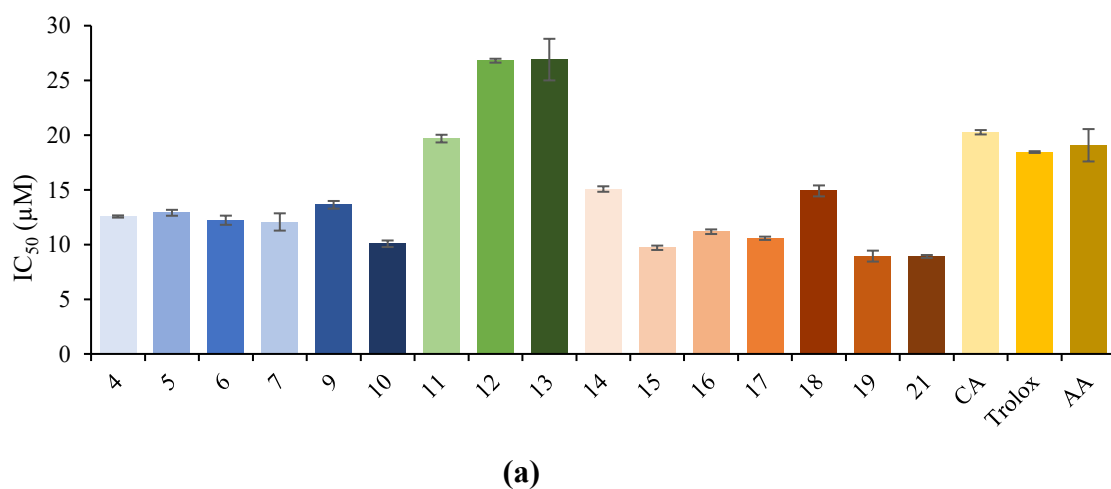
4.6. Evaluation of biological activities of isolated compounds

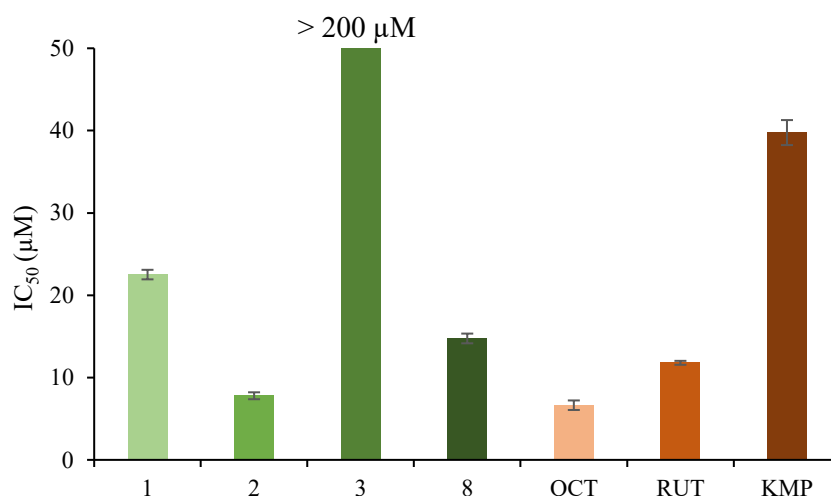
4.6.1. Determination of antioxidant potential

MDiCQAs (**4–7**) exhibited comparable antioxidant activity (IC_{50} 12.0 ± 0.8 μ M– 12.9 ± 0.3 μ M) to DiMDiCQA (**9, 10**) (IC_{50} 10.1 ± 0.3 μ M– 13.6 ± 0.4 μ M) (Figure 11). DiCDA (**11**) exhibited higher inhibitory potency (IC_{50} 19.7 ± 0.4 μ M) compared to its methylated derivatives (**12, 13**) (IC_{50} 26.8 ± 0.18 , 26.9 ± 2 μ M).

Rosmarinic acid (**16**) exhibited stronger antioxidant activity than its glucoside derivative, with IC_{50} values of 11.2 ± 0.2 and 15.1 ± 0.3 μ M, respectively. The isomeric compounds salvianolic acid K (**15**) and salvianolic acid O (**17**), together with the structurally related salvianolic acid P (**19**), displayed comparable antioxidant activities, with IC_{50} values of 9.7 ± 0.2 , 10.6 ± 0.2 , and 8.9 ± 0.5 μ M, respectively. Among the nepetoidins, nepetoidin B (**21**) exhibited higher DPPH radical-scavenging activity than nepetoidin A (**18**), with IC_{50} values of 8.9 ± 0.1 and 14.9 ± 0.5 μ M, respectively.

FMGLs **1, 2**, and **8** showed remarkable antioxidant potential, with IC_{50} values of 22.5 ± 0.6 μ M, 7.8 ± 0.4 , and 14.8 ± 0.6 μ M, respectively. However, FMGL **3** displayed reduced potency, with an IC_{50} value of 233.9 ± 0.9 μ M. The standards ascorbic acid, chlorogenic acid, and Trolox showed IC_{50} values of 19.1 ± 1.5 μ M, 20.3 ± 0.2 μ M, and 18.5 ± 0.1 μ M, respectively.





(b)

Figure 11. The DPPH radical scavenging activity of isolated (a) caffeic acid derivatives (4–19, 21) and (b) flavonoids (1–3, 8) compared with standard antioxidants (chlorogenic acid; CA, ascorbic acid; AA, Trolox, QCT; quercetin, RUT; rutin, KMP; kaempferol).

Source: Author's own work.

The DPPH assay is a well-established screening method for evaluating the free radical-scavenging potential of compounds. However, this assay is based on a simplified chemical system and may not directly reflect cellular antioxidant activity or in vivo effects.

4.6.2. Determination of cytostatic potential

The antiproliferative effects of MDiCQAs (4–7, 9) and FMGLs (1–3, 8) were assessed on five human cancer cell lines, including glioma (U87), colorectal carcinoma (HT-29), hepatocellular carcinoma (HepG2), promyelocytic leukemia (HL-60), and melanoma (A2058). Testing was also performed on non-tumorous Vero E6 cells (derived from African green monkey kidney epithelium) to evaluate the compound selectivity. Two MDiCQAs (4, 9) and the FMGL (3) showed a moderate cytostatic effect, whereas the other compounds showed no activity against the tested cells (Table 16). On the other hand, these compounds (4, 9, 3) also showed cytostatic effects on non-cancerous Vero E6 cells. Especially, MDiCQAs (4, 9) showed a strong cytostatic effect ($IC_{50} < 10 \mu M$) while FMGL (3) had a weaker effect ($IC_{50} \sim 40 \mu M$).

Table 16. Cytostatic effect of FMGI (**3**) and MDiCQA (**4,9**) on Vero E6 Cells and Human Colorectal Carcinoma (HT-29), Glioma (U87), Hepatoblastoma (HepG2), Leukemia (HL-60), and Melanoma (A2058) cells.

Cell line	IC ₅₀ (μM)			
	3	4	9	Daunomycin
Vero E6	39.8	9.9	9.5	1.7
A2058	21.0	13.5	11.9	0.2
U87	86.3	>100	48.5	0.6
HL-60	51.4	29.7	>100	0.16
HepG2	27.5	25.6	>100	0.54
HT-29	>100	>100	47.9	0.9

Note: The IC₅₀ values of compounds **1**, **2**, and **5–8** exceeded 100 μM for all tested cell lines; therefore, they are not included in Table 16. Moreover, these compounds were not tested on HL-60 cells.

The antiproliferative effects of MDiCQAs **10** and CDAs **11–13** remain to be elucidated in future studies.

The cytostatic effect of CDPLAs (**14–17**) and their conversion products (**18**, **19**, **21**) were evaluated on five human cancer cell lines: colorectal carcinoma (HT-29), monocytic leukemia (MM6), colorectal adenocarcinoma (HT-29), glioblastoma (U87 and U251), and lung adenocarcinoma (H838). All the conversion products, namely salvianolic acid P (**19**), nepetoidin A (**18**), and nepetoidin B (**21**), which lack a carboxyl group at the C8' position, showed better cytostatic activity towards the cancer cell lines than the parent compounds rosmarinic acid (**16**), rosmarinic acid glucoside (**14**), salvianolic acid K (**15**), and salvianolic acid O (**17**) containing a carboxyl group at C8' (Table 17).

Table 17. Cytostatic effect of CDPLAs (**14–17**) and their conversion products (**18**, **19**, **21**) on colorectal adenocarcinoma (HT-29), glioblastoma (U87 and U251), lung adenocarcinoma (H838), and monocytic leukemia (MM6) cells.

Compound	IC ₅₀ (μM)				
	MM6	HT-29	U87	U251	H838
14	47.6	>100	>100	>100	60.5
15	21.4	>100	>100	>100	56.6
16	77.4	>100	>100	>100	45.6
17	46.3	>100	>100	>100	77.8
18	24.7	79.6	67.2	88.5	34.5
19	17.6	24.7	34.0	24.7	25.6
20	20.0	19.2	26.3	32.5	22.4

Cytostatic assays provide preliminary information on the antiproliferative activity of compounds. Therefore, further validation by using additional cancer cell lines and comprehensive biological models is required.

5. DISCUSSION

5.1. HPLC-UV-Orbitrap-HR-MS analysis of extracts

Three main classes of metabolites were identified in Apiaceae species, including caffeoylquinic acids (CQAs), caffeoylidaucic acids (CDAs), and flavonoid-malonyl-glucosides (FMGs). Among CQAs, we identified four malonyl dicaffeoylquinic acid (MdiCQA) isomers (**4–7**) and two dimalonyl-dicaffeoylquinic acid (DiMDiCQA) isomers (**9, 10**). These malonyl-substituted CQAs were identified as novel natural products, with only a limited number of structurally related derivatives documented to date (27, 61). Therefore, isolation and structural elucidation of such analogues hold great potential for novel drug discovery. Among CDAs, dicaffeoylidaucic acid (DiCDA) and a feruloyl-caffeoylidaucic acid (FCDA) derivative have been reported (28). Currently, we have identified two isomeric FCDAs (**12, 13**); hence, at least one of these derivatives could be considered a novel compound. Among FMGs, luteolin and kaempferol glucosides substituted with a malonyl group have been reported (126). However, a dimalonyl derivative has not been reported; hence, the currently identified dimalonyl-glucoside derivative of luteolin (luteolin-7-*O*-(2'',6''-di-*O*-malonyl)- β -D-glucoside) (**8**) could be considered a novel compound.

Eight derivatives of caffeoyl-dihydroxyphenyllactic acid (CDHPLAs) were detected in *Salvia* species. Among isomeric compounds (**15, 17**), salvianolic acid K was identified as an already known compound (111). The isomer of salvianolic acid K, named salvianolic acid O, could be considered a novel derivative. The conversion products of salvianolic acid K and O, designated as salvianolic acid P (**19**) and Q (**20**), are also novel compounds.

5.2. Mass fragmentation study to differentiate between isomeric compounds

The isomeric compounds exhibited quite similar fragmentation profiles (Table 2–5); however, the relative intensities of key fragment ions showed compound-specific differences enabling the differentiation of isomers. Two approaches were used for calculating the relative intensity of fragment ions: 1) peak area-based fragment-to-precursor ratio analysis; each fragment's peak area was normalized to the total fragment ion area. This approach was employed for MdiCQAs (**4–7**), DiMDiCQAs (**9, 10**), and

FCDAAs (**12**, **13**), 2) base-peak–normalized MS/MS fragment intensity analysis, where the most intense fragment in the MS/MS spectrum was set to 100%, and all other fragment intensities were expressed relative to it. This approach was employed for isomeric salvianolic acids (**15**, **17**).

5.2.1. Differentiation of malonyl dicaffeoylquinic acid isomers (4–7)

The fragmentation intensities of isomers **4** and **7** were mainly distinguished at 45 eV CID (Table 2). In the MS/MS spectrum of compound **4**, the fragment ion at m/z 173 exhibited relatively lower intensity than the ion at m/z 233 (8% vs 30%, respectively). On the contrary, in compound **7**, the fragment ion at m/z 173 exhibited the highest abundance, followed by the ion at m/z 233 (38% and 28%, respectively). At 25 eV CID energy, the fragment ion at m/z 233 exhibited the highest intensities for both compounds **4** and **7** (65% and 69%, respectively).

The isomers **5** and **6** exhibited significantly different fragment intensities at 25 eV CID. For compound **5**, the ion at m/z 395 showed the highest relative intensity of 35%, while the fragment ion at m/z 233 was barely detectable. In compound **6**, both fragment ions at m/z 395 and m/z 233 showed relatively higher intensities (32% and 36%, respectively).

5.2.2. Differentiation of dimalonyl-dicaffeoylquinic acid isomers (9, 10)

Using 15 eV CID energy, the fragment ions from compound **9** at m/z 275, m/z 437, and m/z 687 were most abundant, exhibiting relative intensities of 21%, 20%, and 18%, respectively (Table 3). On the other hand, the fragment ion from compound **10** at m/z 437 was the most abundant with 45% relative intensity, while the other two fragments were present in trace amounts. Following the application of 30 eV CID energy, the fragment ion from compound **9** at m/z 275 showed significant relative intensity (67%), while the same fragment from compound **10** was barely detectable. At CID energy of 45 eV, the fragment ion from compound **10** at m/z 179 showed a higher relative intensity as compared to that of the same fragment from compound **9** (36% vs 4%, respectively).

5.2.3. Differentiation of feruloyl-caffeoylquinic acid isomers (12, 13)

The MS/MS spectrum of deprotonated compound **12** showed the fragment ions at m/z 379 and m/z 365, with relative intensities of 46% and 16%, respectively, at 10 eV CID energy (Table 4). The MS/MS spectrum of deprotonated compound **13** showed a fragment at m/z 379 with a relative intensity of 83%, whereas the fragment ion at m/z 365

was not detected. Furthermore, compound **12** showed main fragments at m/z 203 and m/z 193 with relative intensities of 20% and 27%, respectively, at 30 eV CID energy. On the other hand, compound **13** mainly possessed the fragment at m/z 185 with a relative intensity of 30%.

5.2.4. Differentiation of isomeric caffeoyl-dihydroxyphenyllactic acids (**15**, **17**) and conversion products (**18–21**)

Because the MS/MS spectra of both nepetoidins A and B (**18**, **21**) displayed identical product ions with comparable relative intensities, the two isomeric compounds could not be distinguished by HPLC-HR-MS/MS analysis.

The relative intensity ratios of the fragment ion pairs m/z 493/359 and m/z 447/313 provide reliable diagnostic markers for distinguishing the isomeric pairs SAAs K/O (**15/17**) and SAAs P/Q (**19/20**), respectively. In the MS/MS spectra of SAA K (**15**), the fragment ion at m/z 493 consistently exhibited lower relative intensity than the ion at m/z 359.08 at CID energies of 10, 12, and 15 eV. By contrast, SAA O (**17**) showed the opposite fragmentation pattern, with the ion at m/z 493 being more abundant than the ion at m/z 359 (Table 5). Accordingly, the intensity ratio I_{493}/I_{359} was consistently <1 for SAA K and >1 for SAA O.

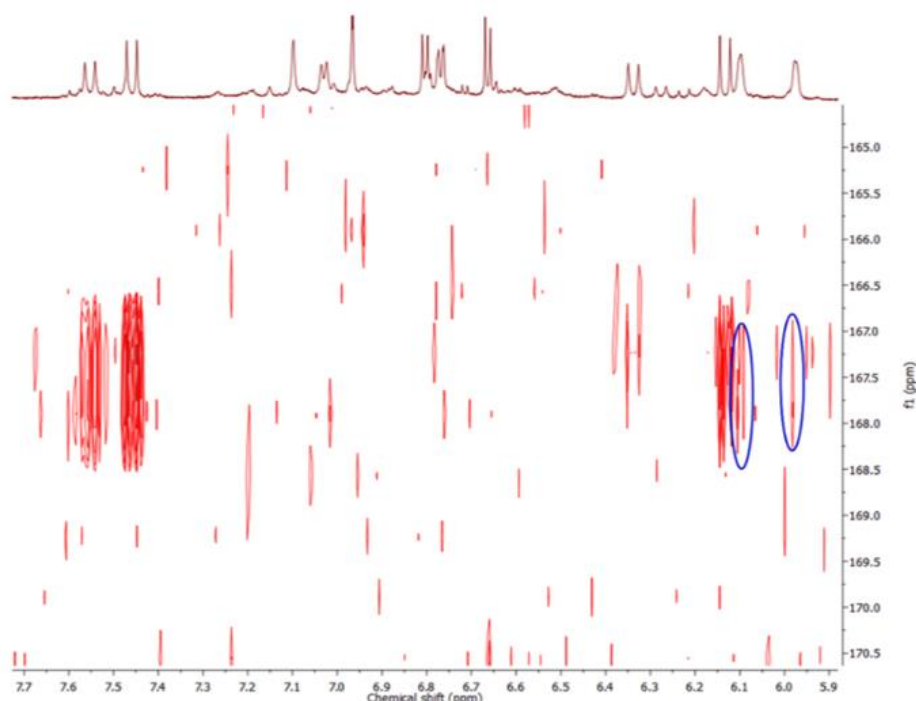
A similar fragmentation pattern was observed for salvianolic acids P (**19**) and Q (**20**). At CID energies of 10, 12, and 15 eV, the intensity ratio I_{447}/I_{313} was consistently <1 for salvianolic acid P and >1 for salvianolic acid Q, providing a reliable diagnostic criterion for differentiating these isomeric compounds.

5.3. Structural characterization of isolated compounds through NMR

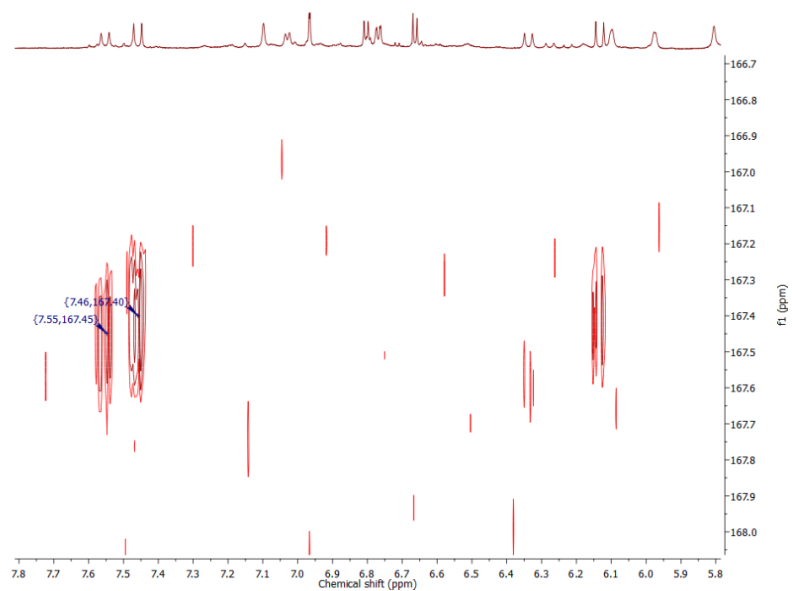
All MdiCQAs (**4–7**) and DiMDiCQAs (**9**, **10**) characterized in the present study were considered as novel compounds. Previously, one related derivative, i.e., 3,5-*O*-dicafeoyl-4-*O*-malonylquinic acid, was isolated from *Centella asiatica* (61). In our case, compound **6** showed similar substitution positions of malonyl and caffeoyl groups; however, the core structure possessed a different stereochemistry and was determined to be an *epi*-quinic acid. A spectroscopic comparison was provided for the differentiation of quinic and *epi*-quinic acid, where H-4 served as a diagnostic proton to decide the relative configuration of 1,3,4,5-tetrahydroxycyclohexanecarboxylic acid. A large trans-diaxial coupling constant ($^3J_{4/5} \approx 9$ Hz) in DMSO- d_6 indicated a quinic acid moiety, while a

smaller coupling constant ($J < 8$ Hz) pointed to an *epi*-quinic acid moiety. A comprehensive analysis was provided for the differentiation of quinic and *epi*-quinic acids for the first time in the present study.

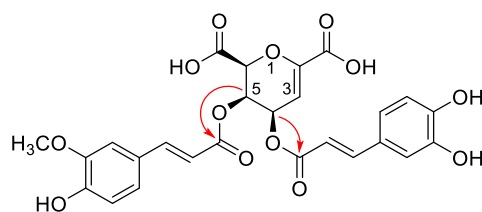
In earlier studies, 4,5-di-*O*-(*E*)-caffeoyl daucic has been identified possessing *xylo* (30) and *lyxo* (28) configurations of daucic acid subunit. In our study, the resonance values and multiplicity patterns of compound **11** align with the *lyxo* configuration (28). Previously, a methylated derivative, i.e., 4-*O*-caffeoyl-5-*O*-feruloyl daucic acid, has been reported by Muarat et al. (28). The NMR data of our compound **13** (4-*O*-(*E*)-feruloyl-5-*O*-(*E*)-caffeoyl daucic acid) align with those of the reported derivative; however, our assigned positions of feruloyl and caffeoyl groups contradict the previous study. We noted that the key correlations from oxymethine protons of daucic acid with carbonyl carbons of feruloyl and caffeoyl groups are missing in the HMBC spectrum provided by Murata et al., suggesting the absence of spectroscopic support for their positional assignments. We were able to attain these diagnostic cross-peaks by recording the HMBC spectra at higher temperature (313 K) (Figures 12, 13), which enabled the correct identification of both isomeric compounds **12** and **13**. Hence, compound **12** is considered a novel derivative, while compound **13** is indicative of an already known analogue, which was mistakenly assigned the name of compound **12** in previous study.



(a)

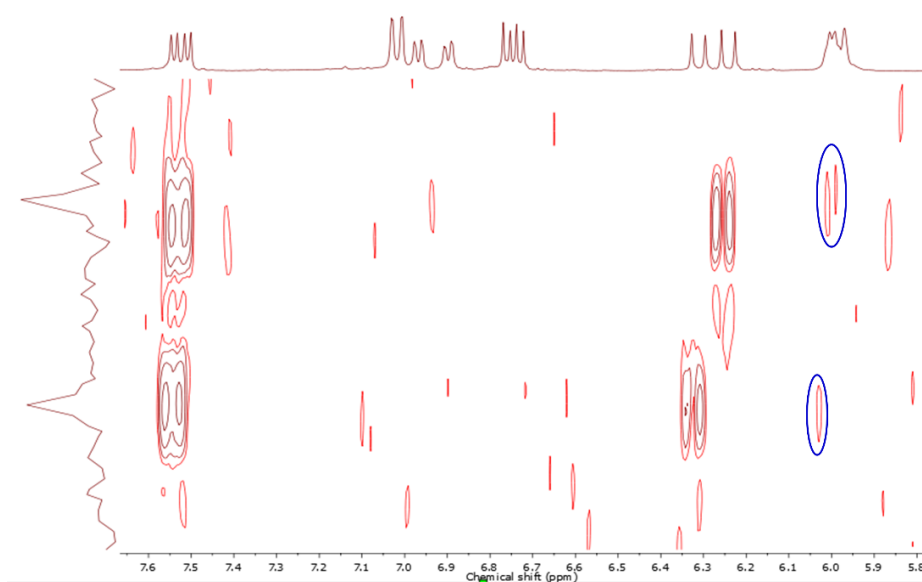


(b)



(c)

Figure 12. Zoomed-in (a) and band-selective (b) ^1H - ^{13}C HMBC (MeOH- d_4 , 700/176 MHz) spectrum of compound **12** recorded at 313 K with highlighted daucic acid proton peaks (c) indicating their correlations with carbonyls of caffeoyl and feruloyl groups.
Source: Author's own work.



(a)

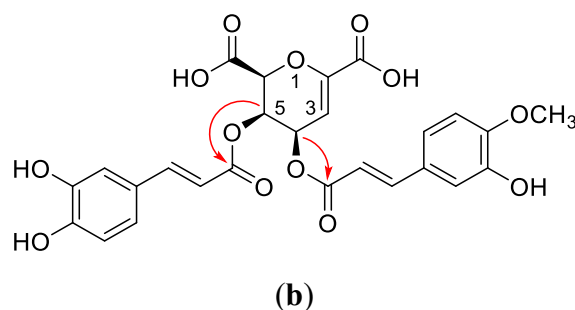


Figure 13. (a) Band-selective ^1H – ^{13}C HMBC (MeOH- d_4 , 500/125 MHz) spectrum of compound **13** recorded at elevated temperature (313 K) with highlighted daucic acid proton peaks (b) indicating their correlations with carbonyls of feruloyl and caffeoyl groups.

Source: Author's own work.

The isomeric compound of salvianolic acid K (**15**) is identified for the first time in our study, which is named as salvianolic acid O (**17**). The conversion product of salvianolic acid K (**15**) also represents a new derivative, which is named salvianolic acid P (**19**). Among FMGIs, luteolin-7-*O*-(6"-*O*-malonyl)- β -D-glucoside (**2**) (129) was previously reported; however, its dimalonyl derivative (**8**), i.e., luteolin-7-*O*-(2",6"-di-*O*-malonyl)- β -D-glucoside, has been revealed for the first time.

5.4. Quantification of metabolites in selected Apiaceae species

Considering the organ- and vegetation phase-specific accumulation of metabolites, optimal tissues containing the highest levels of selected metabolites were identified, enabling their isolation through a one-step preparative HPLC method.

We isolated 1) from *C. bulbosum*: MDiCQAs **4**, **7**, and **9** and FMGIs **1** and **3** from inflorescences, while FMGIs **2** and **8** from leaves collected at the beginning of the vegetation cycle (BVC), 2) from *A. cerefolium*: MDiCQAs **4** and **7** from inflorescences, while diMDiCQA **9** from leaves harvested at the BVC, 3) from *A. sylvestris*: MDiCQAs **4**, **5**, and **6** from leaves collected at the BVC, 4) from *A. caucalis*: diMDiCQA **10** from inflorescences, 5) from *A. nitida*: DiCDA **11** from the roots collected at the post vegetative phase (PVP), 6) from *M. odorata*: DiCDA **11**, FCDA **12** and **13** from the roots collected at the PVP.

5.5. Quantification of metabolites selected in *Salvia* species

Enzymes require an aqueous environment to maintain their native structure and catalytic activity (130). However, exposure to heat and/or organic solvents can degrade

enzymes, thereby preventing their catalytic activity (131). Currently, metabolite transformation occurred upon aqueous incubation of powdered plant tissues at room temperature. In contrast, the metabolites remained intact when the powdered tissues were incubated in hot water at 100 °C. This observation suggests that the aqueous environment activates endogenous enzymes responsible for metabolite conversion, while heat treatment inactivates these enzymes and prevents the transformation of metabolites. Furthermore, the involvement of spontaneous non-enzymatic processes, such as hydrolysis or oxidation, can be excluded based on the lack of transformation following heat treatment. Further mechanistic studies are still required to identify the specific enzymes involved and to clarify the detailed biochemical pathways responsible for these conversions.

Enzymatic conversion of compounds in aqueous media may also be a practical strategy for producing metabolites that are absent or occur only at trace levels in intact plant tissues. Thus, metabolites and their conversion products were isolated from optimal plant tissue after optimized aqueous incubations using a simple one-step preparative HPLC procedure.

Rosmarinic acid (**16**) was present in all selected species and was therefore isolated as a common compound from the untreated root of *S. verticillata* (Sample 8) together with rosmarinic acid glucoside and salvianolic acid O. *S. officinalis* (Sample 2) contained the highest amounts of nepetoidins A and B after 30 min of aqueous incubation. Using this incubation period for preparative-scale isolation, nepetoidins A (**18**) and B (**21**) were obtained in yields of 0.012% and 0.067%, respectively. We could isolate nepetoidins A and B from *S. officinalis* leaves in yields of 0.012% and 0.067%, respectively. These yields are 50-fold and 15-fold higher for nepetoidins A and B, respectively, as compared to previously reported yields of these compounds (132).

Salvianolic acid K (**15**) was isolated from the untreated roots of *S. pratensis* (Sample 2), which contained significantly higher levels of this compound than those of *S. nemorosa* and *S. sclarea*. However, the relatively higher transformation efficacy of salvianolic acid K into salvianolic acid P (**19**) was determined in *S. sclarea* root; hence, this plant tissue was selected for the isolation of salvianolic acid P. Because the highest concentration of salvianolic acid P was detected in *S. sclarea* roots (sample 2) after 5 min

of aqueous incubation, this tissue was selected as the starting material for compound isolation.

Salvianolic acid O (**17**) was isolated from the untreated roots of *S. verticillata* (Sample 8); however, we were unable to isolate its conversion product, salvianolic acid Q (**20**), in pure form because co-eluting impurities could not be separated by preparative HPLC.

5.6. Evaluation of biological activities of isolated compounds

5.6.1. Determination of antioxidant potential

MDiCQAs (**4–7**, **9**, **10**) showed better antioxidant potential than standards, while DiCDA (**11**) exhibited comparable activity. However, FCDA (**12**, **13**) demonstrated relatively lower antioxidant activity as compared to the standards. The superior antioxidant activity of MDiCQAs and DiCDA is ascribed to their catechol moiety, which acts as a hydrogen donor to stabilize the DPPH radical (133). However, in FCDA (**12**, **13**), the methylation of hydroxyl groups hinders the hydrogen-donating sites, resulting in reduced radical-scavenging activity (134). The antioxidant potential of all these compounds has been investigated for the first time, except for DiCDA (**11**). A previous study reported DPPH radical-scavenging activity of DiCDA (**11**) in terms of Trolox equivalents (mM) (30), whereas the present study expresses antioxidant activity as IC₅₀ values (μM).

Consistent with a previous report (135), rosmarinic acid (**16**) exhibited stronger DPPH radical-scavenging activity than its glucoside derivative, with IC₅₀ values of 11.2 ± 0.2 and 15.1 ± 0.3 μM, respectively. This finding is consistent with the established structure–activity relationship of phenolic antioxidants, which identifies catechol moieties bearing free ortho-dihydroxyl groups as key structural determinants of DPPH radical-scavenging activity. Accordingly, glycosylation of one hydroxyl group within the catechol unit reduces antioxidant potency (136).

Salvianolic acid K (**15**), salvianolic acid O (**17**), salvianolic acid P (**19**), together with nepetoidin B (**21**), displayed comparable antioxidant activities. These activities were comparable to those of rosmarinic acid and were consistent with previously reported data for nepetoidin B (137). Notably, all four compounds contain two catechol moieties, whereas nepetoidin A (**18**), which contains only one catechol moiety and one resorcinol-

type aromatic ring, exhibited significantly weaker activity. This comparison further supports the predominant role of catechol units in determining the antioxidant capacity of the isolated phenylpropanoids. Beyond providing the first experimental evidence for the antioxidant activities of salvianolic acid K and nepetoidin A, this study also reveals the antioxidant potential of the newly identified compounds, including salvianolic acid O and salvianolic acid P.

The effective FMGIs (**1**, **2**, and **8**) contain the B ring with *o*-substituted hydroxyls, whereas the least active FMGI (**3**) features only a single hydroxyl group in this ring, thereby limiting its radical-stabilization potential. Our findings for FMGIs **1** and **3** are in close agreement with earlier studies (124, 138). Moreover, the DPPH inhibition potential of FMGI **2** and currently identified FMGI **8** has been demonstrated for the first time.

5.6.2. Determination of cytostatic potential

Both effective MDiCQAs (**4**, **9**) share several comparable structural characteristics: their quinic acid core is substituted with caffeoyl groups at positions 1 and 5, and a malonyl group is present at position 3. The ineffective MDiCQA (**7**) possesses the caffeoyl groups at the same positions, but its malonyl group is substituted at position 4. Likewise, the other ineffective MDiCQAs (**5**, **6**) possess caffeoyl groups at positions 3 and 5, while a malonyl group at position 1 in compound **5** and at position 4 in compound **6**. Based on these findings, we can conclude that the substitution of malonyl group at position 3 and caffeoyl moieties at positions 1 and 5 is essential for the cytostatic activity of MDiCQAs. These results also demonstrated the involvement of isomer-specificity in exhibiting the cytostatic activity of MDiCQAs. Earlier, 1,3-dicaffeoyl-5-malonyl- δ -quinide, a structurally related compound to the MDiCQAs, exhibited non-selective in vitro cytotoxicity against both human leukemia cells (MOLM-13) and non-cancerous rat kidney epithelial cells (NRK) (62). Our study revealed the non-selective cytostatic impact of MDiCQAs (**4**, **9**) on different cancerous cells and non-cancerous Vero E6 cells, thus supporting this previous finding.

The better cytostatic activity of conversion products (**18**, **19**, **21**) as compared to CDPLAs (**14–17**) may be attributed to higher apolarity of these compounds due to the absence of carboxyl groups, which may facilitate cellular uptake and thereby enhance their cytostatic effects. To our knowledge, the cytostatic effect of all the test compounds has been reported for the first time against the specific cancer cell lines.

6. CONCLUSIONS

1) The present dissertation explored the phytochemical profile of various tissues from six Apiaceae species: *A. cerefolium*, *C. bulbosum*, *A. sylvestris*, *A. caucalis*, *A. nitida*, and *M. odorata*, and from five *Salvia* species: *S. officinalis*, *S. nemorosa*, *S. pratensis*, *S. sclarea*, and *S. verticillata*.

2) Metabolites were initially identified in different organs of the analyzed plant species through HPLC-UV-Orbitrap-HR-MS and quantified through an external calibration method. Based on this analysis, optimal tissue and treatment conditions were determined, enabling the isolation of selected compounds via a single-step preparative HPLC method.

3) Thirteen compounds were isolated from Apiaceae species, which were classified as caffeoylquinic acids (CQAs) (4–7, 9, 10), caffeoyldaucic acids (CDAs) (11–13), and flavonoid-malonyl-glucosides (FMGs) (1–3, 8).

4) Seven compounds (14–17) were isolated from *Salvia* species, belonging to the caffeoyl-dihydroxyphenyllactic acids (CDHPLAs) and related derivatives (18, 19, 21).

5) The rapid degradation of rosmarinic acid, accompanied by the transient formation of nepetoidins A and B, followed by their subsequent decomposition, was demonstrated to be a common enzymatic process in *Salvia* species. Furthermore, salvianolic acid K, detected for the first time in the roots of *S. nemorosa*, *S. pratensis*, and *S. sclarea*, and salvianolic acid O, identified as a previously undescribed natural product in the roots of *S. verticillata*, underwent analogous enzymatic transformations in pulverized root tissues. These reactions resulted in the formation of two newly identified compounds, salvianolic acids P and Q, respectively, which were subsequently degraded.

These findings demonstrate that extraction conditions can substantially influence the phytochemical composition and, consequently, the biological properties of extracts prepared from these plants..

6) The structures of all the isolated compounds were confirmed through 1D and 2D NMR experiments, which enabled complete ¹H and ¹³C assignments for these compounds.

7) MS/MS fragment ion profiles of isomeric compounds were evaluated at different CID energies, and energy-dependent differences in diagnostic fragment ion intensities enabled isomer differentiation.

8) Among the twenty isolated compounds, ten compounds were considered novel derivatives, including six CQAs (**4–7, 9, 10**), a CDA (**12**), a FMGI (**8**), a CDHPLA (**17**), and a related derivative (**19**).

9) Both the structure-specific radical scavenging capacity and the cytostatic activities of these compounds were confirmed.

7. SUMMARY

Twenty compounds were isolated from eleven plant species belonging to two taxonomic groups: the Apiaceae family and the genus *Salvia*. The isolated compounds were classified as caffeoylquinic acids (CQAs) (**4–7, 9, 10**), caffeoylidaucic acids (CDAs) (**11–13**), caffeoyl-dihydroxyphenyllactic acids (CDHPLA) (**14–19, 21**), and flavonoid-malonyl-glucosides (FMGLs) (**1–3, 8**). Determining optimal tissues and treatment conditions allowed the isolation of these compounds. Among them, ten compounds were identified as previously undescribed natural compounds, including six CQAs (**4–7, 9, 10**), a CDA (**12**), a FMGL (**8**), a CDHPLA (**17**), and a related derivative (**19**). The structures of the isolated compounds were characterized through HPLC-UV-Orbitrap-HR-MS/MS and NMR analyses. All the isolated compounds showed promising DPPH inhibition potential with IC₅₀ values of 7–27 μ M, except compound **3**, which showed an IC₅₀ value of 233 μ M. CQAs (**4, 9**) and FMGL (**3**) showed non-selective cytostatic effects. The conversion products (**18, 19, 21**) showed better cytostatic activity as compared to CDPLAs (**14–17**).

8. REFERENCES

1. Ogbuagu OO, Mbata AO, Balogun OD, Oladapo O, Ojo OO, Muonde M. Novel phytochemicals in traditional medicine: Isolation and pharmacological profiling of bioactive compounds. *Int J Med All Body Health*. 2022;3(1):63–71.
2. Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. *Nucleus*. 2022;65(3):399–411.
3. Chaachouay N, Zidane L. Plant-derived natural products: a source for drug discovery and development. *Drugs Drug Candidates*. 2024;3(1):184–207.
4. Li Z, Chen K, Rose P, Zhu YZ. Natural products in drug discovery and development: Synthesis and medicinal perspective of leonurine. *Front Chem*. 2022;10:1036329–41.
5. Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnol Rep*. 2019;24:370–80.
6. Teixeira J, Gaspar A, Garrido EM, Garrido J, Borges F. Hydroxycinnamic acid antioxidants: An electrochemical overview. *Biomed Res Int*. 2013;2013(1):251754–65.
7. Sova M, Saso L. Natural sources, pharmacokinetics, biological activities and health benefits of hydroxycinnamic acids and their metabolites. *Nutrients*. 2020;12(8):2190–220.
8. Zielińska D, Zieliński H, Laparra-Llopis JM, Szawara-Nowak D, Honke J, Giménez-Bastida JA. Caffeic acid modulates processes associated with intestinal inflammation. *Nutrients*. 2021;13(2):554–68.
9. Cortez N, Villegas C, Burgos V, Cabrera-Pardo JR, Ortiz L, González-Chavarría I, Nchiozem-Ngnitedem VA, Paz C. Adjuvant properties of caffeic acid in cancer treatment. *Int J Mol Sci*. 2024;25(14):7631–57.
10. Andrade M, Benfeito S, Soares P, Silva DM, Loureiro J, Borges A, Borges F, Simões M. Fine-tuning of the hydrophobicity of caffeic acid: studies on the antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. *RSC Adv*. 2015;5(66):53915–25.
11. Espíndola KMM, Ferreira RG, Narvaez LEM, Rosario ACRS, Da Silva AHM, Silva AGB, Vieira APO, Monteiro MC. Chemical and pharmacological aspects of caffeic acid and its activity in hepatocarcinoma. *Front Oncol*. 2019;9:541–51.

12. Sidoryk K, Jaromin A, Filipeczak N, Cmoch P, Cybulski M. Synthesis and antioxidant activity of caffeic acid derivatives. *Molecules*. 2018;23(9):2199–211.
13. Peng X, Hu T, Zhang Y, Zhao A, Natarajan B, Wei J, Yan H, Chen H, Lin C. Synthesis of caffeic acid sulfonamide derivatives and their protective effect against H₂O₂ induced oxidative damage in A549 cells. *RSC Adv*. 2020;10(17):9924–33.
14. Mao Y, Luo J, Cai Z. Biosynthesis and regulatory mechanisms of plant flavonoids: A review. *Plants*. 2025;14(12):1847–77.
15. Liga S, Paul C, Péter F. Flavonoids: Overview of biosynthesis, biological activity, and current extraction techniques. *Plants*. 2023;12(14):2732–57.
16. Lepiniec L, Debeaujon I, Routaboul JM, Baudry A, Pourcel L, Nesi N, Caboche M. Genetics and biochemistry of seed flavonoids. *Annu. Rev. Plant Biol*. 2006;57(1):405–30.
17. Li Y, Mao J, Liu Q, Song X, Wu Y, Cai M, Xu H, Qiao M. De novo biosynthesis of caffeic acid from glucose by engineered *Saccharomyces cerevisiae*. *ACS Synth Biol*. 2020;9(4):756–65.
18. Zhang H, Stephanopoulos G. Engineering *E. coli* for caffeic acid biosynthesis from renewable sugars. *Appl Microbiol Biotechnol*. 2013;97(8):3333–41.
19. Li Y, Tang W, Chen J, Jia R, Ma L, Wang S, Wang J, Shen X, Chu Z, Zhu C. Development of marker-free transgenic potato tubers enriched in caffeoylquinic acids and flavonols. *J Agric Food Chem*. 2016;64(14):2932–40.
20. Yan C, Li C, Jiang M, Xu Y, Zhang S, Hu X, Chen Y, Lu S. Systematic characterization of gene families and functional analysis of PvRAS3 and PvRAS4 involved in rosmarinic acid biosynthesis in *Prunella vulgaris*. *Front Plant Sci*. 2024;15:1374912–32.
21. Yu J, Xie J, Sun M, Xiong S, Xu C, Zhang Z, Li M, Li C, Lin L. Plant-derived caffeic acid and its derivatives: An overview of their NMR data and biosynthetic pathways. *Molecules*. 2024;29(7):1625–55.
22. Alcázar Magaña A, Kamimura N, Soumyanath A, Stevens JF, Maier CS. Caffeoylquinic acids: Chemistry, biosynthesis, occurrence, analytical challenges, and bioactivity. *Plant j*. 2021;107(5):1299–1319.

23. Clifford MN, Jaganath IB, Ludwig IA, Crozier A. Chlorogenic acids and the acyl-quinic acids: Discovery, biosynthesis, bioavailability and bioactivity. *Nat Prod Rep.* 2017;34(12):1391–1421.
24. Clifford MN, Marks S, Knight S, Kuhnert N. Characterization by LC-MSⁿ of four new classes of *p*-coumaric acid-containing diacyl chlorogenic acids in green coffee beans. *J Agric Food Chem.* 2006;54(12):4095–4101.
25. Clifford MN, Knight S, Surucu B, Kuhnert N. Characterization by LC-MS of four new classes of chlorogenic acids in green coffee beans: Dimethoxycinnamoylquinic acids, diferuloylquinic acids, caffeoyl-dimethoxycinnamoylquinic acids, and feruloyl-dimethoxycinnamoylquinic acids. *J Agric Food Chem.* 2006;54(6):1957–69.
26. Wang D, Bădărau AS, Swamy MK, Shaw S, Maggi F, Da Silva LE, López V, Yeung AWK, Mocan A, Atanasov AG. *Arctium* species secondary metabolites chemodiversity and bioactivities. *Front Plant Sci.* 2019;10:834–61.
27. Ma C, Dastmalchi K, Whitaker BD, Kennelly EJ. Two new antioxidant malonated caffeoylquinic acid isomers in fruits of wild eggplant relatives. *J Agric Food Chem.* 2011;59(17):9645–51.
28. Murata T, Katagiri T, Ishikawa Y, Abe M, Takahashi E, Iwahana R, Sakamoto Y, Sasaki K. Inhibitory effects of phenylpropanoid derivatives from *Oenanthe javanica* on antigen-stimulated degranulation in RBL-2H3 cells. *J Nat Prod.* 2019;82(6):1518–26.
29. Kardon T, Stroobant V, Veiga-da-Cunha M, Schaftingen EV. Characterization of mammalian sedoheptulokinase and mechanism of formation of erythritol in sedoheptulokinase deficiency. *FEBS Lett.* 2008;582(23-24):3330–34.
30. Dini I, Tenore GC, Dini A. New polyphenol derivative in *Ipomoea batatas* tubers and its antioxidant activity. *J Agric Food Chem.* 2006;54(23):8733–7.
31. Zhang X, Feng ZM, Yang YN, Jiang JS, Zhang PC. Phenolic acid derivatives from *Ligusticum chuanxiong*. *Phytochem Lett.* 2019;33:114–8.
32. Du G, Song J, Du L, Zhang L, Qiang G, Wang S, Yang X, Fang L. Chemical and pharmacological research on the polyphenol acids isolated from Danshen: A review of salvianolic acids. *Adv Pharmacol.* 2020;87:1–41.

33. Khodzhaieva RS, Gladkov ES, Kyrychenko A, Roshal AD. Progress and achievements in glycosylation of flavonoids. *Front Chem.* 2021;9:637994–8002.
34. Xiao J, Muzashvili TS, Georgiev MI. Advances in the biotechnological glycosylation of valuable flavonoids. *Biotechnol Adv.* 2014;32(6):1145–56.
35. Essa AF, Teleb M, El-Kersh DM, Gendy AE, Elshamy AI, Farag MA. Natural acylated flavonoids: Their chemistry and biological merits in context to molecular docking studies. *Phytochem Rev.* 2023;22(6):1469–1508.
36. Grujić-Letić N, Rakić B, Šefer E, Rakić D, Nedeljković I, Kladar N, Božin B. Determination of 5-caffeoylquinic acid (5-CQA) as one of the major classes of chlorogenic acid in commercial tea and coffee samples. *Vojnosanit Pregl.* 2015;72(11):1018–23.
37. Alcazar Magana A, Wright K, Vaswani A, Caruso M, Reed RL, Bailey CF, Nguyen T, Gray NE, Soumyanath A, Quinn J. Integration of mass spectral fingerprinting analysis with precursor ion (MS1) quantification for the characterisation of botanical extracts: application to extracts of *Centella asiatica* (L.) Urban. *Phytochem Anal.* 2020;31(6):722–38.
38. Hellström J, Granato D, Mattila PH. Accumulation of phenolic acids during storage over differently handled fresh carrots. *Foods.* 2020;9(10):1515–28.
39. Tajner-Czopek A, Gertchen M, Rytel E, Kita A, Kucharska AZ, Sokół-Łętowska A. Study of antioxidant activity of some medicinal plants having high content of caffeic acid derivatives. *Antioxidants.* 2020;9(5):412–33.
40. Liu L, Zhang J, Zheng B, Guan Y, Wang L, Chen L, Cai W. Rapid characterization of chlorogenic acids in *Duhaldea nervosa* based on ultra-high-performance liquid chromatography-linear trap quadrupole-Orbitrap-mass spectrometry and mass spectral trees similarity filter technique. *J Sep Sci.* 2018;41(8):1764–74.
41. Orlando G, Zengin G, Ferrante C, Ronci M, Recinella L, Senkardes I, Gevrenova R, Zheleva-Dimitrova D, Chiavaroli A, Leone S. Comprehensive chemical profiling and multidirectional biological investigation of two wild Anthemis species (*Anthemis tinctoria* var. *pallida* and *A. cretica* subsp. *tenuiloba*): focus on neuroprotective effects. *Molecules.* 2019;24(14):2582–607.

42. Zhang LN, Wang L, Shi ZQ, Li P, Li HJ. A metabolomic strategy based on integrating headspace gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry to differentiate the five cultivars of *Chrysanthemum* flower. *RSC Adv.* 2018;8(17):9074–82.
43. Arsiningtyas IS, Gunawan-Puteri MD, Kato E, Kawabata J. Identification of α -glucosidase inhibitors from the leaves of *Pluchea indica* (L.) Less., a traditional Indonesian herb: promotion of natural product use. *Nat Prod Res.* 2014;28(17):1350–3.
44. Sharma Y, Velamuri R, Fagan J, Schaefer J. Full-spectrum analysis of bioactive compounds in rosemary (*Rosmarinus officinalis* L.) as influenced by different extraction methods. *Molecules.* 2020;25(20):4599–620.
45. Bakota EL, Winkler-Moser JK, Berhow MA, Eller FJ, Vaughn SF. Antioxidant activity and sensory evaluation of a rosmarinic acid-enriched extract of *Salvia officinalis*. *J Food Sci.* 2015;80(4):711–7.
46. Caleja C, Barros L, Barreira JC, Ciric A, Sokovic M, Calhella RC, Beatriz M, Oliveira P, Ferreira IC. Suitability of lemon balm (*Melissa officinalis* L.) extract rich in rosmarinic acid as a potential enhancer of functional properties in cupcakes. *Food Chem.* 2018;250:67–74.
47. Eghlima G, Aghamir F, Hajizadeh HS, Zarbakhsh S. Role of melatonin in promoting growth attributes, thymol, rosmarinic acid and biochemical properties in *Thymus vulgaris* L. under water deficiency. *BMC Plant Biology.* 2025;25(1):603–21.
48. Sun Y, Zhu H, Wang J, Liu Z, Bi J. Isolation and purification of salvianolic acid A and salvianolic acid B from *Salvia miltiorrhiza* by high-speed counter-current chromatography and comparison of their antioxidant activity. *J Chromatogr B.* 2009;877(8-9):733–7.
49. Ai CB, Li LN. Salvianolic acids D and E: Two new depsides from *Salvia miltiorrhiza*. *Planta Medica.* 1992;58(02):197–9.
50. Li W, Zhou SP, Jin YP, Huang XF, Zhou W, Han M, Yu Y, Yan KJ, Li SM, Ma XH. Salvianolic acids T and U: A pair of atropisomeric trimeric caffeic acids derivatives from root of *Salvia miltiorrhiza*. *Fitoterapia.* 2014;98:248–53.

51. Ai CB, Li LN. Stereostructure of salvianolic acid B and isolation of salvianolic acid C from *Salvia miltiorrhiza*. J Nat Prod. 1988;51(1):145–9.
52. Hou X, Zhou C, Liang Z, Qiu H, Li Z, Wang Y, Qi X, Lu L, Cao Y, Zheng J. Salvianolic acid F suppresses KRAS-dependent lung cancer cell growth through the PI3K/AKT signaling pathway. Phytomedicine. 2023;121:155093–104.
53. Lu Y, Foo LY. Salvianolic acid L, a potent phenolic antioxidant from *Salvia officinalis*. Tetrahedron Lett. 2001;42(46):8223–5.
54. Gong J, Ju A, Zhou D, Li D, Zhou W, Geng W, Li B, Li L, Liu Y, He Y. Salvianolic acid Y: A new protector of PC12 cells against hydrogen peroxide-induced injury from *Salvia officinalis*. Molecules. 2015;20(1):683–92.
55. Zhang HJ, Li LN. Salvianolic acid I: a new depside from *Salvia cavaleriei*. Planta Med. 1994;60(1):70–2.
56. Ai CB, Deng QH, Song WZ, Li LN. Salvianolic acid J, a depside from *Salvia flava*. Phytochemistry. 1994;37(3):907–8.
57. Sotiropoulou NS, Megremi SF, Tarantilis P. Evaluation of antioxidant activity, toxicity, and phenolic profile of aqueous extracts of chamomile (*Matricaria chamomilla* L.) and sage (*Salvia officinalis* L.) prepared at different temperatures. Appl Sci. 2020;10(7):2270–84.
58. He Q, Wu J, Wang X, Yang J. Inhibition Effects of salvianolic acid M and rosmarinic acid in *Salvia deserta* Schang on human aldose reductase. Asian J Agric Res. 2019;11(8):69–70
59. Zhang ZF, Chen HS, Peng ZG, Li ZR, Jiang JD. A potent anti-HIV polyphenol from *Salvia yunnanensis*. J Asian Nat Prod Res. 2008;10(3):252–5.
60. Wang SH, Hu YL, Liu TX. Plant distribution and pharmacological activity of flavonoids. Tradit Med Res. 2019;4(5):269–87.
61. Antognoni F, Perellino NC, Crippa S, Dal Toso R, Danieli B, Minghetti A, Poli F, Pressi G. Irbic acid, a dicaffeoylquinic acid derivative from *Centella asiatica* cell cultures. Fitoterapia. 2011;82(7):950–4.
62. Slimestad R, Rathe BA, Aesoy R, Diaz AEC, Herfindal L, Fossen T. A novel bicyclic lactone and other polyphenols from the commercially important vegetable *Anthriscus cerefolium*. Sci Rep. 2022;12(1):7805–12.

63. Şahin S, Tezcan G, Demir C, Tunca B, Çeçener G, Egeli Ü. Cytotoxic activity of rosmarinic acid isolated from *Prunella vulgaris* L. and *Prunella grandiflora* L. in different tumor cells. *Trak Univ J Nat Sci*. 2017;18(1):9–13.
64. Hao Y, Xie T, Korotcov A, Zhou Y, Pang X, Shan L, Ji H, Sridhar R, Wang P, Califano J. Salvianolic acid B inhibits growth of head and neck squamous cell carcinoma in vitro and in vivo via cyclooxygenase-2 and apoptotic pathways. *Int J Cancer*. 2009;124(9):2200–9.
65. Sha W, Zhou Y, Ling Z-Q, Xie G, Pang X, Wang P, Gu X. Antitumor properties of salvianolic acid B against triple-negative and hormone receptor-positive breast cancer cells via ceramide-mediated apoptosis. *Oncotarget*. 2018;9(91):36331–43.
66. C Maiyo F, Moodley R, Singh M. Cytotoxicity, antioxidant and apoptosis studies of quercetin-3-*O* glucoside and 4-(β-D-glucopyranosyl-1→ 4-α-L-rhamnopyranosyloxy)-benzyl isothiocyanate from *Moringa oleifera*. *Anti-Cancer Agents Med Chem*. 2016;16(5):648–56.
67. Mukhija M, Singh MP, Dhar KL, Kalia AN. Cytotoxic and antioxidant activity of *Zanthoxylum alatum* stem bark and its flavonoid constituents. *J Pharmacogn Phytochem*. 2015;4(4):86–93.
68. Goodarzi S, Tabatabaei MJ, Mohammad Jafari R, Shemirani F, Tavakoli S, Mofasseri M, Tofighi Z. *Cuminum cyminum* fruits as source of luteolin-7-*O*-glucoside, potent cytotoxic flavonoid against breast cancer cell lines. *Nat Prod Res*. 2020;34(11):1602–6.
69. Pirvu LC, Pintilie L, Albulescu A, Stefaniu A, Neagu G. Anti-proliferative potential of cynaroside and orientin-In silico (DYRK2) and in vitro (U87 and Caco-2) Studies. *Int J Mol Sci*. 2023;24(23):16555–79.
70. Mardani M, Badakné K, Szedljak I, Sörös C, Farmani J. Lipophilized rosmarinic acid: Impact of alkyl type and food matrix on antioxidant activity, and optimized enzymatic production. *Food Chem*. 2024;452:139518–28.
71. Zhao GR, Zhang HM, Ye TX, Xiang ZJ, Yuan YJ, Guo ZX, Zhao LB. Characterization of the radical scavenging and antioxidant activities of danshensu and salvianolic acid B. *Food Chem Toxicol*. 2008;46(1):73–81.

72. Panat NA, Amrute BK, Bhattu S, Haram SK, Sharma GK, Ghaskadbi SS. Antioxidant profiling of C3 quercetin glycosides: Quercitrin, quercetin 3- β -D-glucoside and quercetin 3-O-(6''-O-malonyl)- β -Dglucoside in cell free environment. *Free Rad Antiox*. 2015;5(2):90–100.
73. Taiwo FO, Oyedeji O, Osundahunsi MT. Antimicrobial and antioxidant properties of kaempferol-3-O-glucoside and 1-(4-Hydroxyphenyl)-3-phenylpropan-1-one isolated from the leaves of *Annona muricata* (Linn.). *J. Pharm. Res Int*. 2019;26:1–13.
74. Xu T, Wang C, Jiang S, Yang T, Wu X. Glycosylation of luteolin in hydrophilic organic solvents and structure–antioxidant relationships of luteolin glycosides. *RSC Adv*. 2022;12(28):18232–7.
75. Wang XJ, Luo Q, Li T, Meng PH, Pu YT, Liu JX, Zhang J, Liu H, Tan GF, Xiong AS. Origin, evolution, breeding, and omics of Apiaceae: A family of vegetables and medicinal plants. *Hortic Res*. 2022;9:76–100.
76. Lawton BP. Parsleys, Fennels, and Queen Anne's Lace. Oregon: Timber Press; 2011. p. 39–40.
77. Hendawy SF, Hussein MS, El-Gohary AE, Soliman WS. Chemical constituents of essential oil in chervil (*Anthriscus cerefolium* L. Hoffm.) cultivated in different locations. *J Essent Oil-Bear Plants*. 2019;22(1):264–72.
78. Baser K, Ermin N, Demirçakmak B. The essential oil of *Anthriscus cerefolium* (L.) Hoffm.(Chervil) growing wild in Turkey. *J Essent Oil Res*. 1998;10(4):463–4.
79. Stojković D, Drakulić D, Schwirtlich M, Rajčević N, Stevanović M, Soković MD, Gašić U. Extract of herba *Anthrisci cerefolii*: Chemical profiling and insights into its anti-glioblastoma and antimicrobial mechanism of actions. *Pharmaceuticals*. 2021;14(1):55–70.
80. Stojković D, Gašić U, Uba AI, Zengin G, Rajaković M, Stevanović M, Drakulić D. Chemical profiling of *Anthriscus cerefolium* (L.) Hoffm., biological potential of the herbal extract, molecular modeling and KEGG pathway analysis. *Fitoterapia*. 2024;177:106115–29.
81. Bussmann RW, Batsatsashvili K, Kikvidze Z, Paniagua-Zambrana NY, Khutsishvili M, Maisaia I, Sikharulidze S, Tchelidze D. *Anthriscus cerefolium*

- (L.) Hoffm. *Anthriscus sylvestris* (L.) Hoffm. Apiaceae. In: Ethnobotany of the Mountain Regions of Far Eastern Europe: Ural, Northern Caucasus, Turkey, and Iran. Heidelberg: Springer:2020. p. 107–12.
82. Berežni S, Mimica-Dukić N, Domina G, Raimondo FM, Orčić D. *Anthriscus sylvestris*-noxious weed or sustainable source of bioactive lignans? *Plants*. 2024;13(8):1087–127.
 83. Olaru OT, Nițulescu GM, Orțan A, Dinu-Pîrvu CE. Ethnomedicinal, phytochemical and pharmacological profile of *Anthriscus sylvestris* as an alternative source for anticancer lignans. *Molecules*. 2015;20(8):15003–22.
 84. Orčić D, Berežni S, Škorić D, Mimica-Dukić N. Comprehensive study of *Anthriscus sylvestris* lignans. *Phytochemistry*. 2021;192:112958–80.
 85. Dénes A, Papp N, Babai D, Czúcz B, Molnár Z. Wild plants used for food by Hungarian ethnic groups living in the Carpathian Basin. *Acta Soc Bot Pol*. 2012;81(4):381–96.
 86. Molo Z, Tel-Çayan G, Deveci E, Öztürk M, Duru ME. Insight into isolation and characterization of compounds of *Chaerophyllum bulbosum* aerial part with antioxidant, anticholinesterase, anti-urease, anti-tyrosinase, and anti-diabetic activities. *Food Biosci*. 2021;42:101201–11.
 87. Ferrer DB, Venskutonis PR, Talou T, Zebib B, Ferrer JMB, Merah O. Bioactive compounds and antioxidant properties of *Myrrhis odorata* deodorized residue leaves extracts from Lithuania and France origins. *Pharm Chem J*. 2016;3(3):43–8.
 88. Rančić A, Soković M, Vukojević J, Simić A, Marin P, Duletić-Laušević S, Djoković D. Chemical composition and antimicrobial activities of essential oils of *Myrrhis odorata* (L.) Scop, *Hypericum perforatum* L and *Helichrysum arenarium* (L.) Moench. *J Essent Oil Res*. 2005;17(3):341–5.
 89. Dobraval'skytė D, Venskutonis P, Zebib B, Merah O, Talou T. Essential oil composition of *Myrrhis odorata* (L.) Scop. leaves grown in Lithuania and France. *J Essent Oil Res*. 2013;25(1):44–8.
 90. Moein F, Jamzad Z, Rahiminejad M, Landis JB, Mirtadzadini M, Soltis DE, Soltis PS. Towards a global perspective for *Salvia* L.: Phylogeny, diversification and floral evolution. *J Evol Biol*. 2023;36(3):589–604.

91. Yaman C. Lemon balm and sage herbal teas: Quantity and infusion time on the benefit of the content. *CiencAgrotec*. 2020;44:23220–31.
92. Angeli L, Morozova K, Tenuta MC, Bolchini S, Scampicchio M, Ferrentino G. Identification of reactive compounds in *Salvia officinalis* L. extract by high-performance liquid chromatography (HPLC) in presence of 2, 2'-azobis (2-amidinopropane) dihydrochloride (AAPH) radicals. *Appl Food Res*. 2025;5:101249–57.
93. Luca SV, Skalicka-Woźniak K, Mihai CT, Grădinaru AC, Mandici A, Ciocarlan N, Miron A, Aprotosoiaie AC. Chemical profile and bioactivity evaluation of *Salvia* species from Eastern Europe. *Antioxidants*. 2023;12(8):1514–35.
94. Moshari-Nasirkandi R, Alirezalu A, Chamanabad HRM, Amato J, Alipour H, Asghari A, Moshari-Nasirkandi A. Screening of native wild *Salvia nemorosa* populations for chemical compositions, antioxidant activity and UHPLC-HRMS profiling. *Sci Rep*. 2024;14(1):32064–82.
95. Ivanov M, Božunović J, Gašić U, Drakulić D, Stevanović M, Rajčević N, Stojković D. Bioactivities of *Salvia nemorosa* L. inflorescences are influenced by the extraction solvents. *Ind Crops Prod*. 2022;175:114260–71.
96. Srećković N, Mišić D, Gašić U, Matić SL, Stanković JSK, Mihailović NR, Monti DM, D'Elia L, Mihailović V. Meadow sage (*Salvia pratensis* L.): A neglected sage species with valuable phenolic compounds and biological potential. *Ind Crops Prod*. 2022;189:115841–57.
97. Aćimović M, Kiproviski B, Rat M, Sikora V, Popović V, Koren A, Brdar-Jokanović M. *Salvia sclarea*: Chemical composition and biological activity. *J Agron Technol Eng Manag*. 2018;1(1):18–28.
98. Chaita E, Aligiannis N, Kouretas D, Skaltsounis A. Chemical profile characterization of biologically active extracts obtained from *Salvia* species. *Planta Med*. 2009;75(9):53–62.
99. Stanković JSK, Srećković N, Mišić D, Gašić U, Imbimbo P, Monti DM, Mihailović V. Bioactivity, biocompatibility and phytochemical assessment of lilac sage, *Salvia verticillata* L.(Lamiaceae)-A plant rich in rosmarinic acid. *Ind Crops Prod*. 2020;143:111932–43.

100. Ivanova S, Dzhakova Z, Staynova R, Ivanov K. *Salvia verticillata* (L.)- biological activity, chemical profile, and future perspectives. *Pharmaceuticals*. 2024;17(7):859–84.
101. Felegyi-Tóth CA, Garádi Z, Darcsi A, Csernák O, Boldizsár I, Béni S, Alberti Á. Isolation and quantification of diarylheptanoids from European hornbeam (*Carpinus betulus* L.) and HPLC-ESI-MS/MS characterization of its antioxidative phenolics. *J Pharm Biomed Anal*. 2022;210:114554–66.
102. Zou XY, He YJ, Yang YH, Yan XP, Li ZB, Yang H. Systematic identification of bioactive compositions in leaves of morus cultivars using UHPLC-ESI-QTOF-MS/MS and comprehensive screening of high-quality resources. *Separations*. 2022;9(3):76–92.
103. Ahadi H, Shokrpour M, Fatahi R, Naghavi MR, Mirjalili MH. Essential oil, flavonoids and anthocyanins profiling of some Iranian damask rose (*Rosa damascena* Mill.) genotypes. *Ind Crops Prod*. 2023;205:117579–92.
104. Yang M, Tian X, Zhang M, Wei J, Niu Y, Hou J, Jin Y, Du Y. A holistic comparison of flavor signature and chemical profile in different harvesting periods of *Chrysanthemum morifolium* Ramat. based on metabolomics combined with bioinformatics and molecular docking strategy. *RSC Adv*. 2022;12(54):34971–89.
105. Gavrilova V, Kajdzanoska M, Gjamovski V, Stefova M. Separation, characterization and quantification of phenolic compounds in blueberries and red and black currants by HPLC-DAD-ESI-MS. *J Agric Food Chem*. 2011;59(8):4009–18.
106. Valente IM, Moreira MM, Neves P, da Fé T, Gonçalves LM, Almeida PJ, Rodrigues JA. An insight on salting-out assisted liquid-liquid extraction for phytoanalysis. *Phytochem Anal*. 2017;28(4):297–304.
107. Nazli A, Gáborová M, Ausbittel T, Stipsicz B, Toth G, Bősze S, Béni S, Boldizsár I. Malonyl-caffeoylquinic acids and malonyl-flavonoid-glucosides from three edible Apiaceae plants *Anthriscus cerefolium*, *Anthriscus sylvestris*, and *Chaerophyllum bulbosum*. *ACS Omega*. 2025;10(38):43656–68.

108. Sojo LE, Chahal N, Keller BO. Oxidation of catechols during positive ion electrospray mass spectrometric analysis: Evidence for in-source oxidative dimerization. *Rapid Commun Mass Spectrom.* 2014;28(20):2181–90.
109. Öztürk M, Duru ME, Ince B, Harmandar M, Topçu G. A new rapid spectrophotometric method to determine the rosmarinic acid level in plant extracts. *Food Chem.* 2010;123(4):1352–6.
110. Määttä-Riihinen KR, Kamal-Eldin A, Törrönen AR. Identification and quantification of phenolic compounds in berries of *Fragaria* and *Rubus* species (family Rosaceae). *J Agric Food Chem.* 2004;52(20):6178–87.
111. Bouymajane A, Filali FR, El Majdoub YO, Ouadik M, Abdelilah R, Cavò E, Miceli N, Taviano MF, Mondello L, Cacciola F. Phenolic compounds, antioxidant and antibacterial activities of extracts from aerial parts of *Thymus zygis* subsp. *gracilis*, *Mentha suaveolens* and *Sideritis incana* from Morocco. *Chem Biodivers.* 2022;19(3):1–13.
112. Sun H, Wang MY, Huang JQ, Cui DX, Leng L, Gao XM, Li X, Yang WZ. Characterization and identification of the wide-polarity multicomponents from *Prunella vulgaris* by offline two-dimensional liquid chromatography and hydrophilic interaction chromatography coupled to ion mobility-quadrupole time-of-flight mass spectrometry. *Chromatogr A.* 2024;1732:465233–45.
113. Pauli G, Poetsch F, Nahrstedt A. Structure assignment of natural quinic acid derivatives using proton nuclear magnetic resonance techniques. *Phytochem Anal.* 1998;9(4):177–85.
114. Pauli GF, Kuczkowiak U, Nahrstedt A. Solvent effects in the structure dereplication of caffeoyl quinic acids. *Magn Reson Chem.* 1999;37(11):827–36.
115. Könczöl Á, Béni Z, Sipos MM, Rill A, Háda V, Hohmann J, Máthé I, Szántay Jr C, Keserü GM, Balogh GT. Antioxidant activity-guided phytochemical investigation of *Artemisia gmelinii* Webb. ex Stechm.: Isolation and spectroscopic challenges of 3, 5-O-dicaffeoyl (*epi*?) quinic acid and its ethyl ester. *J Pharm Biomed Anal.* 2012;59:83–9.
116. Tolonen A, Joutsamo T, Mattila S, Kämäräinen T, Jalonen J. Identification of isomeric dicaffeoylquinic acids from *Eleutherococcus senticosus* using HPLC-ESI/TOF/MS and ¹H-NMR methods. *Phytochem Anal.* 2002;13(6):316–28.

117. Wang Y, Hamburger M, Gueho J, Hostettmann K. Cyclohexanecarboxylic-acid derivatives from *Psiadia trinervia*. *Helv Chim Acta*. 1992;75(1):269–75.
118. Ono M, Masuoka C, Otake Y, Ikegashira S, Ito Y, Nohara T. Antioxidative constituents from *Tessaria integrifolia*. *Food Sci Technol Res*. 2007;6(2):106–14.
119. Wang Y, Wray V, Tsevegsuren N, Lin W, Proksch P. Phenolic compounds from the Mongolian medicinal plant *Scorzonera radiata*. *Z Naturforsch C*. 2012;67(3-4):135–43.
120. Mailis T, Skaltsa H. Polar constituents of *Salvia willeana* (Holmboe) Hedge, growing wild in Cyprus. *Plants*. 2018;7(1):18–29.
121. Zhou XJ, Yan LL, Yin PP, Shi LL, Zhang JH, Liu YJ, Ma C. Structural characterisation and antioxidant activity evaluation of phenolic compounds from cold-pressed *Perilla frutescens* var. *arguta* seed flour. *Food Chem*. 2014;164:150–7.
122. Ly TN, Shimoyamada M, Yamauchi R. Isolation and characterization of rosmarinic acid oligomers in *Celastrus hindsii* Benth leaves and their antioxidative activity. *J Agric Food Chem*. 2006;54(11):3786–93.
123. Timokhin V, Regner M, Tsuji Y, Grabber J, Ralph J. Synthesis of nepetoidin B. *Synlett*. 2018;29(9):1229–31.
124. Kamata K, Seo S, Nakajima JI. Constituents from leaves of *Apocynum venetum* L. *J Nat Med*. 2008;62(2):160–3.
125. Shimoda K, Otsuka T, Morimoto Y, Hamada H, Hamada H. Glycosylation and malonylation of quercetin, epicatechin, and catechin by cultured plant cells. *Chem Lett*. 2007;36(10):1292–3.
126. Sugawara T, Igarashi K. Identification of major flavonoids in petals of edible chrysanthemum flowers and their suppressive effect on carbon tetrachloride-induced liver injury in mice. *Food Sci Technol Res*. 2009;15(5):499–506.
127. Wald B, Wray V, Galensa R, Herrmann K. Malonated flavonol glycosides and 3, 5-dicaffeoylquinic acid from pears. *Phytochemistry*. 1989;28(2):663–4.
128. Kazuma K, Noda N, Suzuki M. Malonylated flavonol glycosides from the petals of *Clitoria ternatea*. *Phytochemistry*. 2003;62(2):229–37.

129. Feeny P, Sachdev K, Rosenberry L, Carter M. Luteolin 7-*O*-(6''-*O*-malonyl)- β -d-glucoside and trans-chlorogenic acid: Oviposition stimulants for the black swallowtail butterfly. *Phytochemistry*. 1988;27(11):3439–48.
130. Zaks A, Klibanov AM. Enzyme-catalyzed processes in organic solvents. *Proc Natl Acad Sci*. 1985;82(10):3192–6.
131. Daniel RM, Peterson ME, Danson MJ, Price NC, Kelly SM, Monk CR, Weinberg CS, Oudshoorn ML, Lee CK. The molecular basis of the effect of temperature on enzyme activity. *Biochemical journal*. 2010;425(2):353–60.
132. Wu X, Gao H, Sun W, Yu J, Hu H, Xu Q, Chen X. Nepetoidin B, a natural product, inhibits LPS-stimulated nitric oxide production via modulation of iNOS mediated by NF- κ B/MKP-5 pathways. *Phytother Res*. 2017;31(7):1072–7.
133. Anitha S, Krishnan S, Senthilkumar K, Sasirekha V. Theoretical investigation on the structure and antioxidant activity of (+) catechin and (–) epicatechin—A comparative study. *Mol Phys*. 2020;118(17):1745917–30.
134. Lemańska K, Van Der Woude H, Szymusiak H, Boersma MG, Gliszczyńska-Świgło A, Rietjens IM, Tyrakowska B. The effect of catechol *O*-methylation on radical scavenging characteristics of quercetin and luteolin-A mechanistic insight. *Free Radic. Res*. 2004;38(6):639–47.
135. Kim D, Lee J. Comparative evaluation of phenolic phytochemicals from perilla seeds of diverse species and screening for their tyrosinase inhibitory and antioxidant properties. *S Afr J Bot*. 2019;123:341–50.
136. Nie JY, Li R, Wang Y, Tan J, Tang SH, Jiang ZT. Antioxidant activity evaluation of rosemary ethanol extract and their cellular antioxidant activity toward HeLa cells. *J Food Biochem*. 2019;43(7):12851–62.
137. Zhou W, Xie H, Xu X, Liang Y, Wei X. Phenolic constituents from *Isodon lophanthoides* var. *graciliflorus* and their antioxidant and antibacterial activities. *J Funct Foods*. 2014;6:492–8.
138. Zheng CD, Li G, Li HQ, Xu XJ, Gao JM, Zhang AL. DPPH-scavenging activities and structure-activity relationships of phenolic compounds. *Nat Prod Commun*. 2010;5(11):1759–65.

9. BIBLIOGRAPHY OF THE CANDIDATE'S PUBLICATIONS

9.1. Publications related to the thesis

Nazli A, Gáborová M, Ausbuttel T, Stipsicz B, Toth G, Bősze S, Béni S, Boldizsár I. Malonyl-caffeoylquinic acids and malonyl-flavonoid-glucosides from three edible Apiaceae plants *Anthriscus Cerefolium*, *Anthriscus Sylvestris*, and *Chaerophyllum Bulbosum*. **ACS omega**. 2025;10(38):43656–68.

Nazli A, Szögi-Tatár B, Bősze S, Tóth G, Solymosi K, Béni S, Boldizsár I. Aqueous incubation reveals transformation of rosmarinic acid in medicinally important Nepetoideae species and leads to novel bioactive compounds. **Pharmaceutics**. 2026; 18(8):1043–75.

9.2. Publications not related to the thesis

Nazli A, Malanga M, Sohajda T, Béni S. Cationic cyclodextrin-based carriers for drug and nucleic acid delivery. **Pharmaceutics**. 2025;17(1):81–123.

Nazli A, Khan MZI, Rácz Á, Béni S. Acid-sensitive prodrugs; a promising approach for site-specific and targeted drug release. **Eur J Med Chem**. 2024;276:116699–731.

Naseem A, Liu Y, **Nazli A**, Kuang HX, Yang B.Y. An insight into indigenous ethnobotanical and pharmacological potential of Solanaceae family in Pakistan: a review. **J Herb Med**. 2023;42: 100763–73.

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