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How distillation alters bioactivity: Comparative phytochemical and biological evaluation of three Lamiaceae species (*Origanum sipyleum*, *Thymus sipyleus*, and *Sideritis phrygia*) before and after distillation

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ABSTRACT

In the current study chemical profiles and the biological activities of three different plant species (*Origanum sipyleum*, *Thymus sipyleus*, and *Sideritis phrygia*) of the Lamiaceae family native to Turkey were explored considering different approaches leading to extracts with different composition. We adopted for each plant extraction with methanol (Total Methanol), water infusion (W), supercritical CO_2 extract (SCO_2), essential oil (EO). Furthermore, the residues remaining after hydrodistillation and supercritical CO_2 extraction were subsequently extracted with methanol, yielding the Spent HD and Spent SCO_2 extracts for each plant species. To evaluate possible bioactivities several assays were performed. Antioxidant capacities were measured with multiple in vitro assays (ABTS, DPPH, FRAP, CUPRAC, PBD, and metal chelating ability (MCA), along with total phenolic content. Enzyme inhibitory activities of the obtained extracts were evaluated against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, amylase, and glucosidase, important enzymes involved in metabolic and degenerative disorders like diabetes, neurodegenerative and skin disorders. Methanol and water extracts generally exhibited the highest total phenolic content, along with the greatest antioxidant capacities. Among the remaining extracts, the spent fractions (especially spent SCO_2) also demonstrated notable antioxidant activity. Beneath them the extracts spent (especially spent SCO_2) were the most active. The different extraction procedures lead to different chemical composition with limited extraction of phytoconstituents in the SCO_2 and larger presence of phenolics in the other extracts. Salvianolic acid B, rosmarinic acid and kaempferol-hexoside-derivate were the main components in the total methanol extracts of *O. sipyleum*, *T. sipyleus* and *S. phrygia*, respectively. In same order, the main components were alloaromadendrene, valencene and spathulenol in their EOs. The results indicate that the plant materials after HD and SCO_2 are still rich in bioactive compounds and they can be used to extract bioactive compounds. The obtained extracts present different potential bioactivities and can be the starting point to design multidirectional health-promoting products for applications in pharmaceutical and nutraceutical areas.

1. Introduction

Naturally occurring compounds are known to interact with a variety of biological systems [1]. In the recent decade, there has been growing interest in determining natural products' antioxidant and other biological properties [2]. This is partially linked to the perception that dietary intake of a diet high in antioxidants is related to significant health benefits [3,4]. These antioxidants reduce ROS levels in the body and

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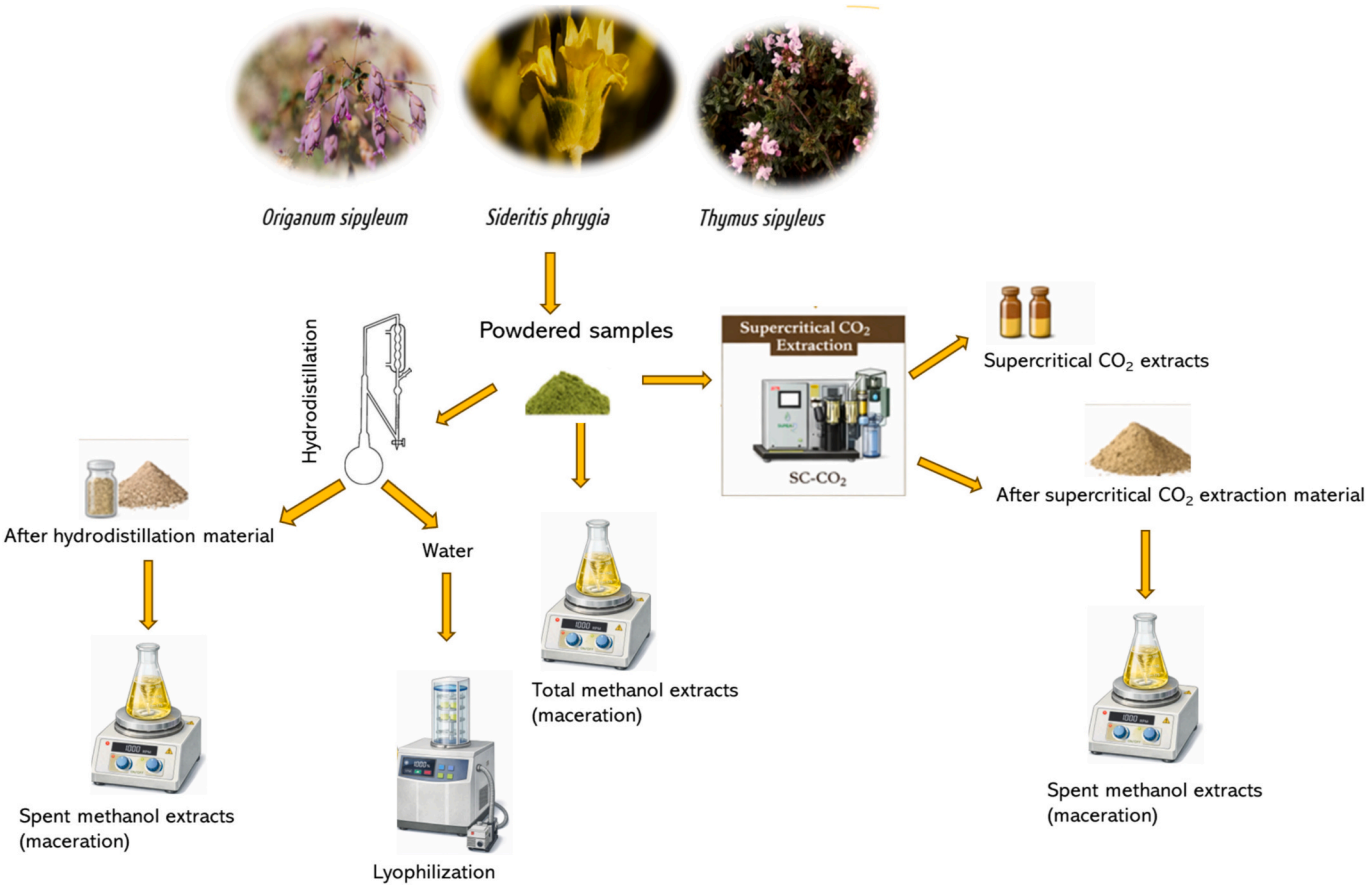


Fig. 1. The flowchart of the extraction procedures in the paper is used.

Table 1
Total phenolic and flavonoid contents in the tested extracts.*

Samples	Extraction	TPC (mg GAE/g)	TFC (mg RE/g)
Origanum sipyleum	Total MeOH	85.51 ± 0.81^{ab}	21.39 ± 0.65^{b}
	Water	82.96 ± 0.66^{b}	28.15 ± 0.11^{a}
	Spent after HD	86.24 ± 2.39^{a}	20.42 ± 0.49^{b}
	SCO_2	8.48 ± 0.15^{c}	8.97 ± 0.34^{d}
	Spent after SCO_2	86.42 ± 0.90^{a}	16.13 ± 0.53^{c}
	EO	–	–
Thymus sipyleus	Total MeOH	76.44 ± 0.75^{b}	75.28 ± 0.88^{a}
	Water	82.61 ± 1.09^{a}	34.29 ± 0.16^{c}
	Spent after HD	74.08 ± 1.25^{c}	67.26 ± 1.80^{b}
	SCO_2	16.19 ± 0.47^{d}	4.49 ± 0.07^{d}
	Spent after SCO_2	77.46 ± 0.68^{b}	77.40 ± 1.24^{a}
	EO	–	–
Sideritis phrygia	Total MeOH	62.96 ± 0.61^{a}	54.22 ± 0.40^{b}
	Water	58.79 ± 0.90^{b}	43.67 ± 0.37^{c}
	Spent after HD	63.02 ± 0.01^{a}	67.24 ± 0.54^{a}
	SCO_2	13.46 ± 0.89^{c}	15.20 ± 0.41^{d}
	Spent after SCO_2	63.76 ± 0.69^{a}	66.28 ± 0.34^{a}
	EO	–	–

* Values are reported as mean ± SD of three parallel measurements. EO: Essential oil; GAE: Gallic acid equivalents; RE: Rutin equivalents. Different letters indicate significant differences among the tested extracts from the same plant species ($p < 0.05$).

modulate processes associated with free-radical damage. Antioxidants can reduce the risk of developing chronic diseases. These diseases include cardiovascular and neurological problems, diabetes, cancer, hypertension, and conditions connected with the skin and blood [5,6].

The Lamiaceae family, popularly known as the mint family, comprises around 6900–7035 species that are usually aromatic and have medicinal and culinary importance, as well as ecological functions. Out of these, *O. sipyleum*, *T. sipyleus*, and *S. phrygia* are important members of this family because they exhibit distinct bioactive properties and are traditionally used in folk medicine [7]. These plant species are endemic to the Mediterranean basin and neighboring countries, especially Turkey, where they grow widely in arid, rocky, and mountainous terrains. Adapting to harsh conditions has enriched them with a variety of phytochemicals; thus, they are important in the pharmaceutical, nutraceutical, and food industries. A common characteristic to all these species is their ability to produce essential oils and bioactive compounds, including phenolics, flavonoids, and terpenoids [8].

Such compounds have been reported to exhibit a wide range of pharmacological properties, including antioxidant, antimicrobial, anti-inflammatory, and enzyme-inhibitory activities [9]. Indeed, *T. sipyleus* and *O. sipyleum* are reported to contain the two phenolic monoterpenes thymol and carvacrol, respectively, which exhibit highly effective antibacterial activities [10]. Also, *S. phrygia*, widely used as "mountain tea," reveals high antioxidant and anti-inflammatory activities, promoting its wide use in herbal prescriptions against respiratory and gastrointestinal disorders because of the existence of diterpenoids and phenylpropanoids [11]. Besides their medicinal value, these plants have an important ecological role: some species are pollinator-friendly, attracting bees and many other beneficial insects. Their flowers are not only a very important factor for biodiversity but also participate in the elaboration of monofloral honey with added therapeutic properties [7].

These plants have traditionally been used in teas, essential oil extraction, and flavoring in Mediterranean and Anatolian cuisine, reflecting their integration into local cultures and economies [12,13]. This increasing demand for natural bioactive compounds has sparked renewed interest in exploring these species using sustainable methods.

Table 2
Chemical characterization of *Origanum sipyleum* extracts (mg/g extract). nd: not detected.

HR-fragments	Identification	Total MeOH	Water	Spent after HD	SCO_2	Spent after SCO_2
	Sucrose	0.11 ± 0.02^{a}	0.42 ± 0.05^{b}	0.42 ± 0.05^{b}	nd	0.01 ± 0.02^{c5}
179,05762;135,03521	Quinic acid	0.16 ± 0.04^{a}	0.17 ± 0.04^{a}	0.45 ± 0.05^{b}	nd	0.12 ± 0.03^{a}
197,04595; 179,03563	Glucosyringic acid isomer 1	13.55 ± 0.93^{a}	12.34 ± 0.63^{b}	9.25 ± 0.74^{c}	0.23 ± 0.10^{d}	15.83 ± 0.59^{d}
197,04595; 179,03563	Glucosyringic acid isomer 2	11.05 ± 0.90^{a}	10.23 ± 0.96^{a}	8.55 ± 0.88^{b}	0.43 ± 0.23^{c}	12.21 ± 0.99^{a}
	1-Hexanol arabinosylglucoside	0.03 ± 0.02^{a}	0.03 ± 0.03^{a}	0.03 ± 0.03^{a}	nd	0.06 ± 0.04^{a}
	3-caffeoyl-quinic acid	0.003 ± 0.002^{a}	0.003 ± 0.002^{a}	nd	0.003 ± 0.002^{a}	0.002 ± 0.002^{a}
179,0216	Caffeoyl hexose	0.11 ± 0.03^{a}	0.10 ± 0.03^{a}	0.09 ± 0.03^{a}	0.05 ± 0.03^{b}	0.12 ± 0.03^{a}
177,01075	Daphnetin-hexoside	0.002 ± 0.002^{a}	0.002 ± 0.002^{a}	0.002 ± 0.002^{a}	nd	0.003 ± 0.002^{a}
	Coumaric acid hexoside	0.22 ± 0.03^{a}	0.20 ± 0.03^{a}	0.13 ± 0.02^{b}	0.005 ± 0.002^{c}	0.20 ± 0.03^{a}
	4-caffeoyl-quinic acid	0.002 ± 0.001^{a}	0.002 ± 0.001^{a}	nd	0.003 ± 0.001^{a}	0.002 ± 0.001^{a}
	5-caffeoyl-quinic acid	0.001 ± 0.001^{a}	0.001 ± 0.001^{a}	nd	nd	0.001 ± 0.001^{a}
225,09813	Epigallocatechin	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.02 ± 0.01^{a}	0.001 ± 0.001^{b}	0.03 ± 0.01^{a}
	Caffeic acid	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	nd	0.03 ± 0.01^{a}
225,09813	Gallocatechin	0.16 ± 0.02^{a}	0.11 ± 0.05^{a}	0.11 ± 0.04^{a}	0.08 ± 0.02^{b}	0.15 ± 0.02^{a}
	Methylepicatechin	0.006 ± 0.003^{a}	0.005 ± 0.003^{a}	0.010 ± 0.010^{a}	0.005 ± 0.003^{a}	0.004 ± 0.003^{a}
225,1097;	Taxifolin	0.015 ± 0.005^{a}	0.014 ± 0.003^{a}	0.014 ± 0.003^{a}	0.007 ± 0.003^{b}	0.013 ± 0.003^{a}
225,1231; 207,10,051	12-Hydroxyjasmonic acid glucoside	0.092 ± 0.004^{a}	0.091 ± 0.004^{a}	0.0882 ± 0.003^{a}	nd	0.1012 ± 0.004^{a}
359,0776	Danshensu C	0.13 ± 0.02^{a}	0.11 ± 0.02^{a}	0.12 ± 0.02^{a}	nd	0.13 ± 0.02^{a}
259,04648; 153,00624	Desmethyloriganoside	13.02 ± 0.92^{a}	9.22 ± 0.99^{b}	8.95 ± 0.99^{b}	1.22 ± 0.42^{c}	14.05 ± 0.92^{a}
153,0191; 109,02894	Origanol A	0.55 ± 0.12^{a}	0.51 ± 0.09^{a}	0.51 ± 0.04^{a}	0.13 ± 0.02^{b}	0.50 ± 0.06^{a}
315,0559; 153,00624	4-[[(3′,4’ Dihydroxybenzoyl)oxy]methyl]phenyl-O-β-D-glucopyranoside	14.25 ± 0.82^{a}	9.67 ± 0.92^{b}	8.53 ± 0.89^{b}	1.56 ± 0.12^{c}	14.68 ± 0.82^{a}
331,0454; 287,05475	Delphinidin 3,5-di(6″-malonylglucoside)	0.22 ± 0.03^{a}	0.20 ± 0.03^{a}	0.17 ± 0.03^{a}	0.06 ± 0.03^{b}	0.22 ± 0.03^{a}
285,0761	Salviaflaside	0.56 ± 0.04^{a}	0.51 ± 0.04^{a}	0.41 ± 0.06^{a}	0.11 ± 0.02^{b}	0.53 ± 0.03^{a}
285,0761	Salviaflaside isomer	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.02 ± 0.01^{a}	0.003 ± 0.002^{b}	0.04 ± 0.02^{a}
	Luteolin 7-O-pentosyl-hexoside 1	0.008 ± 0.001^{a}	0.007 ± 0.001^{a}	0.011 ± 0.001^{a}	nd	0.008 ± 0.001^{a}
	Luteolin 7-O-pentosyl-hexoside 2	0.050 ± 0.001^{a}	0.071 ± 0.001^{a}	0.072 ± 0.002^{a}	nd	0.064 ± 0.001^{a}
359,0776	Sagerinic acid isomer 1	15.66 ± 0.88^{a}	9.34 ± 1.00^{b}	8.14 ± 0.98^{b}	1.22 ± 0.12^{c}	17.42 ± 0.90^{a}
	Origanoside	1.56 ± 0.10^{a}	1.34 ± 0.10^{b}	1.22 ± 0.10^{b}	0.11 ± 0.10^{b}	1.24 ± 0.10^{b}
179,0368; 161,02647; 135,0465	Rosmarinic acid	1.80 ± 0.08^{a}	1.22 ± 0.09^{b}	1.30 ± 0.08^{b}	0.11 ± 0.06^{c}	1.20 ± 0.08^{b}
359,0776	Sagerinic acid isomer 2	1.52 ± 0.08^{a}	1.11 ± 0.03^{b}	1.00 ± 0.04^{b}	0.14 ± 0.02^{c}	1.17 ± 0.08^{b}
295,05929	Salvianolic acid A isomer 1	0.22 ± 0.03^{ab}	0.17 ± 0.03^{a}	0.18 ± 0.03^{a}	0.06 ± 0.01^{a}	0.23 ± 0.03^{a}
	Salvianolic acid B isomer	11.33 ± 0.61^{a}	10.33 ± 0.61^{a}	9.11 ± 0.70^{a}	1.33 ± 0.11^{b}	9.06 ± 0.66^{a}
519,07308; 339,03368	Salvianolic acid B	15.81 ± 0.81^{a}	14.22 ± 0.81^{a}	10.34 ± 0.81^{b}	1.85 ± 0.21^{a}	14.56 ± 0.81^{a}
	Isorhamnetin-3-O-glucoside	0.56 ± 0.11^{a}	0.42 ± 0.11^{a}	0.36 ± 0.11^{a}	0.06 ± 0.02^{b}	0.52 ± 0.11^{a}
295,05929	Salvianolic acid A isomer 2	0.11 ± 0.01^{a}	0.12 ± 0.01^{a}	0.11 ± 0.01^{a}	0.03 ± 0.01^{b}	0.10 ± 0.01^{a}
	Methyl rosmarinic acid isomer 1	0.04 ± 0.01^{a}	0.03 ± 0.01^{a}	0.03 ± 0.01^{a}	0.001 ± 0.001^{b}	0.05 ± 0.01^{a}
179,03416; 135,0443	Methyl rosmarinic acid isomer 2	0.06 ± 0.01^{a}	0.02 ± 0.02^{a}	0.03 ± 0.01^{a}	0.001 ± 0.001^{b}	0.05 ± 0.01^{a}
	Salvianolic acid F	0.012 ± 0.003^{a}	0.009 ± 0.003^{a}	0.012 ± 0.004^{a}	nd	0.008 ± 0.003^{a}
	Litospermic acid	0.24 ± 0.03^{a}	0.22 ± 0.03^{a}	0.20 ± 0.03^{a}	0.112 ± 0.03^{b}	0.22 ± 0.03^{a}

Different letters in the same line indicate significant differences ($p < 0.05$).

Some green chemistry extraction techniques, such as supercritical fluid extraction methods, have been applied to isolate bioactive compounds, including phenolic acids (e.g., rosmarinic acid, salvianolic acid B), flavonoids (e.g., kaempferol and luteolin derivatives), and terpenoids (e.g., carvacrol, spathulenol) from *O. sipyleum*, *T. sipyleus*, and *S. phrygia* with minimal environmental impact [14]. These works align with the current global thrust toward sustainable and eco-friendly production methods in the pharmaceutical and food industries.

This work investigates the phytochemical profiles of *O. sipyleum, T. sipyleus,* and *S. phrygia*, highlighting their bioactive potential and possible therapeutic uses. It will also seek to fill some gaps in knowledge regarding these herbs' chemical and pharmacological properties,

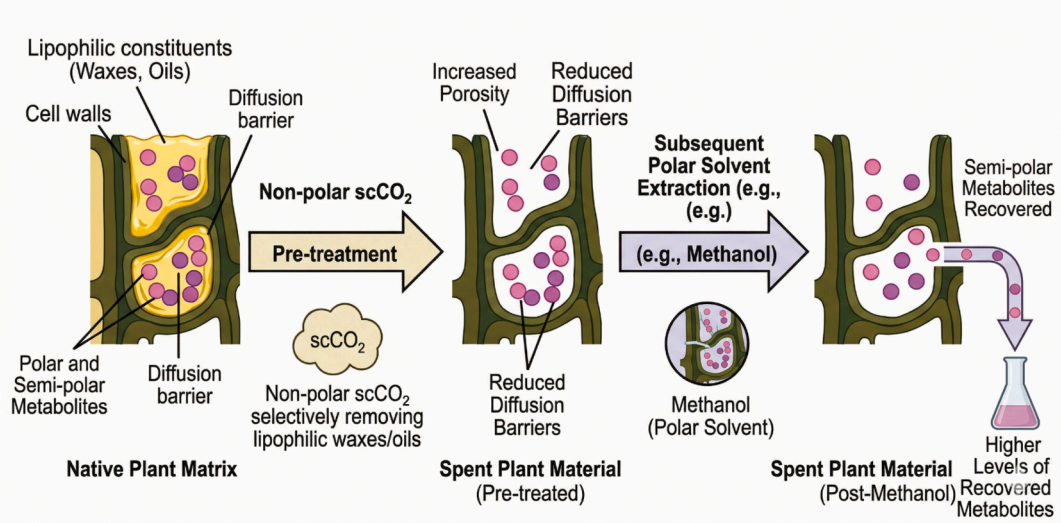


Fig. 2. Possible impact of supercritical CO_2 pre-treatment on the recovery of polar plant metabolites.

Table 3
Chemical composition of *Origanum sipyleum* essential oil.

RT	RIKov	RI	Compound	%
18.79	1096	1099	Linalool	8.87
21.90	1174	1177	Terpinen-4-ol	6.51
22.23	1182	1184	*p*-cymen-8-ol	1.04
22.45	1187	1190	α- terpineol	16.24
24.49	1242	1234	Thymol methyl ether	1.82
24.90	1253	1253	Sabinene hydrate acetate, trans-	0.92
26.10	1285	1294	Dihydroedulan II	3.89
26.23	1289	1288	*p*-cymen-7-ol	1.05
26.57	1297	1290	Thymol	3.52
29.06	1371		Methyl p-anisate	0.21
29.14	1374	1376	α-copaene	1.65
29.45	1383	1384	β-bourbonene	1.69
29.57	1386	1387	β-cubebene	1.41
30.10	1401	1390	β-elemene	0.89
30.59	1417	1420	Caryophyllene	3.55
31.99	1462	1460	Alloaromadendrene	18.81
32.43	1475	1476	ζ-muurolene	2.73
32.65	1482	1481	Germacrene D	0.19
32.72	1484	1486	β-selinene	3.83
32.98	1492	1492	Valencene	4.45
33.14	1497	1498	α-muurolene	1.59
33.56	1511	1513	ζ-Cadinene	2.15
33.87	1522	1523	*cis*-calamenene	3.65
35.51	1577	1576	Spathulenol	4.06
35.69	1583	1581	Caryophyllene oxide	4.20
36.48	1609	1605	Humulene epoxide II	1.11

highlighting their potential applications in nutraceuticals, functional foods, and pharmaceuticals.

2. Material and methods

2.1. Plant collection

Plant specimens were collected in 2025 (Turkey), and the location information is given below. Dr. Selami Selvi carried out the formal botanical classification of the material. A voucher specimen was placed in the Faculty of Science at Balikesir University. After collection, the aerial parts were separated immediately and air-dried at room temperature in a shaded, well-ventilated area. The aerial parts were selected for analysis as they represent the primary plant material used in traditional medicine and are the main source of bioactive compounds, including essential oils and phenolics, in Lamiaceae species [15]. After complete drying, the plant material was milled to fine powder. To ensure chemical integrity and prevent deterioration, the powdered samples were sealed in opaque containers and stored under stable conditions.

Origanum sipyleum L: Balıkesir: Altıeylül, Tayyipler village, Calcareous rocks and slopes, 39°27′8.56"K , 27°50'36.09"D, 370 m, Voucher No: SV 3945.

Thymus sipyleus Boiss.: Afyon: Dinar, Kadılar village, mountain steppes, 38°11′4.14"K, 30°28′8.49"D, 2010 m, Voucher No: SV 3922.

Sideritis phrygia Bornm.: Isparta: Yalvaç, rocky slopes, 38°18′44.39"K, 31°10′58.37"D, 1141 m, Voucher No: SV 3912.

2.2. Extraction procedures

2.2.1. Preparation of supercritical CO_2 extracts

A 100 g plant sample was placed into a high-pressure stainless steel extraction vessel. To keep the sample in place, it was sandwiched between two layers of glass wool—one at the bottom and one at the top of the vessel. The extraction was carried out using a supercritical fluid extraction system (F-500, Qualitec Süper Kritik CO_2, Turkey) at 200 MPa and 40 °C for 3 h. During this time, carbon dioxide was pumped through the system at a constant flow rate of 5 g/min.

2.2.2. Extraction of essential oils by hydrodistillation

For essential oil isolation, 100 g of dried, powdered material was placed in a Clevenger-type apparatus along with 2 L of deionized water. The mixture was distilled for five hours. After distillation, the collected essential oils were dried over anhydrous sodium sulfate, then injected into the GC–MS system for analysis.

2.2.3. Preparation of total, spent, and water extracts

The total extract was obtained by simple maceration: 10 g of plant material was soaked in 200 mL of methanol (99.9%, HPLC grade) at room temperature for 24 h. Meanwhile, the water remaining after hydrodistillation was filtered and freeze-dried to produce the water extracts. After both hydrodistillation and supercritical CO_2 extraction were completed, the remaining plant material (10 g) was dried, then stirred with 200 mL of methanol (99.9%, HPLC grade) at room temperature for another 24 h to obtain the spent extracts.

All extracts were then filtered, and the solvents were removed using a rotary evaporator. The final extracts were stored at 4 °C until analysis. The flowchart of the extraction procedures is given in Fig. 1.

2.2.4. Total phenolic and flavonoid contents

Quantification of total phenolics and total flavonoids in the extracts

Table 4
Chemical characterization of *Thymus sipyleus* extracts (mg/g extract). nd: not detected.

M-H	Identification	Total MeOH	Water	Spent after HD	SCO_2	Spent after SCO_2
341,10,926	Sucrose	3.40 ± 0.02[a]	3.51 ± 0.12[a]	3.09 ± 0.18[b]	0.33 ± 0.02[c]	3.38 ± 0.11[a]
289,0717	Catechin	0.24 ± 0.02[a]	0.24 ± 0.02[a]	0.23 ± 0.02[a]	0.05 ± 0.02[b]	0.22 ± 0.02[a]
315,0716	Protocatecuic acid hexoside	0.41 ± 0.02[a]	0.38 ± 0.02[a]	0.36 ± 0.02[a]	0.02 ± 0.02[b]	0.36 ± 0.02[a]
153,01799	Protocatecuic acid	0.31 ± 0.04	0.28 ± 0.05[a]	0.31 ± 0.02[a]	0.01 ± 0.01[b]	0.29 ± 0.02[a]
447,0919	Kaempferol-hexoside	0.02 ± 0.01[a]	0.02 ± 0.01[a]	0.02 ± 0.01[a]	nd	0.02 ± 0.01[a]
137,0238	4-hydroxybenzoic acid	0.01 ± 0.01[a]	nd	nd	nd	0.01 ± 0.01[a]
179,03395	Caffeic acid	0.79 ± 0.05[a]	0.54 ± 0.09[b]	0.75 ± 0.05[a]	0.11 ± 0.05[b]	0.82 ± 0.05[a]
449,1083	Eriodictyol-galactoside	1.27 ± 0.10[a]	0.53 ± 0.12[b]	1.27 ± 0.15[a]	0.13 ± 0.10[c]	1.32 ± 0.10[a]
449,1083	Eriodictyol-glucoside	1.54 ± 0.09[a]	0.98 ± 0.11[b]	1.59 ± 0.10[a]	0.05 ± 0.03[c]	1.61 ± 0.10[a]
367,1036	Feruloyl quinic acid	2.59 ± 0.19[a]	2.66 ± 0.14[a]	2.25 ± 0.20[a]	0.05 ± 0.10[b]	2.26 ± 0.10[a]
367,1036	Feruloyl quinic acid	14.35 ± 0.85[a]	10.54 ± 2.10[a]	13.64 ± 0.99[a]	0.89 ± 0.11[b]	13.43 ± 1.10[a]
463,0862	Quercetin-3-O-galactoside	10.06 ± 0.80[a]	8.14 ± 0.14[b]	9.85 ± 0.10[b]	3.11 ± 0.13[c]	10.10 ± 0.94[a]
463,0861	Quercetin-3-O-glucoside (Isoquercetin)	10.75 ± 0.90[a]	7.21 ± 0.70[b]	9.46 ± 0.90[a]	1.50 ± 0.20[c]	9.51 ± 0.17[a]
447,0928	Kaempferol 7-O-glucoside	15.86 ± 1.02[a]	11.84 ± 0.90[b]	14.75 ± 1.10[a]	1.31 ± 0.30[b]	14.09 ± 1.10[a]
461,07165	Luteolin 7-O-glucuronide	10.03 ± 0.90[a]	7.21 ± 0.80[b]	10.33 ± 1.10[a]	0.85 ± 0.10[c]	11.21 ± 1.10[a]
447,09235	Luteolin 7-O-galactoside	9.78 ± 0.88[a]	6.82 ± 0.40[b]	9.29 ± 0.80[a]	1.05 ± 0.20[c]	9.15 ± 0.80[a]
448,09235	Luteolin-7-O-glucoside	0.28 ± 0.02[a]	0.26 ± 0.02[a]	0.28 ± 0.02[a]	0.08 ± 0.03[b]	0.30 ± 0.04[a]
521,1298	Rosmarinic acid glucoside	5.02 ± 0.21[a]	3.81 ± 0.71[b]	4.97 ± 0.51[a]	0.61 ± 0.13[c]	4.69 ± 0.21[a]
433,07725	Quercetin 3-O-arabinoside	0.90 ± 0.15[a]	0.29 ± 0.05[b]	0.84 ± 0.11[a]	0.05 ± 0.02[b]	0.94 ± 0.09[a]
625,1403	Quercetin-dihexoside	0.31 ± 0.05[a]	0.22 ± 0.08[a]	0.29 ± 0.02[a]	0.08 ± 0.02[b]	0.28 ± 0.03[a]
555,2231	Lariciresinol-sesquilignan	2.84 ± 0.22[a]	0.81 ± 0.09[b]	2.56 ± 0.27[a]	0.90 ± 0.09[b]	2.80 ± 0.42[a]
359,0776	Rosmarinic acid	31.90 ± 2.22[a]	25.66 ± 2.30[b]	28.71 ± 2.99[b]	3.45 ± 0.02[c]	26.85 ± 1.22[b]
555,2231	Lariciresinol-sesquilignan isomer 2	0.12 ± 0.02[a]	0.10 ± 0.02[a]	0.11 ± 0.03[a]	nd	0.11 ± 0.02[a]
417,082	Luteolin-pentoside	0.19 ± 0.02[a]	0.15 ± 0.02[a]	0.17 ± 0.02[a]	nd	0.19 ± 0.02[a]
461,07165	Kaempferol-glucuronide	3.61 ± 0.23[a]	2.89 ± 0.26[b]	3.68 ± 0.29[a]	0.11 ± 0.02[b]	3.44 ± 0.30[a]
639,11,745	Quercetin-hexoside-glucuronide	0.28 ± 0.02[a]	0.25 ± 0.02[a]	0.27 ± 0.02[a]	0.11 ± 0.02[b]	0.26 ± 0.02[a]
285,0392	Luteolin	3.16 ± 0.32[a]	1.51 ± 0.12[b]	3.21 ± 0.22[a]	2.27 ± 0.29[c]	3.35 ± 0.29[a]

Different letters in the same line indicate significant differences ($p < 0.05$).

was achieved using validated colorimetric techniques, in accordance with established protocols. Standard curves were generated using gallic acid (for phenolic content) and rutin (for flavonoid content), allowing for the calculation of results as GAE and RE per gram, respectively [16].

2.2.5. UPLC-QTOF and GC–MS analysis

The UPLC-QTOF was obtained on a Waters H-class chromatograph coupled with Xevo-QTOF operating in ESI in negative ion mode. Gradient was composed of A- water 0,1% formic acid, B-acetonitrile, and C- methanol flow rate was 0.3 mL/min. The gradient started with 90% A and reach 10% in 16 min. The column was a Waters BEH C18 2.1 × 150 mm (1,7 μm), and the temperature was kept at 65 °C. The spectrometer operates at an electrospray voltage of 3,25 V, 700 l/Hr of drying gas, and a drying-gas temperature of 450 °C. To accurately measure mass, the Lockmass solution (leucine-enkephalin) was sprayed through the Lockspray at a flow rate of 20 μl/min. Continuum data were collected in the *m/z* range 50–1500, then spectra were centroided and *m/z* values were corrected using the lock mass (enkephalin). Samples were prepared by dissolving 10 mg of dried extract in 5 mL of methanol by sonication for 10 min. After sonication, the samples were centrifuged (13,000 rpm for 15 min) and finally filtered through 0,45 PTFE filters. Rutin, Kaempferol-7-O-glucoside, luteolin-7-O-glucoside, rosmarinic acid, chlorogenic acid, catechin, salvianolic acid A, eryodichtiol-glucoside, epicatechin, foristhiside A, verbascoside, and isoverbascoside were used for quantitative purposes.

GC–MS was obtained on a Varian 3800 chromatograph equipped with a Saturn 2000 mass spectrometer with Ion Trap analyser working in EI mode (70 Ev). Spectra were acquired in the 50–550 *m/z* range. Quantification was performed by adding an internal standard (nonanol) and creating calibration curves for the monoterpene (menthol, carvone), sesquiterpene (caryophyllene), and phenolic (thymol) compounds.

2.2.6. Antioxidant assays

Antioxidant activity was determined using a battery of in vitro tests, following the method described by Grochowski et al. [17]. Reducing power was assessed using FRAP and CUPRAC, whereas radical-scavenging activity was measured using DPPH and ABTS. Results from these four assays were converted to Trolox equivalents and expressed as mg TE/g of dried extract. Total antioxidant capacity was additionally quantified via the phosphomolybdenum (PBD) assay (mmol TE/g), and mg EDTA equivalents per gram (mg EDTAE/g) were used to evaluate metal-chelating ability (MCA).

2.2.7. Enzyme inhibitory activity assays

Using established colorimetric protocols [17], the extracts were screened for enzyme inhibition against five relevant targets: acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, α-amylase, and α-glucosidase. To enable comparability across samples, inhibition values were quantified relative to reference standards.

Table 5
Chemical composition of *Thymus sipyleus* essential oil.

RT	RIKov	RI	Compounds	%
13.26	981	980	1-Octen-3-ol	0.81
14.06	995	993	3-Octanol	0.33
17.60	1073	1072	1-Octanol	0.50
17.98	1081	1080	Heptanoic acid	0.35
18.30	1087	1083	Linalool oxide, cis-	0.33
18.81	1097	1099	Linalool	0.85
20.55	1141	1143	Camphor	8.38
21.45	1164	1158	Isoborneol	0.13
21.52	1165	1165	delta-terpineol	4.34
21.92	1175	1177	Terpinen-4-ol	3.57
22.24	1182	1184	*p*-cymen-8-ol	0.36
22.45	1187	1190	α-terpineol	4.10
23.93	1227	1229	Nerol	6.51
25.50	1270	1242	Neral	0.60
26.10	1285	1294	Dihydroedulan II	0.43
26.26	1289	1290	Thymol	0.54
26.58	1298	1300	Carvacrol	0.68
28.26	1348	1351	α-cubebene	0.35
29.15	1374	1376	α-copaene	0.80
29.46	1383	1384	β-bourbonene	0.97
29.56	1386	1387	β-cubebene	0.15
30.44	1412	1412	α-Cedrene	0.21
30.59	1417	1420	caryophyllene, (*E*)-	1.27
30.76	1423	1422	β-Cedrene	0.23
30.91	1428	1431	β-gurjunene	0.83
31.22	1438	1441	Aromadendrene	0.33
31.58	1449	1445	α-himachalene	0.63
31.72	1454	1453	α-humulene	3.33
31.77	1455	1444	*cis*-β-Farnesene	0.34
31.99	1462	1460	Alloaromadendrene	1.22
32.18	1468	1470	β-chamigrene	0.28
32.42	1475	1476	ζ-muurolene	3.90
32.59	1481	1481	Germacrene D	2.36
32.74	1485	1486	β-selinene	6.49
33.02	1494	1492	Valencene	9.18
33.16	1498	1498	α-muurolene	1.26
33.40	1506	1501	β-himachalene	0.31
33.60	1512	1513	ζ-cadinene	3.32
33.87	1522	1523	δ-cadinene	3.32
34.17	1532	1528	Calamenene, trans-	0.80
34.31	1537	1533	α-Cadinene	0.49
34.47	1542	1540	α-Calacorene	0.61
34.79	1553	1554	Elemicin	0.35
35.51	1577	1576	Sphathulenol	3.26
35.69	1583	1581	Caryophyllene oxide	7.99
35.85	1588	1585	Globulol, *epi*-	0.37
35.98	1592	1592	Longiborneol	0.43
36.47	1609	1605	Humulene epoxide II	2.02
36.98	1628	1626	1-Cubenol, epi	0.05
37.12	1632	1630	α-Acorenol	0.01
37.35	1641	1638	α-Cadinol, epi-	2.92
37.51	1646	1643	α-Muurolol	0.44
37.73	1654	1652	α-Cadinol	3.72
38.04	1665	1666	Bulnesol	1.24
38.19	1670	1671	Cadalene	0.41
38.30	1674	1672	β-Bisabolol	0.22
38.47	1680	1683	α-Bisabolol	0.28
38.61	1685	1685	α-Bisabolol, epi-	0.48

Inhibition data were normalized using reference standards, with cholinesterase inhibition expressed as mg GALAE/g, α-amylase and α-glucosidase inhibition as mmol ACAE/g, and tyrosinase inhibition as mg KAE/g.

2.3. Statistical analysis

A one-way analysis of variance (ANOVA) was performed to assess significant differences among samples for each assay ($p < 0.05$), followed by Tukey's post hoc test. Pearson's coefficient higher than 0,9 was defined as statistically significant, since correlations above this threshold corresponded to *p*-values lower than 0.05. The dataset was subsequently scaled, and a distance matrix was computed to evaluate similarities and dissimilarities among samples using the Euclidean distance metric. K-medoids clustering was then applied after determining the optimal number of clusters using the elbow and the average silhouette method. The clustering quality was assessed using the silhouette coefficient (Si), where values closer to 1 indicate better-defined clusters. Finally, a heatmap was generated to visualize the distribution of bioactivities characterizing each identified cluster. All statistical analyses were performed using R software (version 4.1.2).

3. Results and discussion

3.1. Total phenolic content (TPC) and total flavonoid content (TFC)

Phenols are essential components of plants, serving multiple roles including defence against pathogens and herbivores, UV protection, pollinator attraction, and allelopathy, in addition to their well-documented free radical scavenging activity attributed to their hydroxyl groups [18,19]. Therefore, it can be assumed that the phenolic content in plants may directly contribute to its antioxidant activity. Plants from the family Lamiaceae are rich in polyphenolic compounds. The major phenolic compounds identified in Lamiaceae family are carnosic acid, carnosol, rosmarinic acid, rosmadial, methyl carnosate, and epirosmanol [20].The antioxidant potential of phenolic compounds, including flavonoids, is generally attributed to their ability to scavenge free radicals. The higher TPC and TFC values in the different extracts of *O. sipyleum*, especially in "Spent after SCO_2" and "Spent after HD," indicate greater availability of phenolic compounds (Table 1). *Origanum* species are rich in phenolic compounds such as rosmarinic acid and flavonoids, exhibiting strong antioxidant properties [21]. Although *T. sipyleus* has a moderate phenolic and flavonoid content, it performed remarkably well in the SCO_2 extract. This could mean that though the phenolic content is relatively low, the types of phenolic compounds may be highly active as antioxidants. A previous study on *T. sipyleus* reported total phenolic and flavonoid contents in the range of 83.43–127.52 mg GAE/g and 9.41–46.34 mg RE/g, respectively [22]. The values obtained in the present investigation for the methanol and spent extracts (TPC: 74.08–82.61 mg GAE/g; TFC: 67.26–77.40 mg RE/g) are comparable, whereas the SCO_2 extract yielded considerably lower values (TPC: 16.19 mg GAE/g; TFC: 4.49 mg RE/g), confirming that supercritical CO2 is not efficient for extracting polar phenolic compounds from *T. sipyleus*. A previous study on *O. sipyleum* indicated the total phenolics were 203.57 ± 4.62 mg GAE/g, and the flavonoid content was 46.98 ± 0.34 mg QE/g [23]. In contrast, the values obtained from the present investigation were significantly higher, suggesting that SCO_2 is more efficient in extracting bioactive compounds. The TPC and TFC observed in the extracts of *O. sipyleum* were found to correlate with the potent antioxidant capacity measured by subsequent assays, for instance, DPPH and ABTS. The relationship between flavonoids and antioxidant activity has been well documented as flavonoids inhibit oxidative damage by scavenging free radicals and modulating cellular signaling pathways [24,25].

3.2. Chemical composition of the extracts and essential oils

Thirty-nine compounds were identified in the different extracts of *O. sipyleum*, comprising multiple phenolic derivatives as reported in Table 2. Most abundant compounds are the desmethyloriganoside (1.22–14.05 mg/g), the 4-[[(3′,4' Dihydroxybenzoyl)oxy]methyl] phenyl-O-β-D-glucopyranoside (1.56–14.68 mg/g), sagerinic acid isomer I (1.22–17.415 mg/g) and salvianolic acid B (1.85–15.81 mg/g) derivatives. In the tested extracts, the total and spent methanol extracts had nearly identical levels of chemical constituents. Also, the water extract after the distillation process contained significantly higher amounts of the detected compounds than SCO_2. Considering all extracts, the highest levels of the detected metabolites were found in the spent extract after SCO_2, followed by the methanol extract, the water extract,

Table 6
Chemical characterization of *Sideritis phrygia* extracts (mg/g extract). nd: not detected.

M-H	Identification	Total MeOH	WATER	Spent after HD	SCO2	Spent after SCO2
341,10,926	Sucrose	1.48 ± 0.10[a]	1.15 ± 0.23[b]	1.55 ± 0.12[a]	nd	2.33 ± 0.30[b]
191,0593	Quinic acid	1.35 ± 0.13[a]	1.62 ± 0.21[b]	2.17 ± 0.23[c]	0.01 ± 0.01[d]	1.83 ± 0.29[b]
353,07508	3-Caffeoyl-quinic acid	2.76 ± 0.28[a]	2.65 ± 0.23[a]	5.63 ± 0.33[b]	0.05 ± 0.01[c]	0.19 ± 0.13[d]
341,072	Caffeoyl hexose	0.21 ± 0.03[a]	0.21 ± 0.04[a]	0.18 ± 0.02[a]	0.03 ± 0.02[b]	0.11 ± 0.02[a]
353,0752	4-Caffeoyl-quinic acid	4.59 ± 0.50[a]	4.62 ± 0.43[a]	4.11 ± 0.43[a]	0.07 ± 0.01[b]	5.41 ± 0.51[b]
353,0752	5-caffeoyl-quinic acid	1.95 ± 0.12[a]	1.98 ± 0.19[a]	1.13 ± 0.11[b]	0.21 ± 0.05[c]	1.77 ± 0.20[a]
337,0923	Cumaroyl-quinic acid	0.07 ± 0.03[a]	0.05 ± 0.03[a]	0.06 ± 0.03[a]	0.02 ± 0.03[a]	0.07 ± 0.03[a]
649,57,340	Kaempferol-hexoside-derivative	60.25 ± 2.13[a]	59.89 ± 2.03[a]	44.22 ± 2.00[b]	41.22 ± 2.13[b]	61.55 ± 1.93[a]
607,5373	Kaemmpferol-hexoside-methoxydeoxyhexoside	13.23 ± 0.99[a]	14.11 ± 0.98[a]	7.37 ± 0.83[b]	1.54 ± 0.13[c]	9.57 ± 0.93[d]
609,14,556	Isoscutellarein 7-O-allosyl(1 → 2)glucoside	5.41 ± 0.63[a]	5.37 ± 0.67[a]	7.37 ± 0.99[b]	1.65 ± 0.23[c]	6.28 ± 0.99[b]
623,19,760	Verbascoside	14.77 ± 0.93[a]	13.99 ± 0.89[a]	10.77 ± 0.63[b]	0.19 ± 0.03[c]	7.39 ± 0.83[d]
623,19,760	Forsithioside A	21.84 ± 0.90[a]	18.99 ± 0.90[b]	17.34 ± 0.95[b]	0.22 ± 0.03[c]	16.57 ± 0.80[c]
623,19,760	Isoverbascoside	17.68 ± 0.90[a]	15.60 ± 0.80[a]	16.47 ± 0.90[a]	0.29 ± 0.02[b]	12.17 ± 0.50[c]

Different letters in the same line indicate significant differences ($p < 0.05$).

Table 7
Chemical composition of *Sideritis phrygia* essential oil.

RT	RIKov	RI	Compound	%
18.80	1097	1099	Linalool	0.15
20.62	1143	1143	Camphor	0.37
21.46	1164	1158	Isoborneol	0.06
21.92	1175	1177	Terpinen-4-ol	0.46
22.24	1182	1184	*p*-cymen-8-ol	0.47
22.46	1187	1190	α-terpineol	0.72
25.78	1277	1275	Nonanoic acid	1.27
26.25	1289	1290	Thymol	3.04
26.60	1298	1300	Carvacrol	17.30
29.15	1374	1376	α-copaene	0.92
29.45	1383	1386	β-damascenone, trans	0.80
29.56	1386	1387	β-Cubebene	0.34
29.88	1395	1390	β-Elemene	0.62
30.13	1402	1402	Italicene	0.27
30.25	1406	1409	α-Gurujunene	0.50
30.45	1413	1412	α-Cedrene	0.83
30.60	1417	1420	Caryophyllene	4.31
31.66	1451	1452	Geranyl acetone	0.95
31.76	1455	1456	B-Farnesene cis	0.94
31.92	1460	1460	Alloaromandendrene	0.85
32.50	1478	1476	ζ-Muurolene	0.56
32.58	1480	1481	Germacrene D	0.15
32.71	1484	1486	*trans*-β-Ionone	1.54
33.01	1493	1492	Valencene	0.29
33.49	1509	1508	β-Bisabolene, (*Z*)-	2.64
33.73	1517	1518	Myristicin	1.13
33.88	1522	1523	δ-cadinene	5.12
34.06	1528	1532	Dihydroactinidiolide	0.35
34.47	1542	1540	α-Calacorene	1.15
34.78	1553	1553	Farnesene	0.21
35.08	1563	1559	β-calocarene	1.39
35.25	1568	1570	3-Hexenyl benzoate, (3Z)-	0.80
35.52	1577	1576	Spathulenol	17.96
35.71	1583	1581	Caryophyllene oxide	6.93
35.96	1592	1591	Viridiflorol	1.21
36.29	1603	1602	α-Humulene oxide	4.08
36.44	1608	1605	Humullene epoxide II	1.84
36.90	1625	1626	1-Cubenol, epi-	0.15
37.01	1629	1630	α-Acorenol	3.02
37.26	1638	1634	Isospatulenol	1.64
37.38	1642	1638	α-Cadinol, epi-	1.97
37.50	1646	1643	α-Muurolol	0.51
37.65	1652	1650	β-Eudesmol	0.70
37.79	1656	1656	Allomadendrene oxide	0.94
37.99	1663	1663	Sesquiterpene	0.68
38.22	1671	1671	Cadalene	0.78
38.60	1685	1685	α-Bisabolol	3.22
38.78	1691	1690	Bisabolol	2.09
39.60	1721	1723	Farnesol	1.76

the spent extract after CO_2, and finally the SCO_2 extract. This indicates that the phenolic compounds from *O. sipyleum* are not efficiently extracted by SCO_2 alone, whereas methanol extraction after CO_2 extraction is efficient. This outcome may be due to the polar characteristics of the compounds in *O. siypleum* and the limited solubility of CO_2 in substances of this type. The findings were also supported by several researchers who reported that only CO_2 is not suitable for extracting polar compounds from plant matrices [1,26]. Due to the non-polar nature of supercritical CO_2, it primarily removes lipophilic constituents, such as waxes and oils. This pre-treatment increases the porosity of the plant matrix and reduces diffusion barriers, facilitating the subsequent penetration of polar solvents such as methanol. Consequently, higher levels of polar and semi-polar metabolites can be recovered from the spent plant material (Fig. 2). Similar results were also observed for different plant species [27–29]. In the literature, some studies were reported the chemical composition of *O. sipyleum* extracts. For example, Zengin et al. [30] investigated the chemical profile of different extracts of *O. sipyleum,* and in accordance with our results, they found rosmarinic acid derivatives such as salvianolic acid isomers as well as flavonoids. In another study by Ozkan et al. [31], rosmarinic acid was detected as the main constituent in the methanol extract of *O. sipyleum*. Similar finding was also found in the ethanol extract of *O. sipyleum* as reported by Tüzün et al. [32].

Considering the composition of the essential oil of *O. sipyleum*, the constituents are reported in Table 3, with alloaromadendrene (18.81%) and alpha-terpineol (16.24%) as the major constituents. These two compounds accounted for more than 35% of the total identified compounds. Several researchers reported the composition of *O. sipyleum* collected from different locations. For example, Baser et al. [33] investigated the chemical composition of essential oils of *O. sipyleum* collected from different locations and they found that γ-terpinene (10.80–26.60%) and *p*-cymene (3.76–36.60%) were the most abundant compounds in the essential oils. Another study conducted by Bayar and Cinar [34], the main compound was *p*-cymene (69.44–71.94%) in two harvested years (2017 and 2018). Erbas and Fakir [35] also reported that γ-terpinene (45.46%) and p-cymene (24.29%) were the highest among the detected compounds in the essential oil of *O. sipyleum*. The differences between the studies can be explained by factors such as geographical factors, soil composition, and annual rainfall.

In *T. sipyleus* extracts, twenty-seven compounds were identified and quantified (Table 4). In all extracts, the main compound was rosmarinic acid, present at concentrations ranging from 3.45 to 31.90 mg/g extract. After rosmarinic acid, flavonoids including kaempferol-7-*O*-glucoside (1.31–15.86 mg/g) and luteolin-7-*O*-glucuronide (0.85–11.21 mg/g extract) were at significant levels in the tested extracts. A comparison of the total and spent methanol extracts after hydrodistillation revealed that both had similar chemical compositions. For example, the levels of rosmarinic acid were 31.90 mg/g and 28.71 mg/g, respectively. Therefore, we can conclude that plant materials after hydrodistillation are a significant source of bioactive compounds. Similar finding was also reported by Gavaric et al. [36] for *T. vulgaris*. However also in the case of *Thymus* CO_2 extraction appear to be not suitable for extracting phenolics

Table 8
Antioxidant properties of the tested extracts.*

Samples	Extraction	DPPH (mg TE/g)	ABTS (mg TE/g)	CUPRAC (mg TE/g)	FRAP (mg TE/g)	PBD (mmol TE/g)	MCA (mg EDTAE/g)
Origanum sipyleum	Total MeOH	482.90 ± 10.22^{a}	529.83 ± 6.43^{ab}	825.90 ± 8.04^{c}	569.19 ± 2.35^{b}	4.70 ± 0.24^{b}	1.51 ± 0.38^{f}
	Water	390.95 ± 1.71^{b}	495.67 ± 5.57^{c}	814.13 ± 8.34^{c}	512.92 ± 21.66^{c}	6.00 ± 0.07^{a}	11.67 ± 0.28^{b}
	Spent after HD	405.13 ± 3.68^{b}	526.10 ± 4.19^{bd}	926.71 ± 12.08^{a}	576.03 ± 4.00^{ab}	5.97 ± 0.65^{a}	4.73 ± 0.29^{d}
	SCO_2	3.81 ± 0.76^{c}	15.12 ± 0.39^{d}	51.63 ± 1.31^{d}	18.09 ± 0.42^{d}	1.81 ± 0.09^{c}	21.16 ± 0.13^{a}
	Spent after SCO_2	497.08 ± 8.32^{a}	548.57 ± 16.66^{a}	897.77 ± 5.47^{b}	597.62 ± 1.00^{a}	5.01 ± 0.47^{b}	2.47 ± 0.07^{e}
	EO	0.68 ± 0.05^{d}	1.79 ± 0.14^{e}	19.83 ± 0.62^{e}	9.73 ± 0.09^{e}	6.47 ± 0.02^{a}	6.39 ± 0.50^{c}
Thymus sipyleus	Total MeOH	241.10 ± 0.81^{b}	334.66 ± 0.94^{c}	569.63 ± 4.19^{b}	359.08 ± 3.78^{c}	3.49 ± 0.24^{c}	12.03 ± 0.13^{d}
	Water	197.10 ± 2.65^{c}	340.89 ± 9.27^{bc}	624.52 ± 13.87^{a}	395.59 ± 4.09^{a}	4.84 ± 0.69^{b}	16.36 ± 0.46^{b}
	Spent after HD	234.90 ± 4.91^{b}	362.36 ± 15.50^{ab}	576.02 ± 3.30^{b}	374.80 ± 4.46^{b}	3.48 ± 0.18^{c}	11.56 ± 0.24^{d}
	SCO_2	7.02 ± 0.08^{d}	23.26 ± 1.70^{d}	72.69 ± 3.12^{c}	16.24 ± 0.41^{d}	2.07 ± 0.15^{d}	21.94 ± 0.30^{a}
	Spent after SCO_2	268.32 ± 4.61^{a}	372.71 ± 9.81^{a}	612.47 ± 5.28^{a}	396.13 ± 5.74^{a}	3.47 ± 0.10^{c}	13.24 ± 0.22^{c}
	EO	0.26 ± 0.05^{e}	7.75 ± 0.91^{e}	27.91 ± 1.72^{d}	16.37 ± 0.32^{d}	9.08 ± 0.24^{a}	2.97 ± 0.24^{e}
Sideritis phrygia	Total MeOH	140.08 ± 3.78^{c}	193.93 ± 3.33^{b}	362.63 ± 3.41^{b}	221.92 ± 1.94^{b}	3.45 ± 0.02^{cd}	2.81 ± 0.41^{c}
	Water	104.54 ± 5.86^{d}	171.85 ± 4.76^{c}	319.21 ± 4.24^{c}	192.56 ± 4.32^{c}	3.03 ± 0.10^{d}	5.65 ± 0.42^{b}
	Spent after HD	154.03 ± 5.96^{b}	211.37 ± 5.45^{a}	385.80 ± 13.54^{a}	232.73 ± 1.47^{a}	4.38 ± 0.23^{b}	6.04 ± 0.07^{b}
	SCO_2	4.22 ± 1.57^{e}	12.29 ± 1.67^{e}	49.30 ± 1.82^{d}	25.08 ± 0.76^{d}	1.73 ± 0.14^{e}	22.13 ± 0.93^{a}
	Spent after SCO_2	166.70 ± 1.42^{a}	203.09 ± 2.27^{ab}	382.56 ± 5.46^{a}	234.38 ± 1.91^{a}	3.89 ± 0.35^{bc}	5.23 ± 0.36^{b}
	EO	3.12 ± 0.09^{e}	48.92 ± 0.19^{d}	56.29 ± 1.26^{d}	20.90 ± 2.47^{d}	8.02 ± 0.20^{a}	2.12 ± 0.85^{c}

* Values are reported as mean ± SD of three parallel measurements. EO: Essential oil; PBD: Phosphomolybdenum; MCA: Metal chelating Activity; TE: Trolox Equivalent; EDTAE: EDTA equivalent. Different letters indicate significant differences among the tested extracts from the same plant species ($p < 0.05$).

Table 9
Enzyme inhibitory properties of the tested extracts.**

Samples	Extraction	AChE (mg GALAE/g)	BChE (mg GALAE/g)	Tyrosinase (mg KAE/g)	Amylase (mmol ACAE/g)	Glucosidase (mmol ACAE/g)
Origanum sipyleum	Total MeOH	2.29 ± 0.09^{b}	2.04 ± 0.11^{b}	41.37 ± 0.96^{d}	0.18 ± 0.01^{d}	0.24 ± 0.02^{d}
	Water	0.21 ± 0.07^{c}	0.21 ± 0.05^{d}	24.40 ± 0.93^{e}	0.06 ± 0.01^{f}	0.77 ± 0.02^{c}
	Spent after HD	2.38 ± 0.08^{b}	1.76 ± 0.05^{b}	44.18 ± 1.77^{cd}	0.22 ± 0.01^{b}	0.21 ± 0.05^{d}
	SCO_2	2.66 ± 0.13^{a}	2.88 ± 0.31^{a}	53.96 ± 3.01^{a}	0.33 ± 0.01^{a}	2.67 ± 0.22^{b}
	Spent after SCO_2	2.73 ± 0.14^{a}	1.35 ± 0.12^{c}	46.70 ± 0.56^{bc}	0.16 ± 0.01^{e}	3.02 ± 0.18^{a}
	EO	2.87 ± 0.06^{a}	na	50.30 ± 1.25^{ab}	0.19 ± 0.01^{c}	3.28 ± 0.04^{a}
Thymus sipyleus	Total MeOH	2.37 ± 0.20^{b}	0.66 ± 0.09^{d}	42.58 ± 2.32^{d}	0.31 ± 0.01^{b}	1.26 ± 0.03^{cd}
	Water	0.47 ± 0.06^{c}	0.16 ± 0.01^{e}	41.89 ± 0.70^{d}	0.05 ± 0.01^{e}	1.48 ± 0.04^{c}
	Spent after HD	2.89 ± 0.02^{a}	1.13 ± 0.08^{c}	53.48 ± 1.78^{bc}	0.31 ± 0.01^{b}	1.04 ± 0.06^{d}
	SCO_2	2.94 ± 0.06^{a}	2.90 ± 0.15^{b}	62.09 ± 1.35^{a}	0.41 ± 0.01^{a}	2.87 ± 0.01^{b}
	Spent after SCO_2	2.17 ± 0.06^{b}	0.31 ± 0.03^{e}	57.61 ± 2.13^{ab}	0.25 ± 0.01^{c}	2.79 ± 0.21^{b}
	EO	na	3.42 ± 0.01^{a}	50.41 ± 0.94^{c}	0.18 ± 0.01^{d}	3.31 ± 0.04^{a}
Sideritis phrygia	Total MeOH	2.41 ± 0.09^{c}	0.15 ± 0.04^{d}	43.79 ± 3.86^{b}	0.24 ± 0.01^{d}	0.40 ± 0.04^{d}
	Water	0.89 ± 0.08^{d}	0.06 ± 0.02^{e}	35.62 ± 1.17^{c}	0.05 ± 0.01^{e}	0.42 ± 0.02^{d}
	Spent after HD	2.66 ± 0.06^{ab}	0.27 ± 0.05^{c}	52.53 ± 1.43^{a}	0.29 ± 0.01^{c}	1.80 ± 0.48^{c}
	SCO_2	na	2.41 ± 0.22^{b}	54.59 ± 1.60^{a}	0.40 ± 0.02^{b}	2.74 ± 0.01^{ab}
	Spent after SCO_2	2.57 ± 0.10^{bc}	na	53.34 ± 0.44^{a}	0.25 ± 0.01^{d}	2.18 ± 0.36^{bc}
	EO	2.84 ± 0.05^{a}	3.21 ± 0.08^{a}	51.97 ± 1.09^{a}	0.48 ± 0.01^{a}	3.25 ± 0.03^{a}

** Values are reported as mean ± SD of three parallel measurements. EO: Essential oil; GALAE: Galantamine equivalent; KAE: Kojic acid equivalent; ACAE: Acarbose equivalent; na: not active. Different letters indicate significant differences among the tested extracts from the same plant species ($p < 0.05$).

compounds from *T. sipyleus*; results shown that all compound levels were the lowest in the CO_2 extract. After CO_2 extraction, the spent methanol extract contained comparable levels of phenolics as the total methanol extract. In the literature, several *Thymus* species have been characterized with higher levels of rosmarinic acid [37].

We also examined the chemical composition of the essential oil of *T. sipyleus*, and the results are summarized in Table 5. Valencene (9.18%), camphor (8.38%), and caryophyllene oxide (7.99%) were determined as the main components in the essential oil. Similarly, Yigitkan and Firat [38] reported that camphor was detected as the main component (15.08%) in the essential oil of *T. sipyleus*. In another study conducted by Baser et al. [39], the essential oils of *T. sipyleus* collected from different locations in Turkey, as well as geraniol and nerol, were identified as the major constituents in the tested essential oils. Tepe et al. [40] reported that the main compounds in the essential oil of *T. sipyleus* were borneol (11.2%), β-caryophyllene (7.6%) and geranial (7.3%). Again, borneol was found to be the main component in the essential oils of *T. sipyleus* collected from different locations in Turkey, as reported by Yilmaz et al. [41]. Agalar et al. [42] reported that 1,8-cineole, p-cymene, and α-terpineol were the main constituents in the essential oils from *T. sipyleus* collected from three different locations.

As shown in Table 6, thirteen compounds were identified and quantified in the *S. phrygia* extracts. In all extracts, kaempferol-hexoside-derivative was identified as the main component (41.22–61.55 mg/g extract). After this compound, forsithioside A (0.22–21.84 mg/g), isoverbascoside (0.22–21.84 mg/g), and kaempferol-hexoside-methoxydeoxyhexoside (1.54–14.11 mg/g) were determined as other dominant compounds. Similar to *O. sipyleum* and *T. sipyleus*, CO_2 extraction appear to have limited capacity to extracting phenolic constituents from *S. phyrgia*. Interestingly, however, the CO_2 extraction process does not significantly affect the extraction of the main component, the kaempferol-hexoside derivative that appear in high levels in the extract. In addition, the pre-treatment with CO_2 before the spent extract was useful for extracting more compounds than CO_2 alone or spent extract after hydrodistillation. In an earlier study conducted by Tunalier et al. [43], the extract of *S. phrygia* was rich in terms of

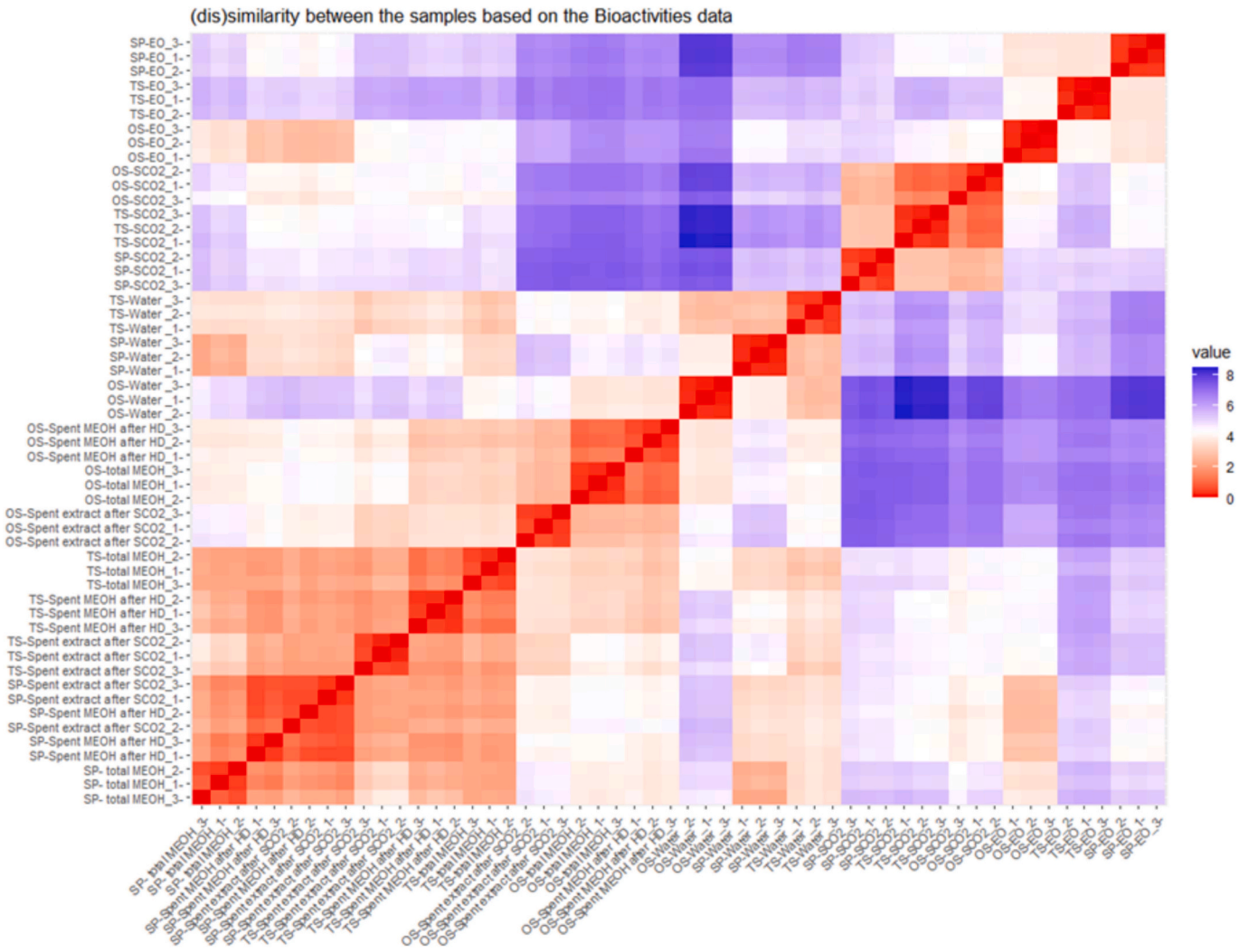


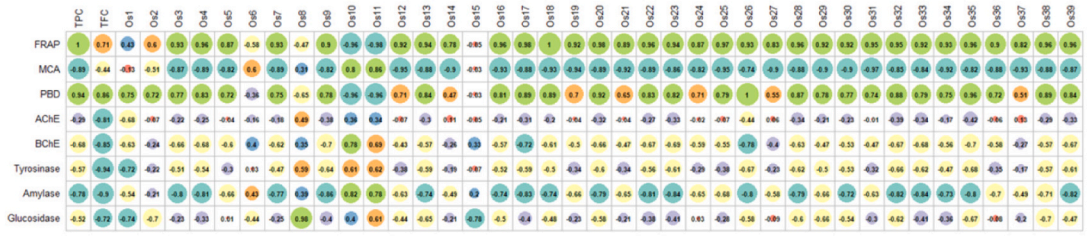
Fig. 3. Distance matrix analysis on the bioactivities *Lamiaceae* sp. (Red color: high similarity. Blue color: low similarity). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

flavonoids. A similar fact was also obtained by Tekeli [44]. The essential oil of *S. phyrgia* was chemically characterized and quantified and the results are tabulated in Table 7. Spathulenol (17.96%), carvacrol (17.30%) and caryophyllene oxide (6.93%) were detected as the main components in the essential oil. In an earlier study by Ozer et al. [45], the main compounds in *S. phrygia* essential oil were caryophyllene oxide (11.4%), limonene (10.6%) and p-cymene (10.1%). Kırımer et al. [46] reported that the essential oil of *S. phyrgia* was rich in monoterpene hydrocarbons.

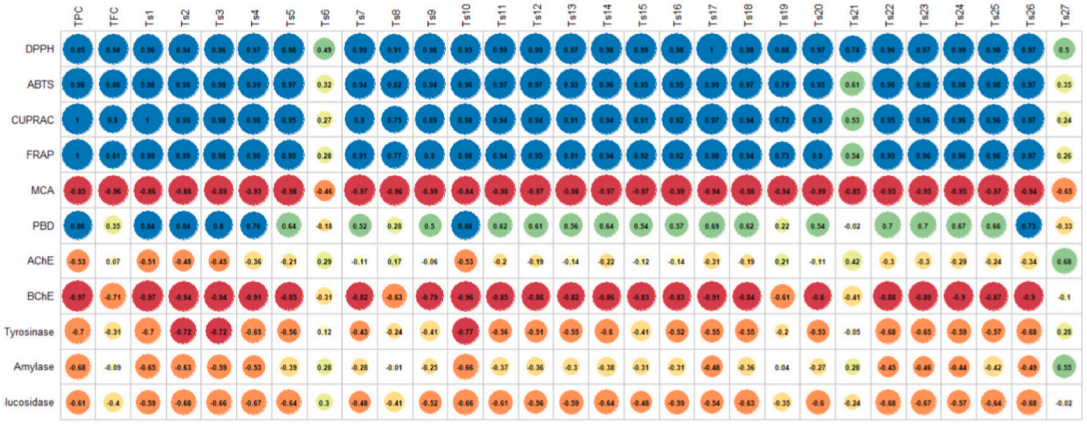
3.3. Antioxidant activities

The DPPH and ABTS assays are widely used to investigate the free radical-scavenging capacity of plant extracts, given their potential to prevent oxidative stress-related diseases [47,48]. The results obtained in the current study revealed that the values for *O. sipyleum* extracts using the DPPH and ABTS assays were high, with the highest activity observed for the Spent after SCO_2 extract (497.08 ± 8.32 and 548.57 ± 16.66 mg TE/g, respectively). At the same time, the other two plant species exhibit low antioxidant activity (Table 8) compared to *O. sipyleum*. The results align with the previous studies regarding the antioxidant properties; a range of extracts of the same *O. sipyleum* have shown scavenger effects against DPPH [49] and hydroxyl radical, reduced peroxide generation via thiocyanate methods, and also reduced molybdenum (VI) to molybdenum(V) within phosphomolybdenum method [50]. Another previous study proved the radical scavenging activity of the *O. sipyleum* with DPPH and ABTS IC_{50} values of 102.75 μg/mL and 88.64 μg/mL, respectively. Although an exact comparison with our results is difficult because the results are expressed in different units, a general understanding of our results, especially with spent SCO_2, revealed the best scavenging activity [23]. This agrees with the general knowledge that antioxidant potential may vary between plant species depending on their polyphenolic content. It is also likely that the difference in scavenging capacity depends on the different phenolic compounds in the plants since the type of phenolic compounds (flavonoids, phenolic acids) influences their ability to neutralize free radicals [51].

The CUPRAC assay estimates the cupric ion-reducing antioxidant capacity and has been a valuable tool in assessing the antioxidant potentials of plant extracts [52]. High CUPRAC values observed for *O. sipyleum* (Spent after HD with a value of 926.71 ± 12.08 mg TE/g) further establish its high antioxidant capacity. The principle of this assay is based on the reduction of Cu^{2+} ions as a measure of electron-donating capacity; the higher the value, the better the reducing capability of metal ions to neutralize free radicals [52,53]. The moderate CUPRAC values for *T. sipyleus* and the low values for *S. phrygia* (Table 8) agree with the trend observed in the assays above. These results have once again emphasized the role of plant species in determining antioxidant capacity since compounds such as flavonoids, tannins, and other



A: *Origanum sipyleum*



B: *Thymus sipyleus*

C: *Sideritis phrygia*

Fig. 4. Correlation between biological activities and identified metabolites in the tested species. The number of compounds is referred to in Tables 2, 4, and 6.

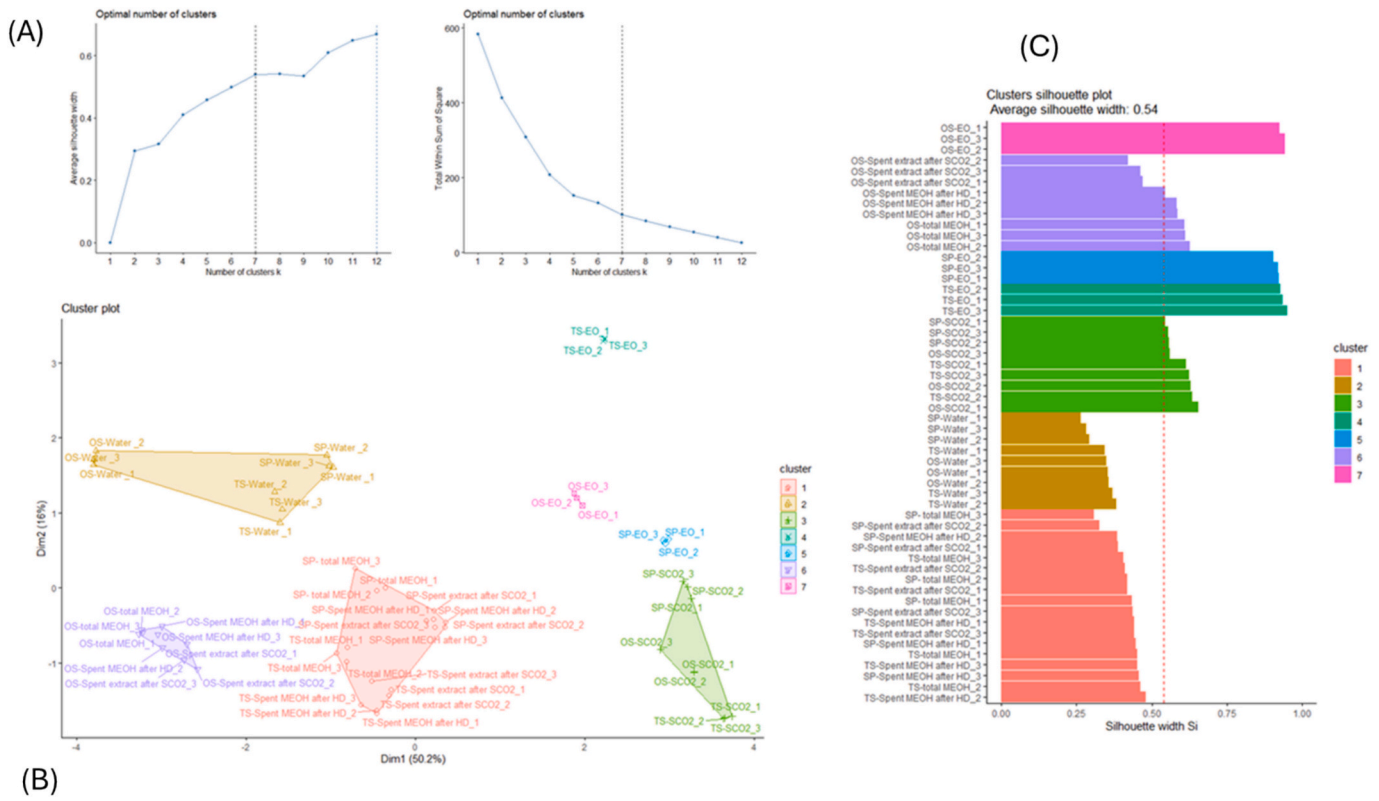


Fig. 5. k-medoids clustering analysis on the bioactivities of the tested extracts. A. Estimation of the optimal number of clusters using the elbow and average silhouette methods. B. Cluster plot showing the different clusters based on the bioactivities. *C. silhouette* plot displaying the average distance between clusters.

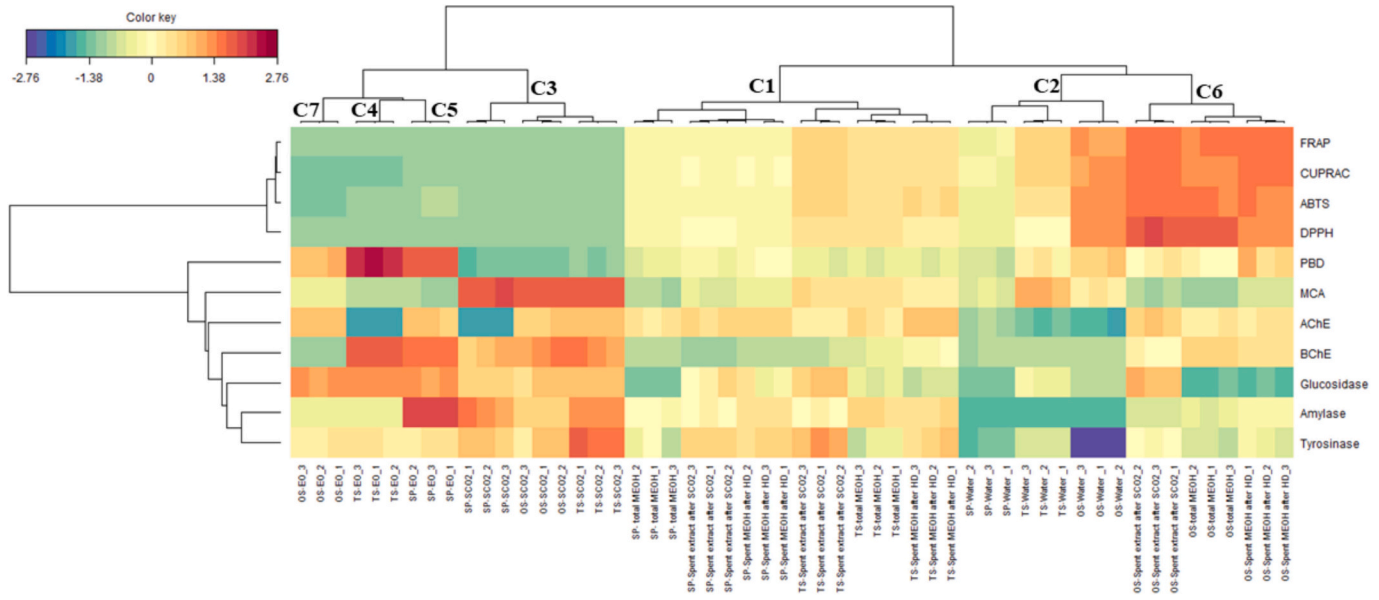


Fig. 6. Heatmap displaying the bioactivities characterizing each cluster (Red color: high activity. Blue color: low activity). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

polyphenols are primarily involved in electron transfer during the reduction process [54].

Based on ferric ion-reducing antioxidant capacity, the FRAP assay [48] showed similar tendencies to the CUPRAC assay. *O. sipyleum* had the highest reducing power (Table 8), reflecting its ability to donate electrons and reduce ferric ions to ferrous ions. The capacity is related to its high phenolic content because phenolics are efficient electron donors in this type of assay [48]. *T. sipyleus* exhibits a moderate FRAP value, consistent with its intermediate antioxidant capabilities. In contrast, *S. phrygia* exhibited relatively weak ferric ion-reducing activity (Table 8).

The PBD assay measures total antioxidant capacity, involving various redox reactions [55]. In the phosphomolybdenum method, the reduction of Mo (VI) to Mo (V) by an antioxidant molecule forms a green

Mo (V) complex with an absorbance maximum at 695 nm [56]. High values in *O. Sipyleum* suggest that its essential oils (6.47 ± 0.02 (mmol TE/g) and water (6.00 ± 0.07 mmol TE/g) extracts may contain various antioxidants, such as flavonoids and other phenolic compounds responsible for broad-spectrum antioxidant activities. Previous reports also suggest a similar trend in the antioxidant potential of different extracts of *O. sipyleum* using the PBD assay [31]. In contrast, the highest values were obtained by EO extracts of *T. sipyleus* (9.08 ± 0.24 mmol TE/g) and *S. phrygia* (8.02 ± 0.20 mmol TE/g), while all other extracts of both species exhibited PBD values in the moderate zone (Table 8). At the same time, the latter showed lower overall antioxidant capacity. These results are supported by the MCA results, which showed *O. sipyleum*'s highest ability to chelate metal ions, which may be very important for reducing oxidative stress. Chelating metal ions is essential because metals like iron and copper can catalyze the formation of ROS, which causes cellular damage.

The findings suggest that *O. sipyleum* exhibits the highest antioxidant activity across the assays employed in this work, particularly in the Spent after SCO_2 and Spent after HD extracts. Antioxidant activities of *T. sipyleus* and *S. phrygia* were recorded at moderate levels, underscoring the importance of selecting an appropriate extraction method to maximize bioactivity and yield.

3.4. Inhibitory properties of tested enzyme extracts

The inhibitive property of the enzyme extracts from *O. sipyleum*, *T. sipyleus*, and *S. phrygia* was studied against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, α-amylase, and α-glucosidase. The results are summarized in Table 9.

Acetylcholinesterase is an enzyme that plays a vital role in neurotransmission by breaking down acetylcholine [57]. Inhibition of acetylcholinesterase is a target for the therapy of neurodegenerative disorders like Alzheimer's disease [58]. *O. sipyleum* EO exhibited a strong AChE inhibiting activity: 2.87 ± 0.06 mg GALAE/g. This observation was in complete agreement with the outstanding antioxidant activity displayed in DPPH and FRAP studies, proving that the contribution of high phenolics should be crucially considered in terms of the role of its volatile components. It was also coupled with significant inhibition exerted by the residual extract after SCO_2 extraction at 2.73 ± 0.14 mg GALAE/g. In a previous study, the aqueous extract of *O. sipyleum* exhibited the highest AChE inhibitory activity (4.87 mg GALAE/g extract), followed by the methanol extract (3.62 mg GALAE/g extract) [30]. Although our values are lower, they indicate the plant's potential against the inhibition of this important enzyme. Interestingly, *T. sipyleus* SCO2 extract showed the highest AChE inhibitory activity (2.94 ± 0.06 mg GALAE/g), possibly because the extraction process maintains bioactive components responsible for demonstrating strong electron-donating ability. *S. phrygia* essential oil extract exhibited quite a significant efficacy of 2.84 ± 0.05 mg GALAE/g, reflecting its high content of volatiles and phenolics.

Another cholinesterase, BChE, which is also involved in acetylcholine degradation, is considered a secondary target for neurodegenerative treatments [59]. Indeed, the SCO_2 extract from *T. sipyleus* had remarkable activity against BChE inhibition (2.90 ± 0.15 mg GALAE/g). *O. sipyleum* also showed considerable inhibitory activity against butyrylcholinesterase for most of its extracts. However, the SCO_2 extract showed the highest inhibitory activity (2.88 ± 0.31 mg GALAE/g), which aligns with previous investigations [30]. *S. phrygia* showed the highest activity in its essential oil extract (3.21 ± 0.08 mg GALAE/g), thus showing that essential oil constituents might contribute to enzyme inhibition activity. Aqueous extracts of all plants exhibited low BChE inhibition, presumably due to the lack of sufficient hydrophobic bioactive constituents.

Tyrosinase is a crucial enzyme implicated in melanin synthesis and is a target in hyperpigmentation conditions [60]. SCO_2 extract of *T. sipyleus* extract exhibited the highest tyrosinase inhibition (62.09 ± 1.35 mg KAE/g), reflecting its excellent antioxidant capacity in ABTS and CUPRAC assays [22]. Similarly, the SCO_2 extract of *O. sipyleum* exhibited significant activity of 53.96 ± 3.01 mg KAE/g, indicating that supercritical extraction is essential in maintaining bioactive phenolics. SCO_2 and "spent after SCO_2" extracts showed remarkable inhibition in *S. phrygia* extracts, with 54.59 ± 1.60 and 53.34 ± 0.44 mg KAE/g (Table 9), respectively, indicating that in this plant both volatile and non-volatile antioxidant molecules play a significant role.

α-Amylase and α-glucosidase inhibitors are excellent ways to decrease postprandial hyperglycemia levels [61]. Various inhibitors of α-amylase and α-glucosidase have been developed from plant sources as alternative therapeutics with high efficiency and lowering the side effects of available drugs [61,62]. Our results (Table 9) indicate that *S. phrygia* essential oil extract exhibited the most effective α-amylase inhibition with 0.48 ± 0.01 mmol ACAE/g. The SCO_2 extract of *O. sipyleum* (0.33 ± 0.01 mmol ACAE/g) and *T. sipyleus* (0.41 ± 0.01 mmol ACAE/g) showed significant α-amylase inhibition. Considering α-glucosidase inhibition activity, the highest value was 3.28 ± 0.04 mmol ACAE/g for *O. sipyleum* essential oil, followed by *T. sipyleus* SCO_2 and EO extracts. In contrast, the EO extract of *S. phrygia* demonstrated 3.25 ± 0.03 mmol ACAE/g. These results are also supported by the previously published reports on the investigated plant species [22,30]. These findings indicate the potential of these plant species for diabetes-related uses.

The enzyme-inhibitory activities observed in this study show good correlation with the antioxidant properties of the extracts. In general, high TPC and TFC values were associated with increased enzyme inhibition, which was more pronounced for SCO_2 and EO extracts. Supercritical CO_2 extraction is highly effective at preserving bioactive molecules responsible for antioxidant and enzyme-inhibitory activities. The results showed that the plants studied, especially *O. sipyleum* and *T. sipyleus,* have very high medicinal potential for treating neurological disorders, hyperpigmentation, and diabetes. Antioxidant and enzyme-inhibitory activities interact; therefore, choosing an appropriate extraction method is necessary to improve bioactivity.

3.5. K-medoids analysis

The distance matrix analysis highlights the degree of similarity and dissimilarity among the studied species based on their bioactivities. The color gradient clearly reveals groups of samples sharing comparable bioactivity profiles, as indicated by the red regions of high similarity, whereas blue regions indicate pronounced dissimilarities between samples (Fig. 3). Such differences may be attributed to variations in phytochemical composition among the studied samples, as we have observed previously. Indeed, Fig. 4 displays the correlation coefficients between biological activity and chemical compounds. As can be seen, several chemical compounds played a significant role in the majority of evaluated biological activities for each species.

The k-medoids clustering analysis further supports the presence of distinct clusters of samples based on their bioactivity profiles. Indeed, the results reported in Fig. 5A suggest that the dataset is optimally partitioned into seven clusters, indicating the presence of well-defined groups of samples sharing similar biological activities. Fig. 5B illustrates the cluster distribution of the samples. The clustering pattern reveals that samples are clearly separated into distinct groups, confirming the heterogeneity in bioactivity among the studied *Lamiaceae* samples. Cluster 1 includes samples of *Thymus sipyleus*, *Sideritis phrygia* derived from MeOH extracts, spent MeOH after HD extraction, as well as the spent extracts after SCO_2 extraction, while cluster 6 included *Origanum sipyleum* samples obtained using the same extraction procedures. Cluster 2 comprises the aqueous extracts of the three studied species, while Cluster 3 consists of the SCO_2 extracts. Clusters 4, 5, and 7 were each represented by the essential oil (EO) extract of *Thymus sipyleus*, *Sideritis phrygia* and *Origanum sipyleum*, respectively. Fig. 5C presents the silhouette plot, which evaluates the quality of clustering. The positive

silhouette values obtained for most samples indicate a satisfactory clustering structure and confirm that the samples are appropriately assigned to their respective clusters. Fig. 5C displays the silhouette plot used to evaluate the quality of the clustering. A S_i value of 0.54 obtained indicates that most samples are well assigned to their respective clusters, confirming the robustness of the clustering structure and the reliability of the identified groups [63]. Finally, the heatmap corroborates the clustering results, revealing that each cluster is characterized by a distinct bioactivity profile. Clusters such as C6 and C3 were associated with stronger antioxidant and enzyme inhibitory activities, respectively, whereas C1 displayed moderate activity across the evaluated bioassays (Fig. 6).

4. Conclusion

Recently, there has been growing interest in using plants or plant products to design health-promoting applications. Many plants, particularly those in the Lamiaceae family, have been used as sources of essential oils. However, the materials remaining after hydrodistillation or supercritical CO_2 extraction are generally considered waste. This paper mainly focuses on the biological potential of these waste materials. To this end, we tested both total and spent extracts. Generally, the spent extracts (after HD or SCO_2 extraction) were richer in bioactive compounds. The spent extracts exhibited greater radical scavenging and reducing power. Regarding the results of enzyme inhibition, different results were obtained for each plant species. These results suggest that raw materials obtained through hydrodistillation or supercritical CO_2 extraction should not be considered waste, as they are a valuable source of bioactive compounds for use in health-promoting applications. These findings support a sustainable, circular approach to plant resource utilization, contributing to waste valorization and the development of novel bioactive products. However, these results provide the first insight, and future studies are needed to confirm their validation with regard to toxicity or pharmacodynamic properties.

CRediT authorship contribution statement

Gokhan Zengin: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Shakeel Ahmed:** Writing – original draft, Visualization, Methodology, Investigation, Conceptualization. **Kouadio Ibrahime Sinan:** Writing – review & editing, Writing – original draft, Visualization, Software, Investigation, Conceptualization. **Stefano Dall'Acqua:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Selami Selvi:** Writing – review & editing, Writing – original draft, Supervision, Resources, Conceptualization. **Umut Kargili:** Writing – original draft, Methodology, Investigation, Conceptualization. **Gunes Ak:** Writing – original draft, Methodology, Investigation, Conceptualization. **Enver Saka:** Writing – review & editing, Writing – original draft, Investigation, Data curation, Conceptualization. **Bengusu H. Akgul:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Milena Terzic:** Writing – review & editing, Writing – original draft, Supervision, Methodology, Investigation, Conceptualization.

Declaration of generative Ai and Ai-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT (OpenAI) and Grammarly to improve the clarity and language of the manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.microc.2026.118739.

Data availability

Data will be made available on request.

References

[1] S. Dall'Acqua, S. Sut, I. Baskose, U. Kargılı, G. Orlando, G. Zengin, Exploring the effects of post-distillation and post-supercritical CO_2 extraction on chemical profile and biological activities of two *Salvia* species (*S. Chrysophylla* and *S. Microstegia*), Microchem. J. 198 (2024) 110183.
[2] S. Ahmed, A. Cvetanović Nilofar, A. Kljakić, B. Stupar, J. Lončar, U. Božunović, E. Gašić, C. Yıldıztugay, G. Zengin Ferrante, Exploring traditional and modern approaches for extracting bioactive compounds from *Ferulago trachycarpa*, Prep. Biochem. Biotechnol. (2024) 1–14.
[3] K.D. Datkhile, S.R. Patil, M.N. Patil, P.P. Durgawale, N.J. Jagdale, V.N. Deshmukh, Studies on phytoconstituents, in vitro antioxidant, antibacterial and cytotoxicity potential of Argemone mexicana Linn, (family: Papaveraceae), Journal of Natural Science, Biology and Medicine 11 (2020) 198–205.
[4] D.A. Korany, N. Nilofar, G. Zengin, O.A. Eldahshan, Chemical constituents, antioxidant, and enzyme inhibitory potentials supported by in-silico studies of the n-hexane extract and essential oil of *Platycladus orientalis* (L.) Franco Leaves, Chemistry & Biodiversity, e202402000, 2026.
[5] G. Bjørklund, S. Chirumbolo, Role of oxidative stress and antioxidants in daily nutrition and human health, Nutrition 33 (2017) 311–321.
[6] J. Zhang, Y. Wu, Y. Li, S. Li, J. Liu, X. Yang, G. Xia, G. Wang, Natural products and derivatives for breast cancer treatment: from drug discovery to molecular mechanism, Phytomedicine 155600 (2024).
[7] E. González-Burgos, M. Carretero, M. Gómez-Serranillos, *Sideritis* spp.: uses, chemical composition and pharmacological activities—a review, J. Ethnopharmacol. 135 (2011) 209–225.
[8] L.R. Ramos da Silva, O.O. Ferreira, J.N. Cruz, C. de Jesus Pereira, T. Oliveira Franco, M.M. dos Anjos, W. Almeida Cascaes, E. da Costa, Helena de Aguiar, M. Santana Andrade, De Oliveira, Lamiaceae essential oils, phytochemical profile, antioxidant, and biological activities, Evid. Based Complement. Alternat. Med. 2021 (2021) 6748052.
[9] H.D. Dorman, S.G. Deans, Antimicrobial agents from plants: antibacterial activity of plant volatile oils, J. Appl. Microbiol. 88 (2000) 308–316.
[10] O. Borugă, C. Jianu, C. Mişcă, I. Goleţ, A. Gruia, F. Horhat, *Thymus vulgaris* essential oil: chemical composition and antimicrobial activity, J. Med. Life 7 (2014) 56.
[11] T.G. Tutin, Flora Europaea: Plantaginaceae to Compositae (and Rubiaceae), Cambridge University Press, 1964.
[12] M.B. Farhat, A. Landoulsi, R. Chaouch-Hamada, J.A. Sotomayor, M.J. Jordán, Characterization and quantification of phenolic compounds and antioxidant properties of *Salvia* species growing in different habitats, Ind. Crop. Prod. 49 (2013) 904–914.
[13] S. Schaffer, S. Schmitt-Schillig, W. Muller, G. Eckert, Antioxidant properties of Mediterranean food plant extracts: geographical differences, Journal of Physiology and Pharmacology. Supplement 56 (2005) 115–124.
[14] F. Chemat, M.A. Vian, G. Cravotto, Green extraction of natural products: concept and principles, Int. J. Mol. Sci. 13 (2012) 8615–8627.
[15] H. Maoz, A. Elalouf, A.Y. Rosenfeld, Pharmacological management of Lamiaceae plants: exploring antioxidant, anti-inflammatory, antimicrobial, and anticancer activities, S. Afr. J. Bot. 192 (2026) 89–108.
[16] N. Bibi Sadeer, D. Montesano, S. Albrizio, G. Zengin, M.F. Mahomoodally, The versatility of antioxidant assays in food science and safety—chemistry, applications, strengths, and limitations, Antioxidants 9 (2020) 709.
[17] D.M. Grochowski, S. Uysal, A. Aktumsek, S. Granica, G. Zengin, R. Ceylan, M. Locatelli, M. Tomczyk, In vitro enzyme inhibitory properties, antioxidant activities, and phytochemical profile of *Potentilla thuringiaca*, Phytochem. Lett. 20 (2017) 365–372.
[18] T. Hatano, R. Edamatsu, M. Hiramatsu, A. Mori, Y. Fujita, T. Yasuhara, T. Yoshida, T. Okuda, Effects of the interaction of tannins with co-existing substances. VI.: effects of tannins and related polyphenols on superoxide anion radical, and on 1, 1-Diphenyl-2-picrylhydrazyl radical, Chem. Pharm. Bull. 37 (1989) 2016–2021.
[19] S. Kumar, M.M. Abedin, A.K. Singh, S. Das, Role of Phenolic Compounds in Plant-Defensive Mechanisms, Plant Phenolics in Sustainable Agriculture: volume 1, Springer, 2020, pp. 517–532.
[20] D. Ivanova, D. Gerova, T. Chervenkov, T. Yankova, Polyphenols and antioxidant capacity of Bulgarian medicinal plants, J. Ethnopharmacol. 96 (2005) 145–150.

[21] E.P. Gutiérrez-Grijalva, M.A. Picos-Salas, N. Leyva-López, M.S. Criollo-Mendoza, G. Vazquez-Olivo, J.B. Heredia, Flavonoids and phenolic acids from oregano: occurrence, biological activity and health benefits, Plants 7 (2017) 2.
[22] E.J. Llorent-Martínez, A. Ruiz-Medina, G. Zengin, G. Ak, S. Jugreet, M. F. Mahomoodally, G. Emre, G. Orlando, M.L. Libero, A. Acquaviva Nilofar, S.C. Di Simone, L. Menghini, C. Ferrante, L. Brunetti, L. Recinella, S. Leone, M.A. Shariati, A.I. Uba, A. Chiavaroli, New biological and chemical evidence of two Lamiaceae Species (*Thymbra capitata* and *Thymus sipyleus* subsp. *rosulans*): In vitro, in silico and ex vivo approaches, Molecules, 27, 2022.
[23] A. Kaska, The phenolic content, antioxidant and cytotoxic activities of *Origanum sipyleum* from Turkey, International Journal of Secondary Metabolite 5 (2018) 343–352.
[24] I.Z. Sadiq, Free radicals and oxidative stress: signaling mechanisms, redox basis for human diseases, and cell cycle regulation, Curr. Mol. Med. 23 (2023) 13–35.
[25] K.I. Nilofar, S. Sinan, S. Dall'Acqua, A.I. Sut, O.K. Uba, C. Etienne, J. Ferrante, G. Ahmad, Zengin, *Ziziphus mauritiana* Lam. bark and leaves: Extraction, phytochemical composition, in vitro bioassays and in silico studies, Plants, 13, 2024, p. 2195.
[26] S.V. Luca, G. Zengin, K.I. Sinan, I. Korona-Glowniak, M. Minceva, K. Skalicka-Woźniak, A. Trifan, Value-added compounds with antimicrobial, antioxidant, and enzyme-inhibitory effects from post-distillation and post-supercritical CO_2 extraction by-products of rosemary, Antioxidants 12 (2023) 244.
[27] K. Tyśkiewicz, M. Konkol, E. Rój, The application of supercritical fluid extraction in phenolic compounds isolation from natural plant materials, Molecules 23 (2018).
[28] G. Zengin, F. Mahomoodally, C. Picot-Allain, A. Diuzheva, J. Jekő, Z. Cziáky, A. Cvetanović, A. Aktumsek, Z. Zeković, K.R. Rengasamy, Metabolomic profile of *Salvia viridis* L. root extracts using HPLC–MS/MS technique and their pharmacological properties: a comparative study, Ind. Crop. Prod. 131 (2019) 266–280.
[29] I. Čechovičienė, K. Kazancev, E. Hallmann, E. Sendžikienė, M. Kruk, J. Viškelis, Ž. Tarasevičienė, Supercritical CO_2 and conventional extraction of bioactive compounds from different cultivars of blackberry (*Rubus fruticosus* L.), Pomace, Plants 13 (2024) 2931.
[30] G. Zengin, C. Ferrante, G. Orlando, D. Zheleva-Dimitrova, R. Gevrenova, L. Recinella, A. Chiavaroli, S. Leone, L. Brunetti, M.Z. Aumeeruddy, A. Aktumsek, M.F. Mahomoodally, P. Angelini, S. Covino, R. Venanzoni, B. Tirillini, L. Menghini, Chemical profiling and pharmaco-toxicological activity of *Origanum sipyleum* extracts: exploring for novel sources for potential therapeutic agents, J. Food Biochem. 43 (2019) e13003.
[31] G. Ozkan, O. Sagdic, L. Ekici, I. Ozturk, M.M. Ozcan, Phenolic compounds of *Origanum sipyleum* L. extract, and its antioxidant and antibacterial activities, journal of food, Lipids 14 (2007) 157–169.
[32] G. Tüzün, C. Hepokur, A. Akkuş, M.O. Faruque, S. Mısır, Antiproliferative effects, Brdu assay and antioxidant activities of *Origanum sipyleum* L. extraction on breast cancer cell lines: investigation *in vitro*, Cumhuriyet Science Journal 47 (2026) 1–8.
[33] K.H.C. Baser, T. Ozek, M. Kurkcuoglu, G. Tümen, Composition of the essential oil of *Origanum sipyleum* of Turkish origin, the, Journal of Essential Oil Research 4 (1992) 139–142.
[34] F. Uysal Bayar, O. Çınar, Kültür koşullarında yetiştirilen farklı *Origanum* spp. türlerinin bazı verim ve kalite parametreleri, Derim 37 (2020) 10–17.
[35] S. Erbaş, H. Fakir, Türkiye'nin Batı Akdeniz Yöresinde doğal olarak yetişen dağ çayı (*Sideritis libanotic*a Labill. subsp. linearis (Bentham) Bornm) ve bayır kekiği (*Origanum sipyleum* L.) türlerinin uçucu yağ oranları ve bileşenlerinin belirlenmesi, SDU Faculty of Forestry Journal 13 (2012) 119–122.
[36] N. Gavarić, N. Kladar, A. Mišan, A. Nikolić, I. Samojlik, N. Mimica-Dukić, B. Božin, Postdistillation waste material of thyme (*Thymus vulgaris* L., Lamiaceae) as a potential source of biologically active compounds, Ind. Crop. Prod. 74 (2015) 457–464.
[37] S.V. Luca, G. Zengin, K.I. Sinan, K. Skalicka-Woźniak, A. Trifan, Post-distillation by-products of aromatic plants from Lamiaceae family as rich sources of antioxidants and enzyme inhibitors, Antioxidants (Basel) 12 (2023).
[38] S. Yiğitkan, M. Fırat, Essential oil contents and biological activities of *Thymus canoviridis* JALAS and *Thymus sipyleus* Boiss, Journal of Faculty of Pharmacy of Ankara University 48 (2024) 1068–1077.
[39] K.H.C. Baser, M. Kürkçüoglu, T. Özek, G. Tümen, A. Akgül, Essential oil of *Thymus sipyleus* Boiss. Subsp. *sipyleus* var. *sipyleus*, J. Essent. Oil Res. 7 (1995) 411–413.
[40] B. Tepe, M. Sokmen, H.A. Akpulat, D. Daferera, M. Polissiou, A. Sokmen, Antioxidative activity of the essential oils of *Thymus sipyleus* subsp. *sipyleus* var. *sipyleus* and *Thymus sipyleus* subsp. *sipyleus* var. *rosulans*, J. Food Eng. 66 (2005) 447–454.
[41] G. Yilmaz, I. Telci, N. Kandemir, N. Kaya, Essential oil contents and compositions of *Thymus sipyleus* growing wild in Central Turkey, Asian J. Chem. 16 (2016) 841–847.
[42] H.G. Ağalar, M. Kürkçüoğlu, K.H.C. Başer, K. Turgut, Volatile constituents of three *Thymus sipyleus* Boiss. Subspecies from different sites in Turkey, Turk. J. Chem. 45 (2021) 1959–1967.
[43] Z. Tunalier, M. Kosar, N. Ozturk, K.H.C. Baser, H. Duman, N. Kirimer, Antioxidant properties and phenolic composition of *Sideritis* species, Chem. Nat. Compd. 40 (2004) 206–210.
[44] Y. Tekeli, Antioxidant activities and phenolic compounds of two endemic taxa of Labiatae *Sideritis*, Rev Chim-Bucharest 63 (2012) 465–469.
[45] Z. Özer, S. Çarıkçı, S. Dereli, D. Acar, A.C. Gören, T. Kılıç, Essential oil composition of five *Sideritis* species endemic to Turkey, Süleyman Demirel Üniversitesi Fen Bilimleri Enstitüsü Dergisi 22 (2018) 301–305.
[46] N. Tabanca, N.E. Kirimer, K. Başer, The composition of essential oils from two varieties of *Sideritis erythrantha* var. *erythrantha* and var. *cedretorum*, Turkish Journal of Chemistry 25 (2001) 201–208.
[47] I.D. Nwachukwu, R.A. Sarteshnizi, C.C. Udenigwe, R.E. Aluko, A concise review of current in vitro chemical and cell-based antioxidant assay methods, Molecules 26 (2021) 4865.
[48] V. Mayakrishnan, S. Veluswamy, K.S. Sundaram, P. Kannappan, N. Abdullah, Free radical scavenging potential of *Lagenaria siceraria* (Molina) Standl fruits extract, Asian Pac. J. Trop. Med. 6 (2013) 20–26.
[49] M. Nakiboglu, R.O. Urek, H.A. Kayali, L. Tarhan, Antioxidant capacities of endemic Sideritis sipylea and *Origanum sipyleum* from Turkey, Food Chem. 104 (2007) 630–635.
[50] A. Karagöz, F.T. Artun, G. Özcan, G. Melikoğlu, S. Anıl, Ş. Kültür, N. Sütlüpınar, In vitro evaluation of antioxidant activity of some plant methanol extracts, Biotechnol. Biotechnol. Equip. 29 (2015) 1184–1189.
[51] A. Acquaviva, A. Nilofar, G. Bouyahya, S.C. Di Zengin, L. Simone, S. Recinella, L. Leone, A.I. Brunetti, U. Cakilcioğlu Uba, Chemical characterization of different extracts from *Artemisia annua* and their antioxidant, enzyme inhibitory and anti-inflammatory properties, Chem. Biodivers. 20 (2023) e202300547.
[52] R. Apak, Electron transfer-based antioxidant capacity assays and the cupric ion reducing antioxidant capacity (CUPRAC) assay, Measurement of antioxidant activity & capacity: recent trends and applications (2018) 57–75.
[53] M. Özyürek, K. Güçlü, E. Tütem, K.S. Başkan, E. Erçağ, S.E. Çelik, S. Baki, L. Yıldız, Ş. Karaman, R. Apak, A comprehensive review of CUPRAC methodology, Anal. Methods 3 (2011) 2439–2453.
[54] A. Guidea, C. Ză̆grean-Tuza, A.C. Moț, C. Sârbu, Comprehensive evaluation of radical scavenging, reducing power and chelating capacity of free proteinogenic amino acids using spectroscopic assays and multivariate exploratory techniques, Spectrochim. Acta A Mol. Biomol. Spectrosc. 233 (2020) 118158.
[55] T. Yang, L. Fang, T. Lin, J. Li, Y. Zhang, A. Zhou, J. Xie, Ultrasonicated sour jujube seed flavonoids extract exerts ameliorative antioxidant capacity and reduces aβ-induced toxicity in *Caenorhabditis elegans*, J. Ethnopharmacol. 239 (2019) 111886.
[56] P. Prieto, M. Pineda, M. Aguilar, Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, Anal. Biochem. 269 (1999) 337–341.
[57] U. Holzgrabe, P. Kapková, V. Alptüzün, J. Scheiber, E. Kugelmann, Targeting acetylcholinesterase to treat neurodegeneration, Expert Opin. Ther. Targets 11 (2007) 161–179.
[58] Ł.J. Walczak-Nowicka, M. Herbet, Acetylcholinesterase inhibitors in the treatment of neurodegenerative diseases and the role of acetylcholinesterase in their pathogenesis, Int. J. Mol. Sci. 22 (2021) 9290.
[59] A. Nordberg, C. Ballard, R. Bullock, T. Darreh-Shori, M. Somogyi, A review of butyrylcholinesterase as a therapeutic target in the treatment of Alzheimer's disease, The primary care companion for CNS disorders 15 (2013) 26731.
[60] S. Zolghadri, M. Beygi, T.F. Mohammad, M. Alijanianzadeh, T. Pillaiyar, P. Garcia-Molina, F. Garcia-Canovas, J. Munoz-Munoz, A.A. Saboury, Targeting tyrosinase in hyperpigmentation: current status, limitations and future promises, Biochem. Pharmacol. 212 (2023) 115574.
[61] T. Matsui, I. Ogunwande, K. Abesundara, K. Matsumoto, Anti-hyperglycemic potential of natural products, Mini Rev. Med. Chem. 6 (2006) 349–356.
[62] H. Matsuda, T. Morikawa, M. Yoshikawa, Antidiabetogenic constituents from several natural medicines, Pure Appl. Chem. 74 (2002) 1301–1308.
[63] T.M. Kodinariya, P.R. Makwana, Review on determining number of cluster in K-means clustering, Int. J. 1 (2013) 90–95.