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Phytochemical analysis, antioxidant, and enzyme inhibition activity of five *Salvia* taxa from Turkey

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ABSTRACT

Salvia L. species are widely used for treatment of different disorders in traditional Turkish medicine. In this study, the methanol extracts of five *Salvia* taxa, including *S. aucheri* subsp. *canescens, S. aytachii, S. heldreichiana, S. viridis,* and *S. wiedemannii* were investigated for their antioxidant activity and enzyme inhibitory potentials which are related with health-benefit properties. In addition, total phenolic and flavonoid contents were determined with Folin- Ciocalteu and aluminum chloride spectrophotometric method, respectively. Screening of enzyme inhibition activity was performed out using ELISA microplate reader. Antioxidant activity was evaluated by 2,2′-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′ -Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical scavenging and iron chelating tests. The *S. aytachii* extract was found with highest total phenol content (77.82 ± 1.76 μg PE/mg) and total flavonoid content (22.33 ± 0.17 μg QE/mg) as dry weight basis. These findings specify that the investigated *Salvia* taxa for anticholinesterase activity can be appealed further phytochemical estimation for spotting its active components.

1. Introduction

Plants have been used as a source of food, medicine and fragrance source for a long time (Sharifi-Rad et al., 2018; Senkal et al., 2019). Lamiaceae family is an important source for different economic industrial areas, such as cosmetics, food, medicine, and perfumery (Senkal et al., 2019; Selvi et al., 2022). *Salvia* L. has ca. 1000 species distributed mainly in Central and South America and Asia and it is the largest genus of Lamiaceae (Abak et al., 2018; Karik et al., 2018; Sharifi-Rad et al., 2018; Celep et al., 2020; Firat, 2020; Eksi and Yilmaz, 2021). Turkey is a center of diversity for *Salvia* with 53 endemics, total 101 *Salvia* species (Firat, 2020; Eksi and Yilmaz 2021).

The healing properties of *Salvia* have been known since the earliest times in the ancient Egypt, and by Anglo-Saxons, Greeks, Romans (Dweck, 2000; Sharifi-Rad et al., 2018; Senkal et al., 2019; Eksi and Yilmaz 2021). *Salvia* species have been used for treating various health issues such as some cancers, bronchitis, diarrhea, asthma, rheumatism, cold, flu, galactorrhoea, headache, some heart diseases, memory enhancement, menopause, muscle pain, night sweats, pharyngitis, skin infections, stomachache, stomatitis, sore throat, toothache, tuberculosis, and wounds since ancient times (Sargin et al., 2013; Paksoy et al., 2016; Abak et al., 2018; Karik et al., 2018; Sharifi-Rad et al., 2018; Senkal et al., 2019; Bakir et al., 2020; Kawarty et al., 2020; Selvi et al., 2022). *Salvia* species contain various secondary metabolites such as sterols, flavonoids, sesquiterpenoids, sesterpenoids, diterpenoids, triterpenoids, essential oils, and flavonoids that have several biological and pharmacological activities such as antioxidant, antimicrobial, antialzheimer, anticancer, antidiabetic, antitumor, antiplasmodial, anti-inflammatory, anti-neurodegenerative, anti-enzymatic (anticholinesterase, anti-urease, anti-tyrosinase, anti-elastase), antipyretic, analgesic, hepatotoxic, cytotoxic, insecticidal activities (Abak et al., 2018; Karik et al., 2018; Sharifi-Rad et al., 2018; Tulukcu et al., 2019; Bakir et al., 2020; Eksi and Yilmaz, 2021; Uysal et al., 2021; Yilmaz et al., 2021).

S. aucheri Benth. subsp. *canescens* (Boiss. & Heldr.) Celep, Kahraman & Dogan is an endemic taxon to Turkey (Celep et al., 2011; Eksi and Yilmaz 2021). *S. aytachii* Vural & Adiguzel is an endemic species to Turkey (Vural and Adiguzel, 1996). It has been reported that *S. aytachii* extracts show antimicrobial effects against yeast cultures (Dulger and Gonuz, 2004). It has been reported that camphor and 1,8-cineole are major compounds for *S. aytachii* with 30.78% and 27.28% respectively (Baser et al., 1997). *S. heldreichiana* Boiss. ex A.

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DC. is endemic to the eastern Mediterranean area (Eksi and Yilmaz, 2021). *S. heldreichiana* extracts have important secondary compounds therfore have high antioxidant and antimicrobial potentials (Ulubelen et al., 1995; Akin et al., 2010; Erdogan et al., 2013a, 2013b; Bardakci et al., 2019; Ozcan et al., 2019).

S. viridis L. is an annual herb distributed to Caucasus, Iran, North Iraq, Cyprus, and the Mediterranean area (Davis, 1988; Dweck, 2000). It has been used as a mouthwash against inflammation and sore gums (Dweck, 2000). The presence of essential oils, diterpenoids and triterpenoids, phenolic acids and flavonoids, which have antibacterial and antihyperitive activities, have been reported in the aerial parts and roots of *S. viridis* (Ulubelen and Brieskorn, 1975; Ulubelen et al., 1977, 2000; Grzegorczyk-Karolak et al., 2018). It has been indicated that ethanolic root extracts of *S. viridis* have potential usage for managing chronic complications of alzheimer, diabetes and skin hyperpigmentation disorders (Zengin et al., 2019). *S. wiedemannii* Boiss. is an aromatic plant that is endemic to central Anatolia (Kaya et al., 2009). The essential oil of *S. wiedemannii* has moderate anti-AChE and anti-BChE enzyme activities and ethanol extracts have significant antiviral activity against HSV-1 (Kunduhoglu et al., 2011; Ustun and Ozcelik, 2016).

In this study, the methanol extracts of five *Salvia* taxa, including *S. aucheri* subsp. *canescens, S. aytachii, S. heldreichiana, S. viridis,* and *S. wiedemannii* were investigated for their antioxidant activity and enzyme inhibitory potentials which are related with health-benefit properties. In addition, total phenolic and flavonoid contents were determined with Folin- Ciocalteu and aluminum chloride spectrophotometric method, respectively.

2. Material and methods

2.1. Plant materials

The plant materials of five *Salvia* taxa were collected and identified by Dr. Gulderen Yilmaz. Voucher specimens have been deposited in the herbarium of the Faculty of Pharmacy at Ankara University (AEF), Turkey. The collecting information and extract yield was given in Table 1.

2.2. Preparation of plant extracts

After finely dried and grounded, the aerial parts of five *Salvia* taxa were extracted with methanol. After filtering by coarse filter paper, the filtrate was concentrated under vacuum with rotary evaporator at 40 °C. The obtained crude extracts were stored at −80 °C until required (Table 1).

2.3. LC-MS/MS analyses

The qualitative and quantitative phytochemical composition of studied *Salvia* species was accomplished according to a previously developed and validated LC-MS/MS method (Yilmaz, 2020). Final concentrations of the extracts were adjusted to 1000 mg/L prior to the LC-MS/MS injection. A Shimadzu-Nexera model UHPLC (ultra-high performance liquid chromatograph) coupled to a Shimadzu-LCMS 8040 model tandem mass spectrometer was used to accomplish quantitative evaluation of 53 phytochemicals in the studied *Salvia* extracts. The reversed-phase U-HPLC was equipped with an autosampler (SIL-30AC model), a column oven (CTO-10ASvp model), binary pumps (LC-30CE model), and a degasser (DGU- 20A3R model). The chromatographic seperation was performed on a reversed phase Agilent Poroshell 120 EC—C18 model (150 mm × 2.1 mm, 2.7 mm) analytical column. The column temperature was set to 40 °C. The elution gradient was composed of eluent A (water + 5 mM ammonium formate + 0.1% formic acid) and eluent B (methanol + 5 mM ammonium formate + 0.1% formic acid). The following gradient elution profile was used: 20–100% B (0–25 min), 100% B (25–35 min), 20% B (35–45 min). Furthermore, the solvent flow rate and injection volume were settled as 0.5 mL/min and 5 mL, respectively (Yilmaz, 2020).

2.4. GC–MS conditions for triterpenoid contents

In order to analyze triterpene contents, the samples were derivatized with *N, O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane with (120 min at 70 °C) and examined by 7890A Model GC/FID with 5977B MS. Chromatographic separation was performed with a nonpolar HP-5MS column (30 *m* × 0.25 mm × 0.25 μm film). The GC oven was started at 200 °C, rapidly at 300 °C/min, and held constant for 15 min at this time (300 °C). The temperature was then brought to 310 °C at a rate of 5 °C/min, which stabilized after 2 min. Fixed helium gas (0.8 mL/min) was installed as the carrier gas. Injection and transfer line temperatures were set to 300 °C. Injections were made in splitless mode. The injection volume was 2.0 μL. The electron ionization/mass spectrometer (EI/MS) was set to an ionization energy of 70 eV. The ion source was set to 230 °C. MS data were acquired by setting the full scan mode and scan *m/z* to a density of 50–650 atomic mass units (amu) (Yigitkan et al., 2022).

2.5. GC–MS conditions for diterpernoids and steroids contents

Analysis was performed using Agilent 7890A Model GC/FID gas chromatography coupled to an Agilent 5977B model mass spectrometer (MS), equipped with a split injection port. Chromatographic separation was performed using an apolar HP-5MS column (30 m x 0.25 mm x 0.25 mm film thickness). Helium was used as the carrier gas with a constant flow (1 mL / min). GC oven temperature started at 150 °C and then ramped to 300 °C by a rate of 5 °C per minute and held at final temperature for 20 min. Samples were injected in splitless mode. The injection volume was 2.0 μL. Injection block and transfer line temperatures were held at 300 °C. The mass spectrometer was operated in the electron impact (EI) mode with an ionization energy of 70 eV. The temperature of the ion source was 230 °C. The mass spectrometer (MS) data were obtained in full scan mode and the scanning mass range *m/z* set to 100–600 atomic mass units (amu). The compounds were identified by comparing their retention times and mass spectra with those obtained from authentic samples and/or the NIST and Wiley spectra as well as data from the published literature. For quantification reconstructed ion chromatograms were used, where usually two fragment ions with greater intensities were selected (Bakir et al., 2020; Akdeniz et al., 2022).

Table 1
The collecting information and extract yield of five *Salvia* taxa.

Plant name	Herbarium /Voucher no.	Locality	Solvent	Extract yield
S. viridis	GY 26761	Antalya - Manavgat Rroad, İbradi 10. Km roadside; 1000 m; 22.04.2016	Ethyl acetate	5.2%
S. wiedemannii	GY 27008	Konya, Cihanbeyli, around the Bolluk lake, 950 m; 21.05.2017	Ethyl acetate	6.08%
S. aytachii	GY 28863	Ankara, between Beypazari-Nallihan 15. Km. roadside slopes, 650–870 m; 16.05.2019	Ethyl acetate	7.8%
S. heldreichiana	GY 28919	Konya, Karaman Yesildere village, slopes, 1300 m; 27.06.2020	MeOH	23%
S. aucheri subsp. *canescens*	GY 28920	Konya, Karaman Yesildere village, slopes, 1300 m; 27.06.2020	MeOH	23.2%

2.6. *In-vitro antioxidant activity*

The total phenolic content of the extracts was determined using Folin-Ciocalteu method described by Clarke et al. (2013). Aluminum chloride colorimetric method was used to determine total flavonoid content in the extracts according to the method by Yang et al. (2011). 2−2′-diphenyl-1-picryhydrazyl (DPPH) radical scavenging potential of the extracts was determined by the method of Clarke et al. (2013). The method of Re et al. (1999) was used to determine ABTS scavenging activities of the plant extracts.

2.7. *Enzyme inhibition activity*

2.7.1. *Anti-aging activity*

Tyrosinase, elastase, and collagenase inhibitory activity assays were used to determine the anti-aging potential of the samples. The method developed by Hearing and Jimenez (1987) for tyrosinase inhibitory activity was applied with minor modifications. Briefly, 150 μL of phosphate buffer (pH=8), 10 μL samples, and 20 μL enzyme solutions (28 nM) were added to all wells and incubated at 37 °C for 10 min. Then, 20 μL of L-DOPA (0.5 mM) was added and incubated

Table 2
Identification and quantification of phenolic compounds of *Salvia* taxa by LC-MS/MS.

No	Analytes	Retention Time (min)	Parent İon (*m/z*)[a]	MS^2 (Collision Energy)[b]	Quantification (mg analyte/g extract)[c]				
					S. viridis	*S. wiedemannii*	*S. aytachii*	*S. heldreichiana*	*S. aucheri* subsp. *canescens*
1	Quinic acid	3.0	190.8	93.0	0.044	0.036	ND	ND	0.159
2	Fumaric acid	3.9	115.2	40.9	2.820	0.089	0.190	ND	ND
3	Aconitic acid	4.0	172.8	129.0	ND	ND	ND	ND	ND
4	Gallic acid	4.4	168.8	79.0	ND	ND	ND	ND	ND
5	Epigallocatechin	6.7	304.8	219.0	ND	ND	ND	ND	ND
6	Protocatechuic acid	6.8	152.8	108.0	0.158	0.054	0.063	0.123	0.109
7	Catechin	7.4	288.8	203.1	ND	ND	ND	ND	ND
8	Gentisic acid	8.3	152.8	109.0	ND	ND	ND	ND	ND
9	Chlorogenic acid	8.4	353.0	85.0	0.132	0.034	ND	ND	0.029
10	Protocatechuic aldehyde	8.5	137.2	92.0	0.126	0.035	0.054	0.104	0.065
11	Tannic acid	9.2	182.8	78.0	ND	ND	ND	ND	0.000
12	Epigallocatechingallate	9.4	457.0	305.1	ND	ND	ND	ND	ND
13	1,5-Dicaffeoylquinic acid	9.8	515.0	191.0	ND	ND	ND	ND	ND
14	4-OH Benzoic acid	10.5	137,2	65.0	ND	ND	ND	ND	ND
15	Epicatechin	11.6	289.0	203.0	ND	ND	ND	ND	ND
16	Vanillic acid	11.8	166.8	108.0	ND	ND	ND	ND	ND
17	Caffeic acid	12.1	179.0	134.0	0.021	0.038	0.039	0.271	0.103
18	Syringic acid	12.6	196.8	166.9	ND	ND	ND	ND	ND
19	Vanillin	13.9	153.1	125.0	ND	ND	ND	ND	ND
20	Syringic aldehyde	14.6	181.0	151.1	ND	ND	ND	ND	ND
21	Daidzin	15.2	417.1	199.0	ND	ND	ND	ND	ND
22	Epicatechingallate	15.5	441.0	289.0	ND	ND	ND	ND	ND
23	Piceid	17.2	391.0	135/106.9	ND	ND	ND	ND	ND
24	*p*-Coumaricacid	17.8	163.0	93.0	ND	ND	ND	ND	ND
25	Ferulic acid-D3-IS[d]	18.8	196.2	152.1	IS	IS	IS	IS	IS
26	Ferulic acid	18.8	192.8	149.0	ND	ND	ND	ND	ND
27	Sinapic acid	18.9	222.8	193.0	ND	ND	ND	ND	ND
28	Coumarin	20.9	146.9	103.1	ND	ND	ND	ND	ND
29	Salicylic acid	21.8	137.2	65.0	ND	0.010	ND	0.011	0.017
30	Cynaroside	23.7	447.0	284.0	0.311	0.673	0.060	0.126	0.642
31	Miquelianin	24.1	477.0	150.9	ND	ND	ND	ND	ND
32	Rutin-D3-IS[d]	25.5	612.2	304.1	IS	IS	IS	IS	IS
33	Rutin	25.6	608.9	301.0	0.024	ND	ND	ND	ND
34	isoquercitrin	25.6	463.0	271.0	ND	0.557	ND	0.210	1.167
35	Hesperidin	25.8	611.2	449.0	0.012	0.020	0.024	0.541	0.114
36	*o*-Coumaric acid	26.1	162.8	93.0	ND	ND	ND	ND	ND
37	Genistin	26.3	431.0	239.0	ND	ND	ND	ND	ND
38	Rosmarinic acid	26.6	359.0	197.0	0.098	5.157	0.268	29.376	27.161
39	Ellagic acid	27.6	301.0	284.0	ND	ND	ND	ND	ND
40	Cosmosiin	28.2	431.0	269.0	0.075	0.191	0.206	0.117	0.167
41	Quercitrin	29.8	447.0	301.0	ND	ND	ND	ND	ND
42	Astragalin	30.4	447.0	255.0	0.025	0.072	0.013	0.047	0.260
43	Nicotiflorin	30.6	592.9	255.0/284.0	0.077	ND	ND	ND	ND
44	Fisetin	30.6	285.0	163.0	ND	ND	ND	ND	ND
45	Daidzein	34.0	253.0	223.0	ND	ND	ND	ND	ND
46	Quercetin-D3-IS[d]	35.6	304.0	275.9	IS	IS	IS	IS	IS
47	Quercetin	35.7	301.0	272.9	0.007	0.007	ND	0.010	0.023
48	Naringenin	35.9	270.9	119.0	0.008	0.002	0.043	0.112	0.012
49	Hesperetin	36.7	301.0	136.0/286.0	ND	ND	0.043	ND	ND
50	Luteolin	36.7	284.8	151.0/175.0	0.365	0.060	0.489	0.513	0.216
51	Genistein	36.9	269.0	135.0	0.004	ND	ND	ND	ND
52	Kaempferol	37.9	285.0	239.0	ND	ND	ND	ND	ND
53	Apigenin	38.2	268.8	151.0/149.0	0.100	0.016	0.242	0.098	0.048
54	Amentoflavone	39.7	537.0	417.0	ND	ND	ND	ND	ND
55	Chrysin	40.5	252.8	145.0/119.0	ND	ND	ND	ND	ND
56	Acacetin	40.7	283.0	239.0	0.940	0.039	2.283	2.445	0.465

[a] Parent ion (*m/z):* Molecular ions of the standard compounds (mass to charge ratio).
[b] MS^2(CE): MRM fragments for the related molecular ions (CE refers to related collision energies of the fragment ions).
[c] Values in *mg*/g (w/w) of plant ethanol extract.
[d] IS: Internal standard, ND: not detected Numbers on the far left raw indicate the standard phytochemical compounds.

again for 10 min at 37 °C, and absorbance values were measured at 475 nm. Alpha-kojic acid was used as the reference.

Elastase inhibitory activity was applied with minor modifications using the method developed by Kraunsoe et al. (1996). Ten microliters of sample and 20 μL of elastase solution were added to 40 μL (0.1 M Tris-Cl, pH=8) buffer solution and incubated for 10 min at 37 °C. Then, 30 μL of 1.015 mM substrate (*N*-succinyl-(Ala)−3-nitroanilide) solution prepared with buffer solution (0.1 M Tris-Cl, pH=8) was added and incubated at 37 °C for 20 min. Then absorbance values were measured at 410 nm. Oleanolic acid was used as the reference.

Collagenase inhibitory activity was performed with minor modifications using the protocol developed by Thring et al. (2009). Twenty microliters of the sample solution prepared in DMSO, 10 μL of collagenase solution (0.8 U/mL), and 50 μL of phosphate buffer (pH:7.5) were added to all wells and incubated at 25 °C for 15 min. Then, 20 μL of substrate solution (*N*-(3-[2-Furyl] acryloyl)-Leu-Gly-Pro-Ala) was added and incubated at 25 °C for 20 min and absorbance values were measured at 340 nm. Epicatechin gallate was used as the reference (Akdeniz et al., 2021).

2.7.2. Anticholinesterase inhibition assay

The plant extracts were tested against the acetylcholinesterase and butyrylcholinestase using Ellman's method with slight modifications (Ellman et al., 1961). Briefly, 140 μL of phosphate buffer (0.1 mM, pH 6.8), 20 μL of acetylcholinesterase (AChE, Type-VI-S, EC 3.1.1.7, 0.22 U/mL) or butyrylcholinesterase (BChE, EC 3.1.1.8, 0.1 U/mL), and 20 μL of the extract solution in various concentrations were mixed in microplate and incubated for 15 min at 25 °C. Subsequently, 10 μL of 0.5 mM DTNB (5, 5′-dithiobis-(2-nitrobenzoic acid)), the reaction was started by the addition of 10 μL of 0.71 mM acetylthiocholine or 10 μL of 0.2 mM butyrylthiocholine chloride for AChE or BChE, respectively. The absorbance was measured using a 96-well microplate reader (Multiscan sky, USA) at 412 nm. Galanthamine was used as a positive control, and methanol was used as a negative control both for AChE and BChE inhibitory effect evaluation.

2.8. Statistical analysis

All the assays were carried out in triplicate. The results were expressed as mean values and standard deviations (mean ± SD). Statistical differences between the extracts were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test (a= 0.05). Correlation analyses were performed using a two-tailed Pearson's correlation test. All the analyses were carried out by using SPSS v22.0 software.

3. Results

3.1. Total phenol (TPC) and flavonoid content (TFC)

The tested extracts' TFC and TPC were determined using the techniques specified in the experimental section. The highest TFC was found in *S. aytachii* ethyl acetate extract with a value of 22.33 ± 0.17 μg QEs/mg, and the highest TPC was found in *S. aucheri* subsp. *canescens* methanol extract with 52.49 ± 1.23 μgPE / mg extract value. Results are shown in Table 2, in line with the result of phytochemical content analyses with LC-MS/MS. The *S. aytachii* ethyl acetate extract contains highest amount of flavonoid compound such as luteolin, heperidin, and naringenin than others. As for phenolic content, quinic acid, protocatechuic acid, caffeic acid, and rosmarinic acid was the dominant compounds in the *S. aucheri* extract.

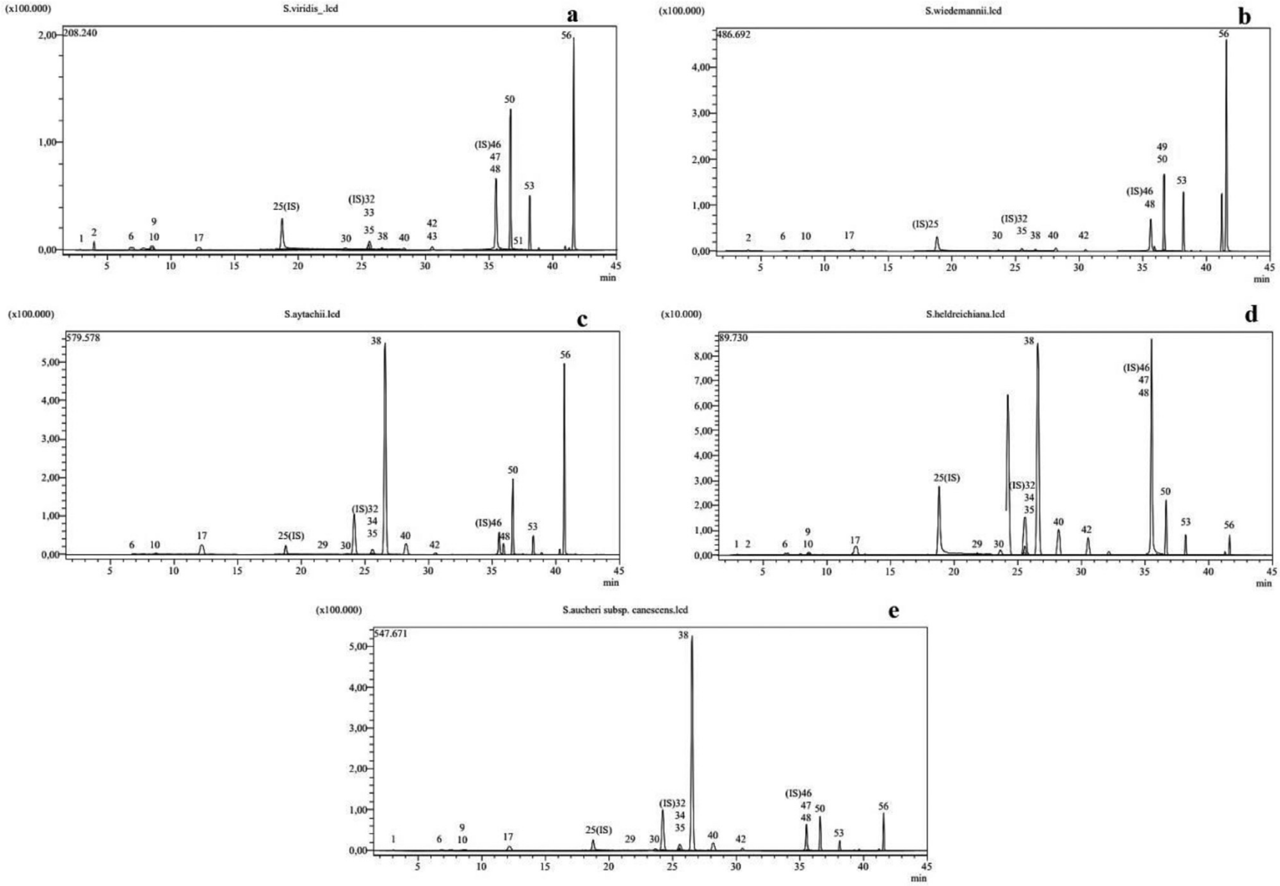

Fig. 1. LC-MS/MS chromatogram of methanol extract of five *Salvia* taxa. a: *S. viridis*; b: *S. wiedemannii*; c: *S. aytachii*; d: *S. heldreichiana*; e: *S. aucheri* subsp. *canescens*.

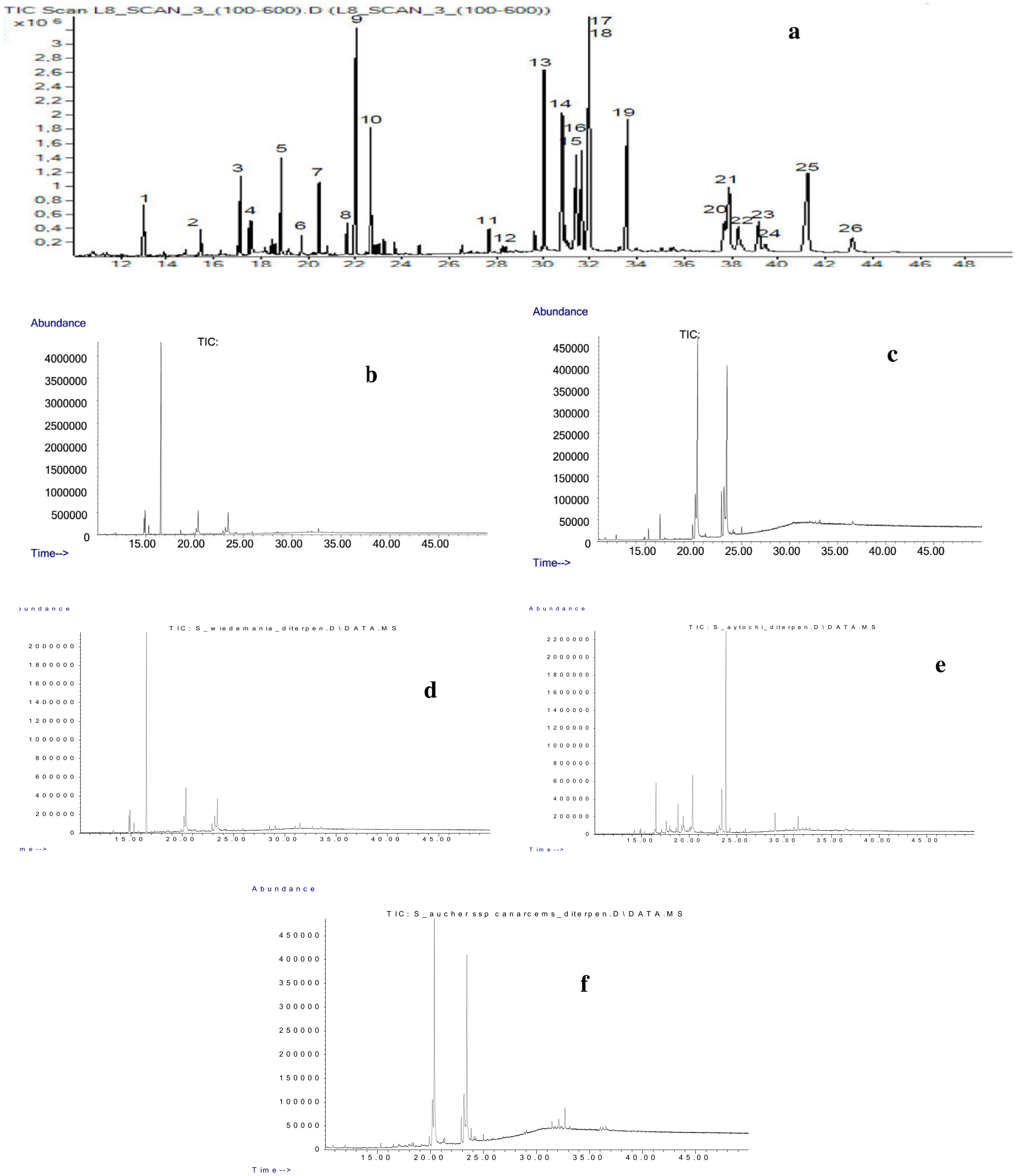

Fig. 2. GC–MS chromatograms of terpenoid-steroid content. **a:** TIC chromatogram of standard chemicals analysed by GC–MS method. 1: Sclareolide, 2:Sclareol, 3:Ferruginol, 4: Cryptanol, 5:6,7-dehydroroyleanone, 6:Suginal, 7:12-Hydroxy abieta-1,3,5(10),8,11,13-hexaen, 8:Sugiol, 9:Inuroyleanone, 10:12-Demetilmulticauline, 11: 7α-Hydroxy-β-sitosterol, 12:Salvigenin, 13: Stigmasterol, 14: β-Sitosterol, 15:Sinensetin, 16: Lupenone, 17: α-Amyrin, 18: Lupeol 19: 3-Acetyl lupeol, 20: 1α,21α-Dihydroxy-2–3-(1′,1′-dimethyl-dioxymethylene)urs-9(11),12-diene, 21: Uvaol, 22: Betulin, 23: Pyxinol, 23: Betulin 3,28-dicetate, 24: Lup-(20)29-ene-2α-hydroxy-3β-acetate, 25: Betulin 3β,28β-diacetate 26: 21α-Hydroxy,2α,3β-diacetoxy urs-9(11),12-diene, **b:** GC–MS chromatogram of the *S. viridis.* **c:** GC–MS chromatogram of the *S. heldreichiana* **d:** GC–MS chromatogram of the *S. wiedemannii* **e:** GC–MS chromatogram of the *S. aytachii.* **f:** GC–MS chromatogram of the *S. aucheri* subsp. *canescens.*

3.2. Quantitative and qualitative analyses by lc-ms/ms

The bioactive compounds of methanol extracts prepared from five *Salvia* taxa were determined by LC-MS/MS in this study. As can be seen from the results, protocatechuic acid, protocatechuic aldehyde, caffeic acid, cynaroside, hesperidin, rosmarinic acid, cosmosiin, astragalin, naringenin, luteolin, apigenin, and acacetin were determined in all extracts (Table 2 and Fig. 1). When the extracts prepared from 5 *Salvia* taxa were evaluated within themselves, overall rosmarinic acid is the most abundant phenolic compound in all extracts. Especially the In the study by (Bakir et al., 2020), *S. hypargeia* leaf extract was rich with rosmarinic acid (38.04 mg/g extract) and

Table 3
Terpenoid-steroid content of methanol extract of five *Salvia* taxa by GC–MS.

Compound	RT[a]	Molecularion-*m/z* (relativeintensity%) (*m/z*)[b]	(μg analyte/g extract)				
			S. viridis	*S. wiedemannii*	*S. aytachii*	*S. heldreichiana*	*S. aucheri* subsp. *canescens*
Sclareolide	13.009	250.38 (1.1)	ND	ND	ND	ND	ND
Sclareol	15.405	308.51 (2.0)	ND	ND	ND	ND	ND
Ferruginol	17.091	286.46 (95.7)	1238.52	ND	8683.06	ND	ND
Cryptanol	17.638	316 (100)	ND	ND	ND	ND	ND
6,7-Dehydroroyleanone	18.821	314.19 (1.1)	1483.65	ND	5890.57	ND	ND
Suginal	19.728	314.43 (2.8)	4940.16	4458.80	2201.07	4382.89	4658.09
9,10-Dihydro-7,8-dimethyl-2-(1-methylethyl) phenanthren-3-ol	20.437	266.15 (48)	ND	ND	ND	ND	ND
Sugiol	21.628	300.44 (69)	ND	ND	1025.52	ND	ND
Inuroyleanone	21.996	346.28 (100)	ND	ND	ND	ND	ND
12-Demethylmulticauline	22.646	264.15 (91.1)	ND	ND	ND	ND	ND
7α-Hydroxy-β-sitosterol	27.700	430.71 (2.5)	2025.61	ND	ND	ND	ND
Salvigenin	28.311	328.09 (100)	ND	20,181.96	ND	ND	ND
Stigmasterol	30.052	412.36 (100)	ND	ND	ND	ND	ND
β-Sitosterol	30.795	414.72 (100)	ND	2700.53	4589.44	ND	1750.39
Sinensetin	31.343	372.37 (19.2)	ND	ND	ND	ND	ND
Lupenone	31.592	424.37 (36.0)	ND	ND	ND	ND	ND
α-Amyrin	31.944	426.73 (8.4)	1241.02	1011.82	1088.24	ND	ND
Lupeol	31.944	426.38 (38.9)	1164.90	ND	ND	ND	ND
3-Acetyl lupeol	33.513	468.40 (22.35)	2257.89	1405.07	1402.69	ND	1636.04
1α,21α-Dihydroxy-2–3-(1′,1′-dimethyl-dioxymethylene)urs-9(11),12-diene	37.636	512.39 (100)	ND	ND	ND	ND	ND
Uvaol	37.800	442.73 (1.1)	ND	ND	1800.67	ND	ND
Betulin	38.269	442.73 (11.1)	ND	ND	ND	ND	ND
Pyxinol	39.160	476.74 (0.62)	ND	ND	ND	ND	ND
Lup-(20)29-ene-2α-hydroxy-3β- acetate	39.467	484 (5.5)	ND	ND	ND	ND	ND
Betulin 3β,28β-diacetate	41.236	526.80 (1.6)	ND	ND	ND	ND	ND
21α-Hydroxy,2α,3β-diacetoxy urs-9 (11),12-diene	43.145	540.38 (100)	ND	ND	ND	ND	ND

[a] RT: Retention time.
[b] Mother ion(*m/z*): Molecular ions of the standard compounds (*m/z* ratio); ND:· Not detected.

isoquercitrin (4.14 mg/g extract). In a study by Bursal et al. (2019), salvigenin (158.64 ± 10.8 mg/kg), fumaric acid (123.09 ± 8.54 mg/kg), and quercetagetin–3.6–dimethylether (37.85 ± 7.09 mg/kg) were detected as major compounds in the ethanol extract, whereas fumaric acid (555.96 ± 38.56 mg/kg), caffeic acid (103.62 ± 20.51 mg/kg), and epicatechin (83.19 ± 8.43 mg/kg) were detected as major compounds in the water extract of *S. eriophora* Boiss. & Kotschy (Bursal et al., 2019). In a study on *S. siirtica* Kahraman, Celep & Doğan, ferruginol (17,721.99 mg/kg) and sugiol (2918.44 mg/kg), were rich in root etanol extract, while salvigenin (33,952.13 mg/kg) and β-sitosterol (16,369.71 mg/kg) were richest constituents in aerial part chloroform extract based on GC–MS and LC-MS/MS results (Fidan et al., 2021).

3.3. *Terpenoid-steroid contents by GC–MS*

Considering the terpenoid-steroid content of the samples, the extracts differ in terms of chemical content (Fig. 2 and Table 3). Results were expressed as μg analyte/g extract. It has been determined that all the extract was rich in suginal. Especially in *S. viridis* samples, a high amount of suginal (4940.16 μg analyte/g extract) was detected. It was followed by *S. aucheri* subsp. *canescence* (4658.09 μg /g extract). The compound β-sitosterol was detected highest in *S. aytachii* (4589.44 μg /g extract), and 2700.53 μg /g extract in *S. wiedemannii* and 1750.39 μg analyte/g extract in *S. aucheri* subsp. *canescence.* It is quite remarkable that ferruginol (8683.06 μg /g extract) and 6,7-Dehydroroyleanone (5890.57 μg /g extract) found richer in *S. aytachii* than *S. viridis*, and not in other species. Salvigenin was only quantified in *S. wiedemannii* (20,181.96 μg/g extract). According to the results, *S. wiedemannii* can be used as a source for obtaining salvigenin. Lupeol was only detected in *S. viridis* (1164.90 μg /g extract) while uvaol was only quantified in *S. aytachii* (1800.67 μg /g extract). These compounds can also be used as markers for the identification of these species or as a source for obtaining lupeol and uvaol.

As for triterpenoid content by GC–MS, all the tested species have the oleanolic acid and ursolic acid (Fig. 3 and Table 4). In terms of content, oleanolic acid was found the highest in *S. aytachii* extract (66,842.46 μg /g extract), followed by *S. wiedemannii* species (40,451.94 μg /g extract). The ursolic acid was detected highest in *S. aytachii* extract (88,167.37 μg /g extract), followed by *S. viridis* species (40,523.08 μg /g extract). Muronic acid was only detected in *S. viridis* species (732.40 μg /g extract), and α-amyrin was highest (1201.60 μg /g extract) in this species than others. For isolation of α-amyrin and moronic acid, it is proporate to choose the *S. viridis* than other tested species as a plant material. *S. aytachii* can also be a good source of oleanolic and ursolic acid. To the best of our knowledge, there is no previous report on terpenoid content analysis by LC-MS/MS and GC–MS.

3.4. *Antioxidant activity*

The antioxidant ability of the methanol extracts obtained from the five *Salvia* taxa were investigated in regard to their scavenging potential of free radicals namely DPPH, ABTS and their capacity to iron chelating. Generally, the *S. aytachii* extract was more promising than other tested *Salvia* extracts for its ability to scavenging the DPPH and ABTS radicals and iron chelating activity (Table 5). Among the extract, *S. aucheri* subsp. *canescens* showed highest DPPH radical scavenging activity (IC_{50}: 168.4 ± 1.32 μg/mL), while *S. heldreichiana* demonstrated the highest ABTS radical scavenging activity (IC_{50}: 0.078 ± 0.18 μg/mL) even than the reference compound trolox (IC_{50}: 248.17 ± 0.66 μg/mL). According to the results, *S. aytachii* extract

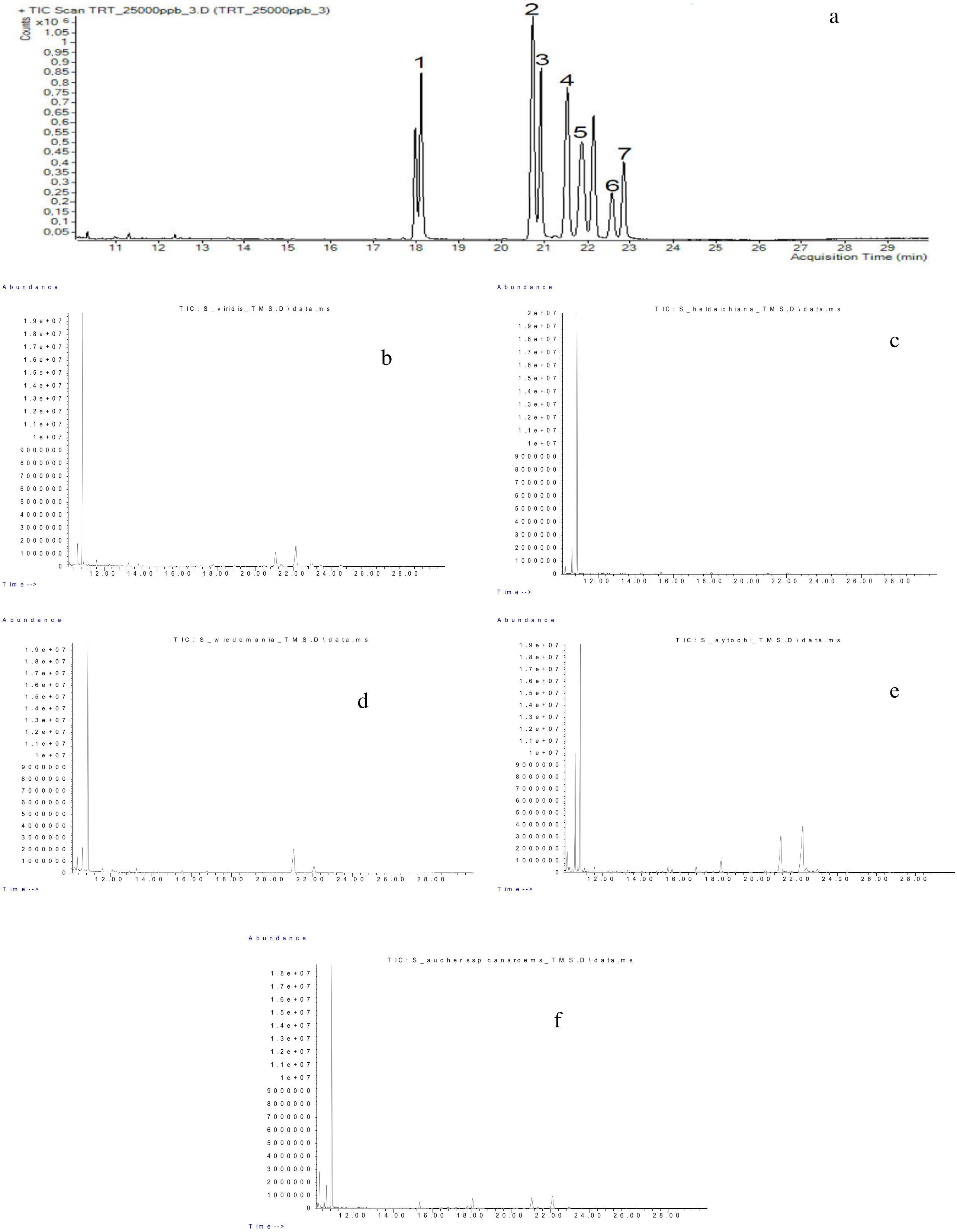

Fig. 3. GC–MS chromatograms of triterpenoid content. **a:** TIC chromatogram of standard chemicals analysed by GC–MS method. **b:** GC–MS chromatogram of the *S. viridis.* **c:** GC–MS chromatogram of the *S. heldreichiana* **d:** GC–MS chromatogram of the *S.wiedemannii* **e:**GC–MS chromatogram of the *S. aytachii.* **f:** GC–MS chromatogram of the *S. aucheri* subsp. *canescens*.

Table 4
Triterpenoid content (μg analyte/g extract) of methanol extract of five *Salvia* taxa by GC−MS.

	α-amyrin	**Moronic acid**	**Oleanonic acid**	**Oleanolic acid**	**Betulinic acid**	**Ursolic acid**	**Ursonic acid**
S. viridis	1201.60	732,40	ND	20,871.26	ND	40,523.08	ND
S. wiedemannii	987.67	ND	ND	40,451.94	ND	13,815.71	ND
S. aytachii	873.03	ND	ND	66,842.46	ND	88,167.37	ND
S. heldreichiana	ND	ND	ND	1330.13	ND	3376.21	ND
S. aucheri* subsp. *canescens	145,50	ND	ND	14,260.55	ND	21,775.37	ND

Table 5
In vitro antioxidant activity of the *Salvia* taxa.

Sample	Radical scavenging activities (IC$_{50}$ μg/mL)		Iron chelating activity (IC$_{50}$ μg/mL)	Total phenol content (μg PEs/mg)[d]	Total flavonoid content (μg QEs/mg)[e]
	DPPH	ABTS			
S. viridis	NA	911.13 ± 7.20	9217.0 ± 0.39	40.03 ± 0.52	6.04 ± 0.02
S. wiedemannii	NA	249.89 ± 1.69	4865.0 ± 0.22	29.79 ± 0.57	11.24 ± 0.12
S. aytachii	1259.08 ± 4.09	232.46 ± 1.56	827.9 ± 0.33	77.82 ± 1.76	22.33 ± 0.17
S. heldreichiana	235.5 ± 1.93	0.078 ± 0.18	–	27.02 ± 0.28	2.76 ± 0.01
S. aucheri subsp. *canescens*	168.4 ± 1.32	5.68 ± 0.38	–	52.49 ± 1.23	5.19 ± 0.02
Reference standard	53.87 ± 4.93[a]	248.17 ± 0.66 [b]	425.3 ± 0.14 [c]		

Results are given as the mean and standard deviation (mean ± SD) of three parallel measurements.

[a] Gallic acid.
[b] Trolox.
[c] EDTA; NA: not active.
[d] Phenolic content equivalent to pyrocatechol (y = 0.0602x + 0.0288, r^2: 0.9953).
[e] Flavonoid content equivalent to quercetin (y = 0.0391x + 0.0637, r^2: 0.9946).

was found more active (IC$_{50}$: 827.9 ± 0.33 μg/mL) than others in iron chelating assay.

3.5. Enzyme inhibition activity

The enzyme inhibitory effects of the extracts obtained from five *Salvia* taxa namely *S. viridis, S. wiedemannii, S. aytachii, S. heldreichiana*, and *S. aucheri* subsp. *canescens* were evaluated and the results are shown in Table 6, the inhibitory activity of extracts compared with positive control drugs at the same concentration. In a result, the *S. aytachii* was found active than the other species. Although the extracts showed lower enzyme inhibition activity compared to the positive control, when they were compared among themselves, it was seen that there was a significant difference in activity between them. This may be due to the difference in the phytochemical content of the compounds and the amount of bioactive compounds as seen in the LC-MS/MS results. The highest AChE inhibitory activity was showed by *S. aytachii* extract (60.11 ± 8.29%), while *S. viridis* demonstrated highest BChE inhibitory activity (66.38 ± 1.08%) than others and it was followed by the *S. aytachii* with the inhibition of 65.95 ± 2.13%. When the inhibition activities on the tyrosinase enzyme were compared, all *salvia* extracts showed almost similar inhibitory activity, while *S. aytachii* showed the highest tyrosinase inhibition activity (16.29 ± 2.80%) among them, but still much lower than the reference substance kojic acid (88.43±1.48%).

4. Discussion

It was reported that polyphenolic compounds have important role in defending oxidative stress. In this context they are known as powerful antioxidants and added in much kind of dietary supplements. In this study, the concentration of phenolic and flavonoids in the extract was expressed as milligrams of gallic acid and milligrams of quercetin equivalents per gram of the extract, respectively. In a previous study, the phenolic content has been found as 214 mg pyrocatechol equivalent and flavonoid demonstrated as 54.2 mg quercetin equivalent per gram of extract from *S. potentillifolia* Boiss. & Heldr. ex Benth (Kivrak et al., 2009). In a study, total phenolic content of ethanol extract prepared form the *S. siirtica* whole plant was found highest (120.23 ± 2.00 mg PEs/mg extract) than the other parts' extract (Fidan et al., 2021). Methanol extracts of *Salvia* species investigated in our study showed near to 50% or more inhibitory activity against these two enzymes, even at very low concentrations (50 μg/mL).

It is generally known that phenolic and flavonoid chemicals have the best antioxidant activity. The total amount and arrangement of

Table 6
Enzyme inhibitory activity of five *Salvia* taxa.

Samples	**AChE**	**BChE**	**Tyrosinase**	**Elastase**	**Collagenase**
S. viridis	40.46 ± 9.75	66.38 ± 1.08	13.28 ± 3.41	29.76 ± 0.15	3.67 ± 0.01
S. wiedemannii	58.23 ± 2.01	41.67 ± 1.64	15.41 ± 4.68	26.76 ± 0.08	9.78 ± 0.06
S. aytachii	60.11 ± 8.29	65.95 ± 2.13	16.29 ± 2.80	28.26 ± 0.17	16.38 ± 0.12
S. heldreichiana	34.58 ± 0.74	30.41 ± 0.68	11.52 ± 1.26	19.49 ± 0.12	NA
S. aucheri $_{\textit{subsp. canescens}}$	49.62 ± 0.57	24.15 ± 0.76	10.18 ± 3.32	21.63 ± 0.11	NA
Galanthamine	85.22 ± 1.78	80.27 ± 1.09	–	–	–
Kojic acid	–	–	88.43±1.48	–	–
Oleanolic acid	–	–	–	59.98±1.06	–
Epicatechin gallate	–	–	–	–	86.01±1.32

[a] Values are given as the mean and standard deviation of three parallel measurements (50 μg/mL).
[b] Reference compound, NA.: not active.

hydroxyl groups in phenolic and flavonoids were found to be major regulators of antioxidant capabilities (Sarian et al., 2017). Plant polyphenols' antioxidant properties play a critical role in protecting the body from oxidative stress, diabetes, cardiovascular disease, and cancer. As a result of its purported safety and nutritional benefits, plant-based medicines are a possible alternative therapy to investigate (Sekhon-Loodu and Rupasinghe, 2019). Based on these investigations, we may conclude that our findings were consistent with those reported in the literature. The highest amount of phenolic (rosmarinic acid and caffeic acid) and flavonoid compound (luteolin, naringenin, and acacetin) determined in *S. aytachi* with LC-MS/MS may be attributed for its highest iron chelating activity. Also, it can be said that the high amount of phenolic compounds in *S. aucheri* subsp. *canescens* extract are responsible for the higher DPPH and ABTS radical scavenging activities.

Different anti-AChE and anti-BChE activities were reported for *Salvia* species before. In a previous study, the inhibition activity of the methanol extracts of *S. eriophora* against AChE and BChE enzymes were reported for with the IC_{50} value of 9.91 ± 0.058 and 5.17 ± 0.043 μg/mL, respectively (Bursal et al., 2019). In another study, the petroleum ether and ethylaceate extract of *S. viridis* displayed more than 90% inhibitory activity against AChE and BChE at the 10 mg/mL concentrations (Rungsimakan, 2011). Nonpolar extracts of some *Salvia* taxa also showed more than 80% inhibition on both enzymes at the concentration of 1 mg/mL Orhan et al., 2007).

Tyrosinase is a is a copper-containing enzyme catalyzes the production of melanin and other pigments from tyrosine by oxidation. Tyrosinase inhibitors are used to treat hyperpigmentation and melasma, among other skin conditions. Collagenase is involved in the breakdown of collagen as well as for the remodeling of the extracellular matrix (ECM). Elastase is the enzyme that breaks down elastin in the extracellular matrix. Because elastin and collagen are crucial for skin elasticity and structural integrity, when they are depleted, undesirable wrinkles, scars, and skin aging will appear (Karatoprak et al., 2022). The above mentioned three enzyme inhibitors are associated with various skin conditions and wound healing activity. On tyrosinase inhibitory activity assay, PBS used as a blank control, while kojic acid served as a positive control. As shown in Table 6, generally the *S. aytachii* extract displayed stronger inhibitory effect than the other extract, it was followed by *S. wiedemannii*, which indicated that a phytochemical compound presents in *S. aytachii* extract may play a role on the inhibition of tyrosinase activity. Therefore, LC-MS/MS analysis of extract was used as the research object for identifying the active compounds responsible for tyrosinase activity different from the other extracts.

Among the extracts, *S. aytachii* extract showed higher collagenase inhibition activity (16.38 ± 0.12%) than the other two species which was lower than the positive control epicatechin gallate (86.01± 1.32%) at the concentration of 50 μg/mL, but no activity was detected in *S. heldreichiana* and *S. aucheri* subsp. *canescens* species. As for elastase assay, *S. viridis* and *S. aytachii* displayed higher inhibitory activity than other, however, they showed lower activity than the reference drug oleanolic acid (59.98 ± 1.06%). In a previous study, the *S. fruticosa* extract showed 18.76 ± 2.13% inhibition against elastase (Deniz et al., 2021).

5. Conclusion

The results presented in this work are the first report on the antioxidant, and screening enzyme inhibition activities of five *Salvia* species. Among the tested species, the highest activity was observed for *S. aytachii* for iron chelating and ABTS radical scavenging activity. It also showed DPPH radical scavenging activity, while others have shown no activity. As can be seen from results, there is a significant correlation between phenolic contents and free radical scavenging activity especially on the DPPH radical. In this study, all the tested extract demonstrated strong anticholinesterase inhibitory activity nearly same with reference standard galanthamine at the higher concentrations. Therefore, the results of this study proved that the plant is potential AChE and BChE inhibitor and can be used as natural medicinal drugs. In conclusion, the results of this study can provide scientific research data for *Salvia* taxa used in rational phytotherapy. Further experiments should be conducted on *in-vivo* tests parallel with bioactivity-guided isolation of active compounds and on the mechanism of action.

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.

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