South African Journal of Botany 125 (2019) 72–80

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

A comparative investigation on phenolic composition, antioxidant and antimicrobial potentials of *Salvia heldreichiana* Boiss. ex Bentham extracts

H. Bardakci [a,*], E. Celep [b], T. Gözet [c], I. Kurt-Celep [d], I. Deniz [e], B. Şen-Utsukarci [f], G. Akaydin [g]

[a] Acibadem Mehmet Ali Aydınlar University, Faculty of Pharmacy, Department of Pharmacognosy, 34752 Ataşehir, Istanbul, Turkey
[b] Yeditepe University, Faculty of Pharmacy, Department of Pharmacognosy, 34755 Ataşehir, Istanbul, Turkey
[c] Altınbas University, Faculty of Pharmacy, Department of Pharmaceutical Chemistry, 34144 Bakırkoy, Istanbul, Turkey
[d] Yeditepe University, Faculty of Engineering, Department of Genetics and Bioengineering, 34755 Ataşehir, Istanbul, Turkey
[e] Yeditepe University, Faculty of Pharmacy, Laboratories of Microbiological Analysis, 34755 Atasehir, Istanbul, Turkey
[f] Istanbul University, Faculty of Pharmacy, Department of Pharmacognosy, 34116 Fatih, Istanbul, Turkey
[g] Hacettepe University, Department of Biology Education, 06800 Beytepe, Ankara, Turkey

ARTICLE INFO

Article history:
Received 6 February 2019
Received in revised form 7 May 2019
Accepted 2 July 2019
Available online 16 July 2019

Edited by AR Ndhlala

Keywords:
Salvia heldreichiana
Antioxidant
Antimicrobial
LC–MS/MS
HPTLC
Rosmarinic acid

ABSTRACT

The purpose of the present work is to screen phenolic composition and evaluate the antioxidant and antimicrobial activities of the main extract and subextracts of *Salvia heldreichiana*, an endemic plant from Turkey. The aerial parts have been powdered and extracted with MeOH initially and then partitioned with chloroform ($CHCl_3$), ethyl acetate (EtOAc) for the generation of subextracts along with the remaining water (R-H_2O). Total phenolic, phenolic acid, flavonoid and proanthocyanidin contents present in the MeOH, $CHCl_3$, EtOAc and R-H_2O extracts were measured. Presence of 22 phenolic metabolites were confirmed by utilizing LC–MS/MS in MRM scan mode and then Rosmarinic acid (RA) contents of each extract were quantified by HPTLC-densitometry, since the biological effects of many medicinal plants, including *Salvia* sp. is attributed to RA content. Antioxidant capacities of the aforementioned extracts were estimated using several procedures including free radical scavenging and metal-associated activity and then disc diffusion method was employed to designate their antibacterial and antifungal activities. The results obtained from the current study has revealed a positive correlation between the phenolic composition and the antioxidant profile as well as the antimicrobial activities of the extracts. Among the tested extracts, EtOAc subextract showed the highest antioxidant and antimicrobial activities consistent with the highest phenolic and RA content.

1. Introduction

The genus *Salvia* belonging to the family Lamiaceae has been disturbed widely in several regions of the world, especially in the Mediterranean region and Asia, with more than 1000 species (Kalaycıoglu et al., 2018). There are 100 species of *Salvia* in Turkey, 57 of which are endemic (Sen-Utsukarci et al., 2019). Although, studies related with the chemistry of *Salvia* have been predominantly limited to diterpenoids, especially tanshinones (Chang et al., 1990; Zhang et al., 1990; Lu and Foo, 2002), a broad array of compounds such as phenolics, terpenics and alkaloids were isolated from *Salvia* species (Ghorbani and Esmaeilizadeh, 2017). Previous studies have indicated that monoterpenes, triterpenoids and flavonoids are particularly found in aerial organs, however phenolic acids and diterpenoids are found roots (Topcu, 2006). It has been shown that various *Salvia* species have antioxidant (Er et al., 2013), memory-enhancing (Senol et al., 2010), antimicrobial (Akın et al., 2010; Erdogan et al., 2013a), cytotoxic (Erdogan et al., 2013b), cardioprotective (Xu et al., 2018), anti-inflammatory, anticancer, antimutagenic (Ghorbani and Esmaeilizadeh, 2017) and antinociceptive effects, and demonstrate α-glucosidase enzyme inhibitory activity (Kalaycıoglu et al., 2018). Indeed, many members of *Salvia* genus have been utilized as analgesics, for promoting the removal of blood stasis and blood circulation, and for regulating menstruation especially in Traditional Chinese Medicine (Lu and Foo, 2002; Xu et al., 2018). Similarly, *Salvia* species have been employed for their antiseptic, antibacterial, diuretic, hemostatic, spasmolytic, carminative and wound healing properties in Turkish folk medicine (Baytop, 1999). One of the endemic member of *Salvia* genus from Turkey is *S. heldreichiana*, which has been studied mainly with respect to essential oil composition (Basalma et al., 2007; Akın et al., 2010; Erdoğan, 2014), however, secondary metabolite composition of *S. heldreichiana* has not been studied in details except Ulubelen et al. (1995) who have indicated the

* Corresponding author at: Acıbadem Mehmet Ali Aydınlar University, Faculty of Pharmacy, Department of Pharmacognosy, 34752 Istanbul, Turkey.
E-mail address: hilal.bardakci@acibadem.edu.tr (H. Bardakci).

https://doi.org/10.1016/j.sajb.2019.07.010
0254-6299/

existence of several diterpenoids such as isopimaric acid, wiedelactone and wiedemannic acid.

Emergence and progression of various chronic disorders has been mainly attributed to oxidative stress. It has been suggested that oxidative damage induced by reactive oxygen species (ROS) could be potentially inhibited either by promoting the body's endogenous antioxidant defense mechanisms or incorporation of antioxidant exogenously (Kasote et al., 2015). Indeed, it has been shown that including antioxidant rich foods to diet was critical in the treatment of several diseases such as cancer and cardiovascular diseases together with inflammatory diseases (Krishnaiah et al., 2011). In addition to complex enzymatic systems (superoxide dismutase, catalase, gluthatione peroxidase and gluthatione reductase), plants contain non-enzymatic antioxidants (ascorbic acid, carotenoids, etc.) and phenolic and polyphenolic secondary metabolites such as phenolic acids, flavonoids and tannins which play a crucial role in antioxidant defense mechanisms for destroying the hazardous effects of free radicals (Chanda and Dave, 2009; Kasote et al., 2015).

Infectious diseases is one of the major causes of morbidity and mortality worldwide. Currently, many infections with no or limited treatment are usually induced by resistant microorganisms. Resistance to microorganisms have been developed because antimicrobials are accessed readily and used for various purposes, including food production (Hayashi et al., 2013). Natural products from diverse sources (microorganisms, marine organisms, plants etc.) have been used substantially in fighting with microbes for centuries. Indeed, about half of the pharmaceuticals in broad spectrum of use are compounds derived from natural products (Clark, 1996). According to the World Health Organization 25% of modern medicines are prepared from traditionally utilized medicinal plants. However, modernization of traditional medicines is required in this century. The aim of the medicinal plant studies is not only to screen bioactive or marker components from the extracts for new drug development, but also to standardize and quality control of raw herbal materials in order to ensure their safety and efficacy. Quality control of herbal medicines are challenging because of the diverse chemical structures of plant constituents (Liu, 2011), therefore, analytical tools powerful in separation, characterization and quantification are required in plant metabolomics studies (Jorge et al., 2016). Various analytical techniques such as high performance liquid chromatography (HPLC), high performance thin layer chromatography (HPTLC), and gas chromatography (GC) are utilized for the identification and quantification of plant metabolites.

HPTLC is an irreplaceable analytical tool used in the plant metabolomics studies due to the advantages of generating visual (compound specific colored) data, low running cost, high versatility, high throughput, flexibility, accuracy and reproducibility (Reich and Schibli, 2007). HPLC with ultraviolet (UV) and diode array dedector (DAD) are frequently used in quantitation of secondary metabolites. Furthermore, HPLC combined with mass selective detector, i.e. Mass Spectrometry (MS) in LC–MS/MS configuration offers high sensitivity, accuracy and selectivity in identification and quantification. Additionally, if the multiple reaction monitoring (MRM) mode is applied, the sensitivity will be even higher (Wang, 2015).

Efficient extraction of antioxidants from plants are crucial in exploration of the plant based sources rich with potential antioxidants and promotion of natural products use as pharmaceuticals and food additives (Xu et al., 2017). In this study, antioxidant and antimicrobial activities of total extract and subextracts of *S. heldreichiana* Boiss. ex Bentham will be investigated. Then, the phenolic compounds in the extract and subextracts will be identified by LC–MS/MS based on MRM and rosmarinic acid (RA) will be quantified by HPTLC.

Table 1
Spectrophotometric determination of phenolic profile of *S. heldreichiana* extract/subextracts.

Analysis	MeOH	$CHCl_3$	EtOAc	R-H_2O
Total phenolic content[A]	313.60 ± 20.06[ac]	302.09 ± 24.17[a]	420.46 ± 4.6[b]	352.32 ± 4.76[c]
Total flavonoid content[B]	15.15 ± 1.29[a]	14.87 ± 1.16[a]	53.60 ± 9.64[b]	17.81 ± 4.64[a]
Total phenolic acid content[C]	340.67 ± 2.36[a]	472.33 ± 9.43[b]	405.67 ± 7.07[c]	225.67 ± 7.70[d]
Total proanthocyanidin content[D]	4.08 ± 0.05[a]	11.92 ± 0.02[b]	0.55 ± 0.07[c]	0.16 ± 0.03[d]

a–e Different letters in the same row indicate significance ($p < .05$).
[A] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg gallic acid equivalents (GAE) in 1 g sample.
[B] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg quercetin equivalents (QE) in 1 g sample.
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg caffeic acid equivalents (CAE) in 1 g sample.
[D] Total proanthocyanidin content was expressed as mg epigallocathecin gallate equivalents (EGCG-E) per g dried extract ± SD.

2. Materials and methods

2.1. Chemicals

All chemicals, and reference materials with analytical grade were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Table 2
Retention times, MRM transitions and optimized fragmentor voltage (FV) and collision energy (CE) of phenolic compounds.

No	Compound	Molecular Weight	Retention Times	MRM Transitions	FV	CE
1	*p*-coumaric acid	164.16 g/mol	21.89 min	163 m/z → 119 m/z	90 V	10 V
2	Gallic acid	170.12 g/mol	7.99 min	169 m/z → 119 m/z	110 V	10 V
3	Caffeic acid	180.159 g/mol	17.88 min	179 m/z → 135 m/z	120 V	10 V
4	Ferulic acid	194.186 g/mol	22.04 min	193 m/z → 134 m/z	100 V	15 V
5	Apigenin	270.240 g/mol	33.82 min	269 m/z → 117 m/z	150 V	30 V
6	Naringenin	272.256 g/mol	30.82 min	271 m/z → 151 m/z	140 V	15 V
7	CAPE	284.311 g/mol	38.10 min	283 m/z → 179 m/z	160 V	10 V
8	Catechin	290.271 g/mol	13.96 min	289 m/z → 245 m/z	130 V	5 V
9	Epicatechin	290.271 g/mol	17.65 min	289 m/z → 245 m/z	150 V	10 V
10	Quercetin	302.238 g/mol	30.53 min	301 m/z → 151 m/z	150 V	15 V
11	Chlorogenic acid	354.311 g/mol	15.39 min	353 m/z → 191 m/z	100 V	10 V
12	Rosmarinic acid	360.318 g/mol	26.02 min	359 m/z → 161 m/z	100 V	10 V
13	Apigenin-7-*O*-glucoside	432.381 g/mol	26.04 min	431 m/z → 268 m/z	200 V	30 V
14	Vitexin	432.381 g/mol	22.56 min	431 m/z → 311 m/z	130 V	15 V
15	Quercitrin	448.38 g/mol	26.72 min	447 m/z → 301 m/z	160 V	15 V
16	Luteolin-7-*O*-glucoside	448.38 g/mol	23.91 min	447 m/z → 285 m/z	190 V	20 V
17	Isoorientin	448.380 g/mol	21.29 min	447 m/z → 327 m/z	170 V	15 V
18	Hyperoside	464.379 g/mol	24.61 min	463 m/z → 300 m/z	170 V	25 V
19	Rutin	610.521 g/mol	24.62 min	609 m/z → 151 m/z	210 V	25 V
20	Verbascoside	624.592 g/mol	24.26 min	623 m/z → 161 m/z	210 V	25 V
21	Leucoseptoside A	638.619 g/mol	24.22 min	637 m/z → 461 m/z	240 V	20 V
22	Martynoside	652.646 g/mol	27.13 min	651 m/z → 175 m/z	230 V	25 V

Table 3
Phenolic profiling of *S. heldreichiana* extract/subextracts (100 μg/mL) by ESI- LC–MS/MS.*, **

No	Bileşik/Bitki	*S.held.* MeOH	*S.held.* $CHCl_3$	*S.held.* EtOAc	*S. held.* R-H_2O
1	*p*-coumaric acid	trace	+	+	trace
2	Gallic acid	–	–	trace	–
3	Caffeic acid	+	+	+	+
4	Ferulic acid	–	trace	trace	
5	Apigenin	+	+	+	trace
6	Naringenin	–	–	–	–
7	CAPE	+	trace	–	–
8	Catechin	–	–	–	–
9	Epicatechin	–	–	–	–
10	Quercetin	trace	trace	+	–
11	Chlorogenic acid	+	trace	+	+
12	Rosmarinic acid	+	+	+	+
13	Apigenin-7-glucoside	+	+	+	+
14	Vitexin	trace	trace	+	trace
15	Quercitrin	trace	–	+	–
16	Luteolin-7-glucoside	+	trace	+	+
17	Isoorientin	trace	trace	+	trace
18	Hyperoside	+	trace	+	+
19	Rutin	–	–	–	–
20	Verbascoside	–	–	–	–
21	Leucoseptoside A	–	–	–	–
22	Martynoside	–	–	trace	–

* Cut-off point 10^3 (intensity, cps).
** Peaks under cut off point were evaluated as trace.

Standards used in optimization of LC–MS/MS parameters were also purchased from Sigma except isoorientin, verbascoside, leucoseptoside A and martynoside which were previously isolated with a purity grade of >95% (Kırmızıbekmez et al., 2004; Bardakci et al., 2015; Kırmızıbekmez et al., 2018).

2.2. Plant material

Aerial parts of *Salvia heldreichiana* were collected from Konya (Turkey) in July 2010 by Dr. Hilal Bardakcı, and identified by one of the authors (Dr. Galip Akaydın). The voucher specimen is stored at the Herbarium of the Faculty of Education, Hacettepe University, Ankara (Akaydın 13,366). The shade-dried upper ground parts of plant sample were kept at room temperature in air-tight containers until further use.

2.3. Extraction and solvent fractionation

The powdered plant materials (315 g) were extracted with MeOH (4 L) and left to macerate at dark for four days, extracted at 45 °C for four hours each day. Subsequently, the macerates were filtered using a filter paper and pooled solvent was completely removed under reduced pressure and lyophilized to give crude total MeOH extract (29 g, yield: 9.20%). This procedure was repeated twice. A small part of the extract has been separated and the remaining part was dissolved in H_2O (300 mL) and partitioned with $CHCl_3$ (3 × 300 mL) and EtOAc (3 × 300 mL), respectively. Each solvent and the R-H_2O were separately evaporated under reduced pressure and lyophilized. Eventually, subextracts of $CHCl_3$ (8.46 g, yield 33.80%), EtOAc (4.29 g, yield 17.12%), R-H_2O (9.46 g, yield 37.78%) were obtained, respectively.

2.4. Quantitative investigation of the phenolic content

2.4.1. Total phenolic content

The samples were investigated in terms of total phenolic content in accordance with the method described earlier (Singleton and Rossi, 1965). The samples are initially diluted and then mixed with Na_2CO_3 (20%) and Folin Ciocalteu reagent in proper ratios. Then, the samples are incubated at 45 °C and subsequently, the absorbances of the mixtures were read at 765 nm. The results were expressed as gallic acid equivalents (GAE) per dried extract (DE).

2.4.2. Total flavonoid content

Total flavonoid content of the samples were measured spectrophotometrically as explained by Celep et al. (2012). Properly diluted extracts were added to the mixture of $AlCl_3$ and sodium acetate, and incubated at room temperature for 30 min. The absorbances were measured at 415 nm. Total flavonoid contents were expressed as mg quercetin equivalents (QE) per DE.

2.4.3. Total phenolic acid content

Total phenolic acid content of the extracts was determined at 490 nm spectrophotometrically, which is based on the interaction of phenolic acids with sodium molybdate–sodium nitrite complex (Mihailovic et al., 2016). The results were given as caffeic acid equivalents (CAE) in 1 g DE.

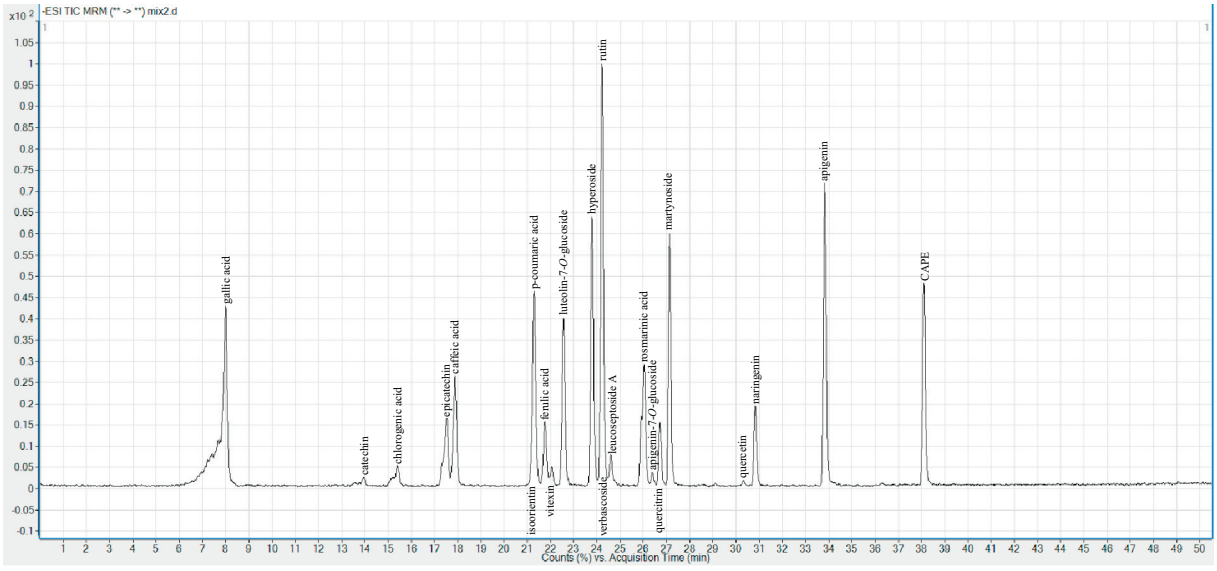

Fig. 1. HPLC chromatogram of standard compounds.

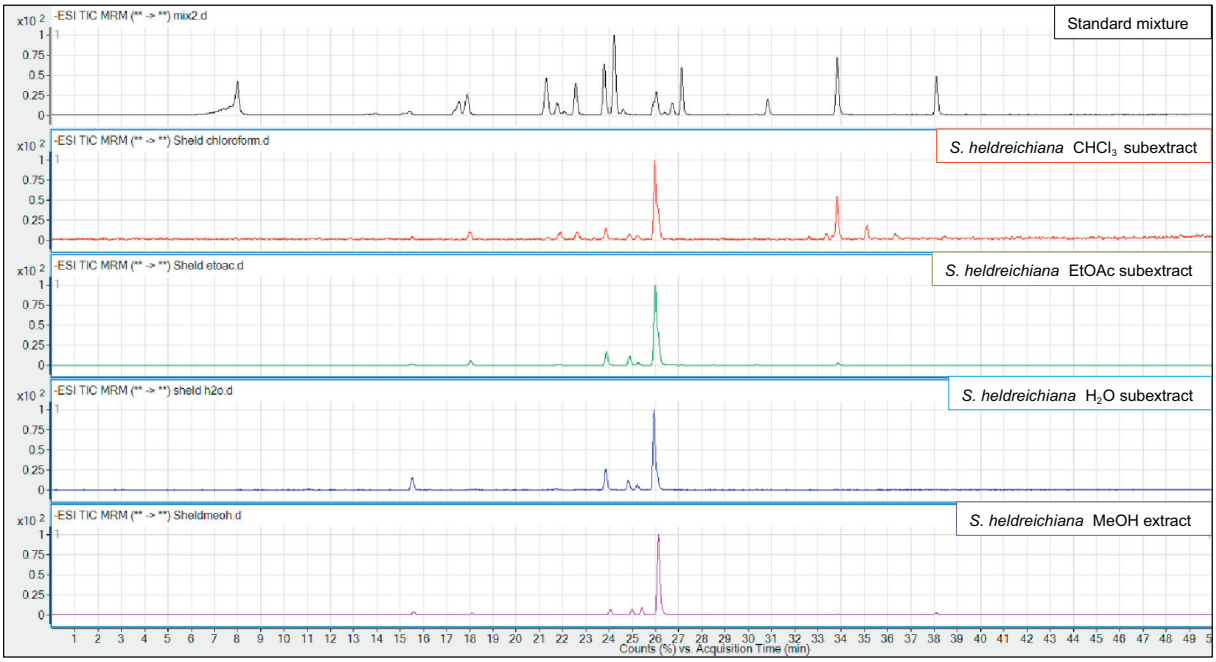

Fig. 2. Overlapped chromatographic profiles of standard compounds and *S. heldreichiana* extract/subextracts.

2.4.4. Determination of total proanthocyanidin content

Total proanthocyanidin content of each sample were estimated by employing the method illustrated by Ariffin et al. (2011). Accordingly, 1% vanillin HCl (9 M) in methanol were mixed with 1 mL of each extract and then incubated at 30 °C for 20 min. Subsequently, the absorbance was taken at 500 nm, and the results were expressed as mg epigallocatechingallate equivalents (EGCG-E) per g DE.

2.5. Identification of phenolic compounds by LC/ESI-MS–MS

Phenolic compounds in *S. heldreichiana* extracts were identified by Agilent 6420 triple quadrupole mass spectrometer equipped with electrospray ion source (ESI) and Agilent 1260 infinity series HPLC system consisting of a vacuum degasser, a binary pump, an autosampler. For the data analysis Agilent Mass Hunter software program was used. 5–10 μg/mL individual standard solutions of phenolic compounds are prepared by dissolving pure standards in MeOH. Mixture standard solution of phenolic compounds was prepared from the combination of individual standard compounds. Both individual standard solutions and mixture standard solution were used for optimization of instrument parameters and the confirmation of the identity of the peaks from the extracts.

Standard solutions are directly injected to the ion source of the mass spectrometer without using column for optimization of ion source and tandem mass spectrometer parameters. The mass range

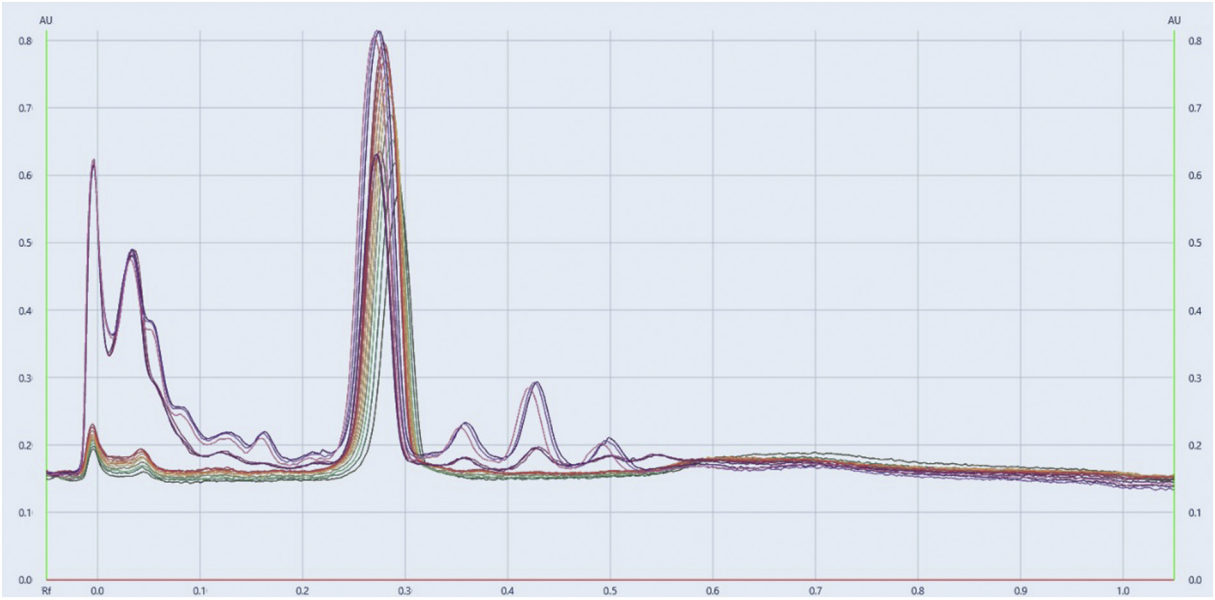

Fig. 3. Separation and R*f* value of RA in *S. heldreichiana* extract/subextracts.

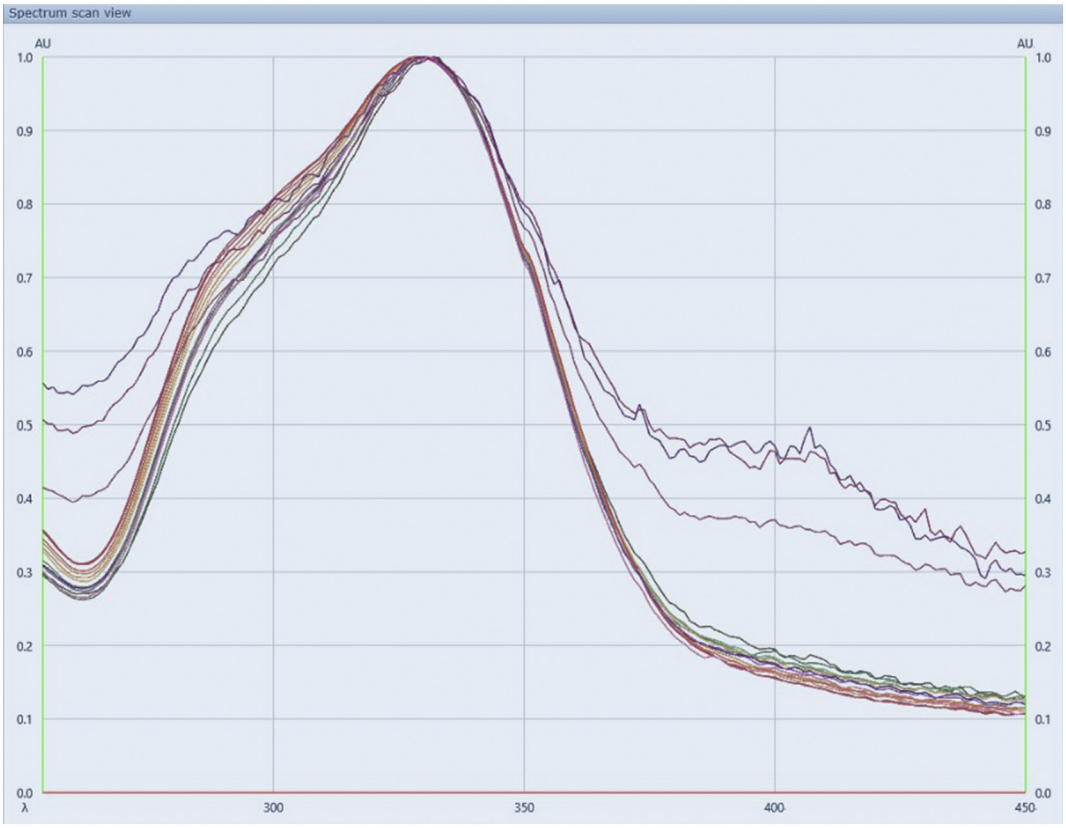

Fig. 4. Calibration curve for RA standard.

was adjusted to 10–1200 m/z for mass scan (MS) in negative ion mode for the generation of deprotonated ions of phenolic compounds, $[M-H]^-$. Optimized ion source parameters were as follows: 250 °C (N_2 drying gas temperature), 11 l/min (drying gas flow), 40 psi (nebulizer) and 4000 V (capillary voltage).

Fragmentation of the precursor ions in product ion (MS/MS) and multiple reaction monitoring (MRM) scan modes have been achieved by collision-induced dissociation (CID) using N_2 as the collision gas. MS/MS scans of the observed deprotonated ions, $[M-H]^-$ were performed for generation of the most intense fragment ions and the precursor and product ion pairs i.e. MRM transitions, for each compound. Then, collision energy (CE) and the fragmentor voltage (FV) of the MRM transitions were optimized.

The combination Inertsil ODS column (Zorbax Eclipse 4.6 × 150 mm i.d., 3.5 μm particle size) from Agilent with gradient flow using mobile phase A MeOH:H_2O: FA(formic acid) (10:89:1, *v/v/v*) and mobile phase B MeOH:H_2O:FA (89:10:1, *v/v/v*) were used for separation of phenolic compounds (Agalar et al., 2018). Flow rate was adjusted to 0.4 mL/min. The gradient started with 0% B and then changed to 10% B within 5 min. and then increased to 100% B in 45 min. and kept there for 5 min. The elution of all analytes was accomplished within 38 min. and the total run time was 50 min. including column washing. The column is flushed with 10 mL MeOH and conditioned with the initial composition of the mobile phase for at least 5 min. after each run. Column temperature was not controlled, and room temperature was kept constant. Injection volume was optimized as 10 μL, since higher volumes caused peak distortions significantly.

Table 4
RA contents (*w/w%*) of different *S. heldreichiana* extract/subextracts (n = 3).*, **

Extract	RA content (w/w%)	CV%
MeOH extract	9.68	0.63
EtOAc subextract	23.68	1.26
$CHCl_3$ subextract	2.32	3.43
R-H_2O subextract	2.61	3.15

* RA: Rosmarinic Acid.
** CV: Coefficient of Variation.

2.6. Quantification of rosmarinic acid by HPTLC

Rosmarinic acid contents were determined by using the previously validated method published by Bardakci-Altan et al. (2014). The standard solution of RA (250 μg/mL) was prepared in MeOH. Then, 15 mg of total extract and each fraction were dissolved in 10 mL MeOH. Each extract were filtered through a 0.45 μm syringe filter. 5 μL of MeOH and EtOAc extracts, 15 μL of $CHCl_3$ and R-H_2O extracts and 1–7 μL of standard RA were measured in triplicate. The spots of the sample and standard solutions were marked as bands with 8 mm length on silica gel glass HPTLC plates $60F_{254}$ with Camag Automatic TLC Sampler IV. A constant application rate was applied. The spaces between the tracks were adjusted to 10 mm. Densitometric screening was performed by using Camag TLC Scanner IV and VisionCATS software in absorbance mode at 330 nm. The slit dimension was kept at 5 × 0.2 mm, micro and the scanning speed was set at 20 mm/s. RA contents were obtained by comparing AUCs with the calibration curve of standard RA. The coefficient of variation (CV%) was under 1.00 and the correlation coefficient (R) of the calibration curve was 0.999. The mobile phase was toluene:ethyl acetate:formic acid (5:4:1) (*v/v%*). Developments were carried out in Camag Automatic Developing Chamber (ADC-2). Chamber was saturated for 20 min and the plate is preconditioned for 5 min before the development. The humidity was controlled by ADC-2 using $MgCl_2$ (33% RH) for 10 min. The presence of RA in extracts was assured by comparing the retention factors (R_f) and overlaying UV spectra of each extract and standard RA.

2.7. Appraisal of antioxidant potential depending on free radical-scavenging capacity

2.7.1. DPPH radical-scavenging activity

DPPH radical-scavenging activity test was performed according to the method defined earlier (Celep et al., 2013). Each extract diluted properly is individually mixed with 100 μM methanolic DPPH solution. The mixture was stored at room temperature, away from light, and the loss in absorption was measured at 517 nm. Butylated hydroxytoluene (BHT) was used as reference compound.

2.8. Appraisal of antioxidant potential depending on metal-linked activity

2.8.1. Cupric reducing antioxidant capacity (CUPRAC)

The method modified by Apak et al. (2004) was performed to investigate the CUPRAC activity of the extracts. Equivalent volumes of neocuproine, $CuSO_4$ and ammonium acetate buffer were mixed. Then, the extracts are added to mixture and then kept at room temperature for 1 h, and the absorbance was taken at 450 nm. The results were expressed as mg ascorbic acid equivalent per g DE.

2.8.2. Ferric reducing antioxidant power (FRAP)

FRAP is determined spectrophotometrically according to the method described by Benzie and Strain (1996). Correspondingly, diluted extracts were mixed with FRAP solution to the final volume of 0.3 mL. Following an incubation period of 30 min., the absorbance readings were taken at 593 nm. BHT was used as reference. The results were expressed as mM $FeSO_4$ per g DE.

2.8.3. Determination of total antioxidant capacity by phosphomolybdenum method

Total antioxidant capacity were determined by phosphomolybdenum method previously described by Prieto et al. (1999). Diluted extracts were mixed with the reaction mixture composed of sulfuric acid, ammonium molybdate and sodium phosphate monobasic. After incubation at 95 °C for 90 min., the absorbance was read at 695 nm. Total antioxidant capacity was expressed as mg ascorbic acid equivalent per g DE.

2.9. Estimation of antimicrobial activity

2.9.1. Standard microbial cultures

Antibacterial activity has been assessed against strains of both Gram-positive [*Staphylococcus aureus* (ATCC 6538)] and Gram-negative bacteria [*Pseudomonas aureginosa* (ATCC 15442), *Escherichia coli* (ATCC 11229)]. The antifungal activity against *Candida albicans* (ATCC 10231) strain was also examined.

2.9.2. Disc diffusion method

Antimicrobial activities of samples were performed at 2000 μg/mL concentration by disc diffusion method described by Atay-Balkan et al. (2017). Standard discs of Ofloxacin 0.50 μg (antibacterial agent) and Nystatin discs (100 units) (antifungal agent) were employed as positive controls. The bacterial and fungal suspensions which fulfilled the turbidity of the 0.50 McFarland standards were inoculated to Mueller-Hinton Agar (bacteria) or Sebouraud %2 Dextrose Agar (fungal) with sterile ecuvion sticks. Blank discs (6 mm in diameter) were impregnated with 20 μl of the extracts and sub-extracts placed on the inoculated plates. The diameter of inhibition zones were monitored following an incubation period of 18–24 h at 37 °C to determine the antimicrobial activity of the extract/sub-extracts.

2.9.3. Minimum inhibitory concentration

Minimum inhibitory concentration was determined by serial tube dilution technique given by Atay-Balkan et al. (2017). Ten screw cap test tubes were taken and serially labeled from 1 to 7 for extracts and the rest three was labeled as TM for medium, TMI for medium & inoculum and TMS for medium & DMSO, respectively. Nutrient broth medium (1 mL) were taken in all test tubes and the MeOH extract (2000 μg/mL) was added to the no 1 labeled tube only and the tube was shaken gently for proper mixing of the content. The content (1 mL) form the first tube was added to the no. 2 marked tube and repeated up to the no.7 marked tubes, after proper mixing 1 mL content form the 7 marked tube was discarded. 10 μL of the bacterial and fungal suspensions that achieved the turbidity of the 0.50 McFarland standards were added to the tubes labeled 1–7 and TMI. 1 mL of DMSO was added to TMS labeled tube, after shaking 1 mL of the mixture was discarded from the tube. TM labeled tube contained only 1 mL of medium. All the test tubes were subjected to incubation at 37 °C for 18–24 h.

2.10. Statistics

The data were recorded as mean ± standard deviation (SD) or mean ± standard error of the mean (SEM) of triplicate and duplicate (antimicrobial tests) experiments. These results were statistically evaluated by ANOVA test. The multiple comparisons were achieved by Tukey–Kramer post hoc test. Statistically significant difference was defined as $p < .05$.

3. Results

3.1. Quantitative evaluation of phenolic profile of S. heldreichiana extracts/subextracts

Total phenolic, total phenolic acid, flavonoid, and proanthocyanidin contents of *S. heldreichiana* extracts/subextracts were demonstrated in Table 1. The highest phenolic content was found in EtOAc subextract (420.46 ± 4.6 mg GAE/1 g extract) followed by R-H_2O subextract (352.32 ± 4.76 mg GAE/1 g extract), MeOH extract (313.60 ± 20.06 mg GAE/1 g extract), and $CHCl_3$ subextract (302.09 ± 24.17 mg GAE/1 g extract). Total flavonoid content of the extracts were similar, highest for EtOAc subextract (53.60 ± 9.64 mg QE/1 g extract), followed by remaining R-H_2O subextract (17.81 ± 9.64 mg QE/1 g extract), MeOH extract (15.15 ± 1.29 mg QE/1 g extract), and $CHCl_3$ subextract (14.87 ± 1.16 mg QE/1 g extract). $CHCl_3$ subextract displayed the highest total proanthocyanidin content (11.92 ± 0.02 mg EGCG-E/1 g extract) followed by MeOH extract (4.08 ± 0.05 mg EGCG-E/1 g extract). Eventually, $CHCl_3$ extract exhibited richest phenolic acid composition (472.33 ± 9.43 mg CAE/1 g DE) followed by EtOAc, MeOH and R-H_2O extracts.

3.2. Identification of phenolic compounds by LC/ESI-MS–MS

Compounds were identified based on both MRM transitions and retention times. Isobaric compounds (compounds with identical m/z) have been distinguished by either difference in their retention times or product ions or both. Catechin and epicatechin eluted at significantly different retention times (14 and 17 min., respectively). Apigenin-7-*O*-glucoside and vitexin with m/z 431 were identified based on their retention times at 26 and 22 min., and MRM transitions of 431 → 268 m/z and 431 → 311 m/z, respectively. Luteolin-7-*O*-glucoside, isoorientin and quercitrin with m/z 447 eluted at 21, 22 and 26 min. with MRM transitions of 447 → 285 m/z and 447 → 327 m/z and 447 → 301 m/z, respectively. As a result, combination of retention time and MRM transitions characteristic to each compound facilitated their accurate identification from plant material (Table 2). LC–MS/MS results and chromatograms of standard compounds are shown in Table 3 and Fig. 1, respectively. Commonly, *p*-coumaric acid, caffeic acid, chlorogenic acid, rosmarinic acid, apigenin, apigenin-7-*O*-glucoside, vitexin, isoorientin, luteolin-7-*O*-glucoside and hyperoside were detected in whole *S. heldreichiana* extracts and subextracts (Fig. 2).

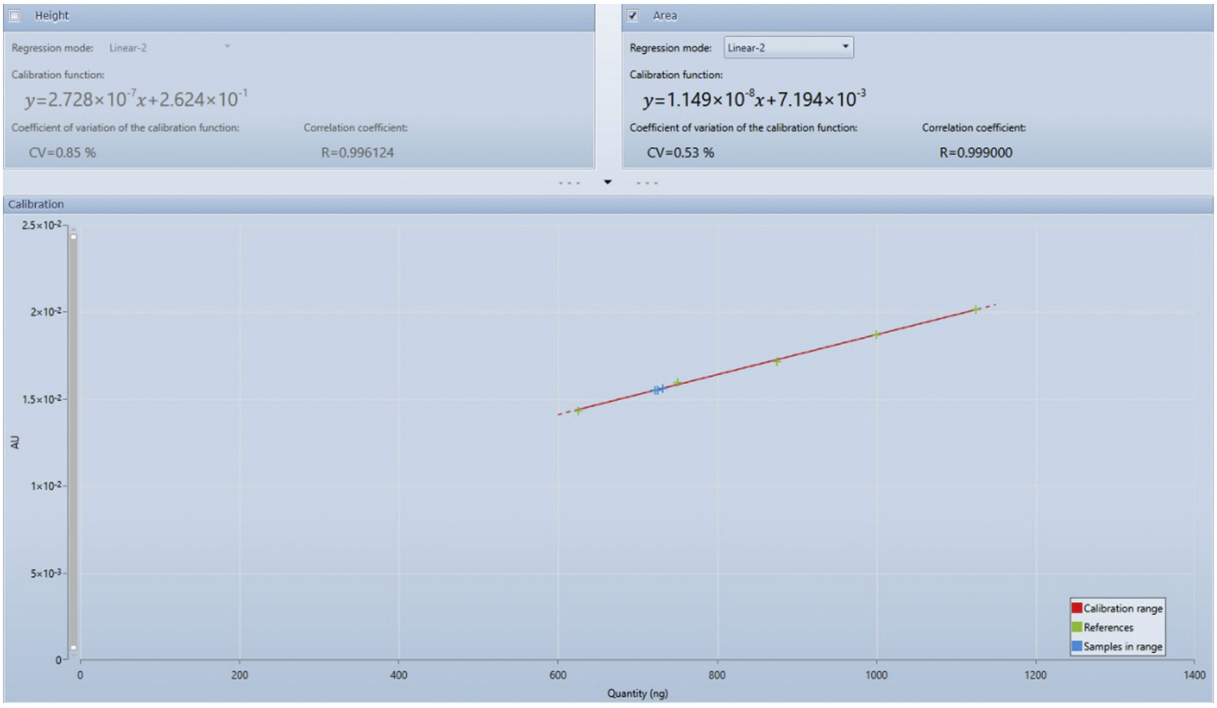

Fig. 5. Overlapped UV spectra of RA standard and *S. heldreichiana* extract/subextracts at 330 nm.

3.3. Rosmarinic acid content of extracts

Rosmarinic acid, which is an ester of caffeic acid and 3,4-dihydroxyphenyllactic acid, is one of the major component of *Salvia* species. By using HPTLC, RA in plant extracts were calculated. The R_f value for RA was calculated as 0.290 (Fig. 3). Quantification was afforded by using the calibration curve of RA with 0.53 CV% and 0.999 R (Fig. 4). The quantification data were shown in Table 4. The RA contents of extracts were calculated as 9.68%, 23.68%, 2.61%, 2.32% with the CV values 0.63%, 1.26%, 3.15% and 3.43% in MeOH, EtOAc, R-H_2O and $CHCl_3$ extracts, respectively. The overlaid chromatogram and UV spectra of compounds and RA was given in Fig. 5**.** Results obtained by HPTLC are consistent with the findings result of LC–MS/MS MRM method and bioactivity studies.

Table 5
In vitro antioxidant activity of *S. heldreichiana* extract/subextracts.

Analysis	MeOH	$CHCl_3$	EtOAc	R-H_2O
DPPH scavenging activity[A]	606.56 ± 1.74[a]	2310 ± 42.10[b]	593.70 ± 2.78[a]	609.44 ± 1.17[a]
FRAP[B]	3.54 ± 0.13[a]	0.52 ± 0.02[b]	6.48 ± 0.44[c]	4.12 ± 0.40[a]
CUPRAC[C]	501.44 ± 4.44[a]	170.8 ± 8.78[b]	830.3 ± 2.62[c]	531.58 ± 1.41[d]
Total antioxidant capacity[C]	78.73 ± 4.46[a]	87.68 ± 1.16[b]	98.73 ± 0.74[c]	70.84 ± 0.10[d]

P.S. 1) EC_{50} value of the reference compound "BHT" in DPPH scavenging activity is found to be 350 ± 10 μg/mL. 2) FRAP activity of the reference compound "BHT" is found to be 4.24 ± 0.48 mM FeSO4 eq. in 1 g sample.
a–e Different letters in the same row indicate significance ($p < .05$).
[A] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and DPPH activity was expressed as EC50 in μg/mL equivalents.
[B] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as Mm FeSO4 equivalents in 1 g sample.
[C] Results were expressed as the mean of triplicates ± standard deviation (S.D.) and as mg ascorbic acid equivalents (AAE) in 1 g sample.

3.4. Estimation of antioxidant capacity

Antioxidant spectra of the extracts were evaluated by DPPH radical and metal-chelating assays in addition to antioxidant capacity assay. The results are summarized in Table 5**.** DPPH scavenging, FRAP, and CUPRAC activities were highest for EtOAc subextract followed by R-H_2O subextract, MeOH extract and $CHCl_3$ subextract. In terms of total antioxidant capacity, EtOAc showed the highest activity similar to other assays, followed by $CHCl_3$, MeOH and R-H_2O.

3.5. Estimation of antimicrobial potential

The antibacterial activity of the crude MeOH extract and subextracts were evaluated by the disc diffusion method, in addition to the minimum inhibitory concentrations calculated. According to the results shown in Tables 6 and 7, the most promising activity was seen against *E. coli* (ATCC 11229) with 13 mm inhibition zone by EtOAc and R-H_2O subextracts, followed by crude MeOH extract and $CHCl_3$ subextract with the inhibition zones 11 and 9 mm, respectively. MeOH and R-H_2O extracts inhibited *S. aureus* equally with 12 mm inhibition zone, followed by EtOAc and $CHCl_3$ with the inhibition zones 10 and 8 mm, respectively. Similar inhibitions were observed against *P. aeruginosa* by EtOAc, MeOH and R-H_2O, which were followed by $CHCl_3$. Eventually, the antifungal activities of the extract/subextracts were tested against

Table 6
Antibacterial and antifungal activities of *S. heldreichiana* extract/subextracts (the activity is expressed as diameter of inhibition zone, mm).*

Microorganisms	MeOH (2000 μg/mL)	$CHCl_3$ (2000 μg/mL)	EtOAc (2000 μg/mL)	R-H_2O (2000 μg/mL)	Ofloxacin (5 μg)	Nystatin (100 units)
S. aureus	12	8	10	12	31	–
P. aureginosa	12	11	12	12	28	–
E. coli	11	9	13	13	32	–
C. albicans	12	12	15	15	–	30

* These values were the mean values of two replicates ± standard deviation.

Table 7
Antibacterial and antifungal activities of *S. heldreichiana* extract/subextracts (MIC values).

Extracts	MIC (μg/mL)			
	S. aureus	*P. aureginosa*	*E. coli*	*C. albicans*
MeOH	500	500	1000	500
$CHCl_3$	2000	500	2000	500
EtOAC	1000	500	500	250
R-H_2O	500	500	500	250

C. albicans using Nystatin as a standard drug. The highest antifungal activity against *C. albicans* was observed in EtOc and R-H_2O subextracts with 15 mm inhibition zones. Since data obtained were promising, serial dilution method was also employed for the determination of MIC values for whole strains. The determined MIC values were 500 μg/mL for crude MeOH extract and EtOAc, $CHCl_3$, R-H_2O subextracts for *P. aeruginosa* and 250 μg/mL for EtOAc and R-H_2O subextracts for *C. albicans*.

4. Discussion

Nature is an important and precious source of primary and secondary metabolites, which have potential in treatment of acute and chronic diseases. Utilization of the plants could be considered as economical way of producing antioxidants, antimicrobials, anti-inflammatory and chemopreventive agents. Primarily, phenolic compounds and then phenolic acids, flavonoids and phenylpropanoids are the most important secondary metabolites because of exhibiting significant antioxidant effects both in vivo and in vitro (Kasote et al., 2015). Besides, epidemiological data stated the importance of consuming an antioxidant-rich diet and antioxidant supplements in decreasing the risk of several chronic diseases, especially cardiovascular diseases (Rice-Evans, 2001). In addition to these benefits, the importance of natural sources in terms of their antimicrobial activity is also evident that approximately, two or three new antibiotics arisen from natural sources are introduced each year (Clark, 1996). Furthermore, among 109 new antibacterial drugs approved between 1981 and 2006, 69% of which originated from natural products. Similarly, 21% of antifungal drugs were natural derivatives or compounds simulating natural products (Savoaia, 2012). Majority of antimicrobial phytochemicals in plants contain a phenolic ring or phenolic ring with additional –OH groups are considered as the functional group responsible for their inhibitory action and antioxidant activity. Moreover, toxicity to microorganisms increases as the number of –OH groups in the structure of natural compound increases. Therefore, hydroxylated phenolic compounds, flavonoids are found to be effective against many microbial pathogens (Lai and Roy, 2004).

LC–MS/MS is used for identification and detection of a few dozens of phenolic compounds simultaneously. Alternatively, HPLC with UV or DAD detector are used mostly. However, different compounds may potentially have identical retention time and absorption wavelength (Gray et al., 2010). It has been proven that LC–MS/MS provides screening of plant metabolites with higher sensitivity and selectivity from complex mixtures compared to LC-DAD or LC-UV (Wu et al., 2012). In addition to screening a few authentic metabolites from plants (Wu et al., 2012), the use of LC–MS/MS for simultaneous screening of large number of drugs has been very popular and dates back to beginning of 2000's (Gergov et al., 2003) and retain its popularity today (Odoardi et al., 2015) in the field of clinical toxicological and forensic studies (Yao et al., 2008). HPTLC technique also offers several advantages in plant metabolomics studies, because it is faster, easier, and more flexible. Most importantly, many samples that can be analyzed simultaneously by HPTLC rapidly and accurately (Reich and Schibli, 2007). Therefore, LC–MS/MS in MRM mode is utilized for the identification or fingerprinting analysis of extracts, and HPTLC is applied for the quantitation in this study. The separation capability of an analytical method is critical in quantification. Especially, if the sample is composed of too many components, while baseline separation of all substances required for accurate quantification but it is not necessary in fingerprinting analysis. HPTLC with scanning densitometry, especially fluorescence detection offers an advantage of precise determination and quantification of chemical markers. Hence, HPTLC for the routine or conventional analysis of many herbal medicines industrially has gained increased popularity (Reich and Schibli, 2007). According to literature, many noteworthy members of *Salvia* genus used in folk medicines and for culinary purposes owing to their prosperous phenolic composition. Especially, in Turkey *Salvia* species are constant members of cuisines and medicine cabinets. In this study, main and subextracts of an endemic member *S. heldreichiana* was examined in terms of its phenolic composition, antioxidant and antimicrobial activity.

It has been demonstrated that apigenin–luteolin type flavones are highly encountered in Turkish *Salvia* species according to a review by Ulubelen and Topçu (1998). Similarly, our LC–MS/MS results have shown that apigenin, apigenin-7-*O*-glucoside and luteolin-7-*O*-glucoside were identified in all the extracts. Moreover, LC–MS/MS data have indicated that EtOAc subextract contains *p*-coumaric acid, gallic acid, caffeic acid, ferulic acid, apigenin, quercetin, chlorogenic acid, rosmarinic acid, apigenin-7-*O*-glucoside, vitexin, quercitrin, luteolin-7-*O*-glucoside, isoorientin, hyperoside and martynoside as phenolic metabolites. Indeed, these findings by LC–MS/MS is confirmed by the significantly higher GAE total phenolic content (420.46 ± 46) of EtOAc subextract compared to other extracts. In addition, according to the results HPTLC analysis, RA content of EtOAc subextract is 23.68%, which is considerably higher amounts than the other extracts.

Essential oil composition (Basalma et al., 2007; Akın et al., 2010; Erdoğan, 2014), and the isolation of few diterpenoids (Ulubelen et al., 1995) from *S. heldreichiana* has been reported in literature. Previously, DPPH radical scavenging and FRAP activity tests of CH_2Cl_2, EtOAc and MeOH extracts from *S. heldreichiana* has also been documented. It has been found out that EtOAc extract demonstrated highest DPPH scavenging activity, whereas CH_2Cl_2 extract showed the highest activity in FRAP (Senol et al., 2010).

According to our study, EtOAc subextract displayed the highest DPPH radical scavenging, FRAP, CUPRAC and total antioxidant capacity among the crude extract and subextracts. EtOAc subextract was also found to possess the highest total phenolic and total flavonoid contents. Furthermore, *P. aeruginosa*, *E. coli* and *C. albicans* strains were inhibited by EtOAc subextract more compare to other extracts.

Consequently, EtOAc subextract of *S. heldreichiana* showed the highest bioactivity among the tested extracts. The results of antioxidant and antimicrobial activity tests were consistent with the results of both LC–MS/MS and HPTLC. LC–MS/MS data indicated that EtOAc subextract is rich in phenolic composition and similarly, EtOAc subextract contains the highest RA content in comparison to other extracts according to HPTLC data.

References

Agalar, H.G., Çiftçi, G., Göger, F., Kırımer, N., 2018. Activity guided fractionation of *Arum italicum* Miller tubers and the LC/MS-MS profiles. Rec. Nat. Prod. 12, 64–75.

Akın, M., Demirci, B., Bagci, Y., Baser, K.H.C., 2010. Antibacterial activity and composition of the essential oils of two endemic *Salvia* sp. from Turkey. Afr. J. Biotechnol. 9, 2322–2327.

Apak, R., Güçlü, K., Özyürek, M., Karademir, S.E., 2004. Novel total antioxidant capacity index for dietary polyphenols and vitamins C and E, using their cupric ion reducing capability in the presence of neocuproine: CUPRAC method. J. Agric. Food Chem. 52, 7970–7981.

Ariffin, F., Chew, S.H., Bhupinder, K., Karim, A.A., 2011. Antioxidant capacity and phenolic composition of fermented *Centella asiatica* herbal teas. J. Sci. Food Agric. 91, 2731–2739.

Atay-Balkan, I., Taşkın, T., Doğan, H.T., Deniz, I., Akaydın, G., 2017. A comparative investigation on the in vitro anti-inflammatory, antioxidant and antimicrobial potentials of subextracts from the aerial parts of *Daphne oleoides* Schreb. subsp. *oleoides*. Ind. Crop Prod. 95, 695–703.

Bardakci, H., Skaltsa, H., Milosevic-Ifantis, T., Lazari, D., Hadjipavlou-Litina, D., Yesilada, E., Kırmızıbekmez, H., 2015. Antioxidant activities of several *Scutellaria* taxa and bioactive phytoconstituents from *Scutellaria hastifolia* L. Ind. Crops Prod. 77, 196–203.

Bardakci-Altan, H., Akaydın, G., Kırmızıbekmez, H., Yesilada, E., 2014. Validated HPTLC method for the quantitative analysis of rosmarinic acid in several *Salvia* sp. Turk. J. Pharm. Sci. 11, 245–254.
Basalma, D., Gürbüz, B., Sarıhan, E.O., Ipek, A., Arslan, N., Duran, A., Kendir, H., 2007. Essential oil composition of *Salvia heldreichiana* Boiss. ex Bentham described endemic species from Turkey. Asian J. Chem. 19, 2130–2134.
Baytop, T., 1999. Türkiye'de Bitkilerle Tedavi. 2nd ed. Nobel Tıp Kitabevleri, Istanbul.
Benzie, I.F.F., Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: the FRAP assay. Anal. Biochem. 239, 70–76.
Celep, E., Aydın, A., Yesilada, E., 2012. A comparative study on the *in vitro* antioxidant potentials of three edible fruits Cornelian cherry, Japanese persimmon and cherry laurel. Food Chem. Toxicol. 50, 3329–3335.
Celep, E., Aydın, A., Kırmızıbekmez, H., Yesilada, E., 2013. Appraisal of *in vitro* and *in vivo* antioxidant activity potential of cornelian cherry leaves. Food Chem. Toxicol. 62, 448–455.
Chanda, S., Dave, R., 2009. *In vitro* models for antioxidant activity evaluation and some medicinal plants possessing antioxidant properties: an overview. Afr. J. Microbiol. Res. 3, 981–996.
Chang, H.M., Cheng, K.P., Choang, T.F., Chow, H.F., Chui, K.Y., Hon, P.M., Lau Tan, F.W., Yang, Y., Zhong, Z.P., Lee, C.M., Sham, H.L., Chan, C.F., Cui, Y.X., Wong, H.N.C., 1990. Structure elucidation and total synthesis of new tanshinones isolated from *Salvia miltiorrhiza* Bunge (Danshen). J. Org. Chem. 55, 3537–3543.
Clark, A., 1996. Natural products as a resource for new drugs. Pharm. Res. 13, 1133–1141.
Er, M., Tugay, O., Özcan, M.M., Ulukuş, D., AL-Juhaimi, F., 2013. Biochemical properties of some *Salvia* L. species. Environ. Monit. Assess. 185, 5193–5198.
Erdoğan, E.A., 2014. Lamiaceae Familyasına Ait Bazı Bitkilerin Uçucu Yağ İçeriklerinin Belirlenmesi, Antimikrobiyal ve Antimutajenik Aktivitelerinin Araştırılması. Doktora Tezi. Mersin Üniversitesi.
Erdogan, E.A., Everest, A., Kaplan, E., 2013a. Antimicrobial activities of aqueous extracts and essential oils of two endemic species from Turkey. Ind. J Tradit. Know. 12, 221–224.
Erdogan, E.A., Everest, A., Martino, L., Mancini, E., Festa, M., Feo, V., 2013b. Chemical composition and *in vitro* cytotoxic activity of the essential oils of *Stachys rupestris* and *Salvia heldreichiana*, two endemic plants of Turkey. Nat. Prod. Com. 8, 1637–1640.
Gergov, M., Ojanpera, I., Vuori, E., 2003. Simultaneous screening for 238 drugs in blood by liquid chromatography–ionspray tandem mass spectrometry with multiple-reaction monitoring. J. Chromatogr. B 795, 41–53.
Ghorbani, A., Esmaeilizadeh, M., 2017. Pharmacological properties of *Salvia officinalis* and its components. J. Tradit. Complement. Med. 7, 433–440.
Gray, M.J., Chang, D., Zhang, Y., Liu, J.X., Bensoussan, A., 2010. Development of liquid chromatography/mass spectrometry, methods for the quantitative analysis of herbal medicine in biological fluids: a review. Biomed. Chromatogr. 24, 91–103.
Hayashi, M., Bizerra, F., Da Silva, P., 2013. Antimicrobial compounds from natural sources. Front. Microbiol. 4, 195.
Jorge, T.F., Mata, A.T., António, C., 2016. Mass spectrometry as a quantitative tool in plant metabolomics. Phil. Trans. R. Soc. A 374, 20150370.
Kalaycıoglu, Z., Uzas, S., Dirmenci, T., Erim, B., 2018. α-Glucosidase enzyme inhibitory effects and ursolic and oleanolic acid contents of fourteen Anatolian *Salvia* species. J. Pharm. Biomed. Anal. 155, 284–287.
Kasote, D., Katyare, S., Hedge, M., Bae, H., 2015. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int. J. Biol. Sci. 11, 982–991.
Kırmızıbekmez, H., Çalış, İ., Piacente, S., Pizza, C., 2004. Phenolic compounds from *Globularia cordifolia*. Turk. J. Chem. 28, 455–460.
Kırmızıbekmez, H., İnan, Y., Reis, R., Sipahi, H., Gören, A.C., Yeşilada, E., 2018. Phenolic compounds from the aerial parts of *Clematis viticella* L. and their *in vitro* anti-inflammatory activities. Nat. Prod. Res. https://doi.org/10.1080/14786419.2018.1448815 In-press.
Krishnaiah, D., Sarbatly, R., Nithyanandam, R., 2011. A review of the antioxidant potential of medicinal plant species. Food Bioprod. Process. 89, 217–233.
Lai, P.K., Roy, J., 2004. Antimicrobial and chemopreventive properties of herbs and spices. Curr. Med. Chem. 11, 1451–1460.
Liu, W.J.H., 2011. Introduction to traditional herbal medicines and their study. In: Liu, W. (Ed.), Traditional Herbal Medicine Research Methods. Wiley, New Jersey.
Lu, Y., Foo, Y., 2002. Polyphenolics of *Salvia* - a review. Phytochemistry. 59, 117–140.
Mihailovic, V., Kreft, S., Benkovic, E.T., Ivanovic, N., Stankovic, M.S., 2016. Chemical profile, antioxidant activity and stability in stimulated gastrointestinal tract model system of three *Verbascum* species. Ind. Crops Prod. 89, 141–151.
Odoardi, S., Fisichella, M., Romolo, F.S., Strano-Rossi, S., 2015. High-throughput screening for new psychoactive substances (NPS) in whole blood by DLLME extraction and UHPLC-MS/MS analysis. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 1000, 57–68.
Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal. Biochem. 269, 337–341.
Reich, E., Schibli, A., 2007. High-Performance Thin-Layer Chromatography for the Analysis of Medicinal Plants. Thieme, New York.
Rice-Evans, C., 2001. Flavonoid antioxidants. Curr. Med. Chem. 8, 797–807.
Savoaia, D., 2012. Plant-derived antimicrobial compounds: alternatives to antibiotics. Future Microbiol. 7, 979–990.
Senol, F., Orhan, I., Celep, F., Kahraman, A., Dogan, M., Yilmaz, G., Şener, B., 2010. Survey of 55 Turkish *Salvia* taxa for their acetylcholinesterase inhibitory and antioxidant activities. Food Chem. 120, 34–43.
Sen-Utsukarci, B., Gurdal, B., Bilgin, M., Satana, D., Demirci, B., Tan, N., Mat, A., 2019. Biological activities of various extracts from *Salvia cassia* Sam. ex Rech.f. and chemical composition of its most active extract. Rec. Nat. Prod. 13, 24–36.
Singleton, V.L., Rossi, J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.
Topcu, G., 2006. Bioactive triterpenoids from *Salvia* species. J. Nat. Prod. 69, 482–487.
Ulubelen, A., Topçu, G., 1998. Chemical and biological investigations of *Salvia* species growing in Turkey. In: Atta-ur-Rahman (Ed.), Studies in Natural Products Chemistry. 20, pp. 659–668.
Ulubelen, A., Topçu, G., Tan, N., 1995. Diterpenoids from *Salvia heldreichiana*. Phytochemistry. 40, 1473–1475.
Wang, G., 2015. LC-MS in plant metabolomics. In: Qi, X., Chen, X., Wang, Y. (Eds.), Plant Metabolomics. Chemical Industry Press, Beijing and Springer Science + Business Media, Dordrecht, pp. 45–61.
Wu, J., Shen, H., Xu, J., Zhu, L., Jia, X., Li, S., 2012. Detection of sulfur-fumigated Paeoniae alba radix in complex preparations by high performance liquid chromatography tandem mass spectrometry. Molecules. 17, 8938–8954.
Xu, D.P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J., Zhang, J.J., Li, H.B., 2017. Natural antioxidants in foods and medicinal plants: extraction, assessment and resources. Int. J. Mol. Sci. 18, 96.
Xu, J., Wei, K., Zhang, G., Lei, L., Yang, D., Wang, W., Han, Q., Xia, Y., Bi, Y., Yang, M., Li, M., 2018. Ethnopharmacology, phytochemistry, and pharmacology of Chinese *Salvia* species: a review. J. Ethnopharmacol. 225, 18–30.
Yao, M., Ma, L., Humphreys, G.W., Zhu, M., 2008. Rapid screening and characterization of drug metabolites using a multiple ion monitoring–dependent MS/MS acquisition method on a hybrid triple quadrupole-linear ion trap mass spectrometer. J. Mass Spectrom. 43, 1364–1375.
Zhang, K.Q., Bao, Y., Wu, P., Rosen, R.T., Ho, C.-T., 1990. Anti-oxidative components of tanshen (*Salvia miltiorrhiza* Bunge). J. Agric. Food Chem. 38, 1194–1197.