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Research Article

Antimicrobial and antioxidant activities of *Sideritis lanata* L. extracts

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ABSTRACT

Sideritis L. (Lamiaceae) is represented by 45 species in Flora of Turkey. *Sideritis lanata* L. (Hairy ironwort) is located in the north-west, west, south-west and central Anatolia. Mountain tea (*Sideritis plants*) is used as a traditional remedy against common cold and gastrointestinal disorders. Its beneficial properties are attributed to its rich bioactive constituents. In this study, the antioxidant activities of the extracts of Sideritis lanata prepared using different solvents (*n*-hexane, ethyl acetate, and 70% ethanol) from the aerial parts were determined by DPPH· scavenging effect assay. Also; determined of the antimicrobial activity of S. lanata extracts against 4 bacteria and 5 *Candida* species. Dried aerial parts of *Sideritis* were subjected to hydrodistillation and the oil obtained was analyzed by using GC and GC/MS. *S. lanata* extracts prepared with 70% ethanol was found to have a higher DPPH· scavenging activity (IC_{50}= 0.241 mg/mL) than extracts prepared with other solvents. All extracts showed generally weak inhibitory effects (500-8000 µg/mL, MIC) against bacterial strains. Inhibitory doses against the tested *Candida* species were determined between 125-4000 µg/mL. The 70% ethanol extract of *S. lanata* inhibited *Candida tropicalis* (ATCC 750) (MIC: 125 µg/mL).

Keywords: Antimicrobial Activity, Antioxidant activity, Essential oil, GC-GC/MS, *Sideritis lanata*

1. INTRODUCTION

There are 45 species (60 taxa) of *Sideritis* in Turkey, and the endemism rate is quite high with approximately 80% [1]. *Sideritis* species has been used in traditional medicine for cough, cold, gastrointestinal disorders, antiseptic, anti-inflammatory, antirheumatic, antimicrobial and insecticidal [2]. *Sideritis* genus contains chemical components such as flavonoids, terpenes, coumarins, essential oils, lignans, iridoids, and sterols. Diterpenes, flavonoids, and essential oils are found in almost every species of *Sideritis* and are the main compounds responsible for *in vivo* and *in vitro* pharmacological activities [3]. *Sideritis lanata* L. (Lamiaceae) is a species that spreads mainly in the Mediterranean region. The plant is locally known as "İpek çayı" in Turkey [4]. The chemical composition of *S. lanata* has been extensively studied in 2000. The major components of *S. lanata* were found as hexadecanoic acid (10.67%) and spathulenol (9.45%) [5]. With spectroscopic methods; 7-*O*-β-D-glucopyranosylchrysoeriol, 7-*O*-[(6‴-O-acetyl)-β-D-allopyranosyl (1→2)-β-D-glucopyranosyl] hypolaetin , 7-*O*-[(6‴-O-acetyl)-β-D-allo pyranosyl (1→2)-β-D-glucopyranosyl] hypolaetin-3′-methyl

ether and 7-*O*-[(6‴-O-acetyl)-β-D-allopyranosyl (1→2)-β-D-glucopyranosyl] isoscutellarein and a new iridoid diglucoside 10-0-(*E*)-p coumaroylmelittoside and a new flavone glucoside and a new flavone glucoside 7-O- (6″-O-Acetyl-)-β-glucopyranosylchrysoeriol have been identified by Alipieva et al. [6]. The antimicrobial activity of *S. lanata* essential oil was studied by the disc diffusion method. The essential oil has been shown to have a strong antimicrobial effect against Gram-positive bacteria, especially methicillin-resistant *Staphylococcus aureus* (MRSA) and oxacillin-resistant coagulase-negative *Staphylococcus epidermidis*. *Bacillus cereus, Bacillus subtilis*, and *Micrococcus luteus* were found sensitive bacteria to the essential oil of *S. lanata* [7]. As far as we know when the literature was reviewed, the antioxidant effect of *S. lanata* has not been reported before. Our study, *S. lanata* with $DPPH^{\cdot}$ scavenging activity method, can be considered the first report in terms of antioxidant activity. In addition, in our study, the antibacterial effect was studied by selecting different strains from other antimicrobial activity experiments.

2. MATERIALS AND METHODS

2.1. The Plant Material

S. lanata was collected from Kutlu, Bolvadin/ Afyonkarahisar in Turkey on June 2021. The voucher specimen has been deposited at the Herbarium in the Anadolu Unıversity, Eskisehir, Turkey (Voucher specimen no: ESSE 15820). The plant material was identified by Prof. Dr. Yavuz Bulent KOSE.

2.2. Isolation of Essential Oil

Essential oil of the aerial parts was extracted by hydrodistillation Clevenger apparatus method.

2.3. GC Analysis

GC analyses were performed using an Agilent 6890N GC system. FID temperature was set to 300°C and the same operational conditions were applied to a triplicate of the same column used in GC/MS analyses. Simultaneous auto-injection was employed to obtain equivalent retention times. Relative percentages of the separated compounds were calculated from integration of the peak areas in the GC-FID chromatograms.

2.4. GC/MS Analysis

The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). Innowax FSC column (60m x 0.25mm, 0.25μm film thickness) was used with helium as carrier gas (0.8 mL/min.). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted 40:1. The injector temperature was at 250°C. The interphase temperature was at 280°C. MS were taken at 70 eV. Mass range was from *m/z* 35 to 450.

2.5. Identification of Compounds

The components of essential oils were identified by comparison of their mass spectra with those in the in-house Baser Library of Essential Oil Constituents, Adams Library [8], MassFinder Library [9], Wiley GC/MS Library [10], and confirmed by comparison of their retention indices. These identifications were accomplished by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of n-alkanes. Alkanes were used as reference points in the calculation of relative retention indices (RRI) [11]. Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

2.6. Extract Preparation

Three extracts of the species with 70% ethanol, *n*-hexane, and ethyl acetate were prepared. The aerial parts of the plant were cut into small pieces. A weight of 18 grams was taken from the sample and 200 mL of solvent was added. Samples were left to maceration in a shaker (Orbital) at 150 rpm at room temperature for 48 hours. After the macerated were filtered through filter paper, their solvents were removed with a rotavapor under reduced pressure.

70% ethanolic extract was obtained by lyophilizer. The extracts, freed from their solvents, were stored in the refrigerator at +4 ºC until use.

2.7. Antioxidant activity

The antioxidant activities of the extracts of *S. lanata* prepared using different solvents (*n*-hexane, ethyl acetate and 70% ethanol) from the aerial parts were determined by DPPH· radical scavenging effect assay. The methods of Kumarasamy et al. were used for the determination of DPPH· scavenging effect [12].

2.8. Determination of Total Phenolic Content Using the Folin-Ciocalteu Reagent (FCR)

The extract's total amount of phenol was determined as equivalent to mg Gallic Acid using the Folin-Ciocalteu reagent method. The method based on the Folin-Ciocalteu reagent (FCR), known as the total phenolic reagent, determines the reducing capacity of the sample. Gallic acid is usually used as the standard compound and results are given as gallic acid equivalent (mg/mL). Different solutions of gallic acid were prepared in the concentration range of 1-0.03 mg/mL, absorbance values were recorded at 760 nm, and the calibration equation was determined by drawing the concentration absorbance graph of gallic acid. Samples absorbance measurements at 760 nm were taken and compared with the gallic acid calibration curve. TFC (Total Phenolic Content) was calculated as gallic acid equivalents (GAE) in mg/g dry weight of plant material. A reagent mixture without extract and solvent was used as a control. Three parallel experiments were performed and the results were given as mean values [13,14].

2.9. Antimicrobial activity

Determination of the antimicrobial activity of *S. lanata* extracts against 4 bacteria *(Staphylococcus aureus (*ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853), *Serratia marcescens* (NRRL B-2544), *Klebsiella pneumoniae* (NCTC 9633) and 5 *Candida* species (*Candida utilis* (NRRL Y- 900), *Candida albicans (*ATCC 90028), *Candida tropicalis* (ATCC 750), *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 6258). The minimum inhibitory (MIC) concentrations of the extracts were determined using standard protocols (CLSI M7-A7 and M27-A2).

3. RESULTS AND DISCUSSION

3.1. Chemical composition of the essential oil

The yield of essential oil obtained from the aerial parts of the plant was found to be 1.05%. The essential oil was analyzed by GC and GC/MS. 27 compounds representing 89.1% of the essential oil was characterized with unknown (29.7%), Phytol (15.7%), 9-geranyl-p-cymene (7.5%) and β-caryophyllene (6.1%) as major constituents. The chemical components of *S. lanata* essential oil analyzed by GC-GC/MS are shown in Table 1. In a study on *Sideritis* in Greece, it was observed that the essential oil was quite dense (46.3%) in terms of sesquiterpenes. It has fewer components in terms of monoterpenes (3.5%) and diterpenes (10.9%). The major components were spathulenol (12.7%), β-phellandrene (3.5%), and ent-2α hydroxy-8(14),5-pimaradiene (9.7%), respectively [15]. In a study on some *Sideritis* species (*S. scardica, S. raeseri, S. syriaca, S. taurica* and *S. lanata*) in the Balkan Peninsula the most abundant hydroxycinnamic acid derivative in *Sideritis* was 5-caffeoylquinic acid. In most samples, including *S. lanata*, 5-caffeoylquinic acid was the only hydroxycinnamic acid detected [16]. The aerial parts of *S. albiflora* Hub.-Mor. essential oil were analyzed simultaneously by GC-FID and GC-MS. 88 compounds representing 88.2% of the essential oil were characterized by germacrene D (23.5%), β-caryophyllene (13.6%), caryophyllene oxide (8.0%), and hexadecanoic acid (3.8%) as major constituents [17].

3.2. Antimicrobial activity

All extracts showed generally weak inhibitory effects (500-8000 µg/mL, MIC) against bacterial strains (Table 2). Inhibitory doses against the tested *Candida* species were determined between

125-4000 µg/mL. A 70% ethanol extract of *S. lanata* inhibited *Candida tropicalis* (ATCC 750) at a dose of 125 µg/mL (MIC) (Table 3).

In a study done in 2005, the essential oil of *S. lanata* (5 µl) had no effect on Gram-negative bacteria, whereas it was observed to have an inhibitory effect on all Gram-negative organisms at 25 µL. 10 and 25 µl essential oils of *S. lanata* were observed to have a significant effect on all Gram-positive bacteria used in the study [7].

Table 1. The chemical components of *Sideritis lanata* essential oil

RRI	Compounds	Sideritis %	Grup	IM
1400	Nonanal	0.5	D	MS
1452	1-Octen-3-ol	0.5	D	t_R, MS
1495	Bicycloelemene	1.0	ST	MS
1541	Benzaldehyde	2.0	D	MS
1589	β-Ylangene	0.5	ST	MS
1612	β-Caryophyllene	6.1	ST	t_R, MS
1722	2-Undecanol	0.2	D	MS
1726	Germacrene D	1.3	ST	MS
1755	Bicyclogermacrene	3.0	ST	t_R, MS
1800	Octadecane	0.4	D	t_R, MS
1958	(*E*)-β-Ionone	1.4	D	MS
2096	Elemol	3.2	OST	MS
2131	Hexahydrofarnesyl acetone	1.5	D	MS
2144	Spathulenol	0.8	OST	t_R, MS
2227	Unknown*	29.7	DT	MS
2239	Carvacrol	tr	OMT	t_R, MS
2255	α-Cadinol	0.7	OST	t_R, MS
2300	Tricosane	0.3	D	t_R, MS
2312	9-Geranyl-p-cymene	7.5	D	MS
2384	Farnesyl acetone	0.6	D	MS
2486	8-α-Acetoxyelemol	1.4	D	MS
2500	Pentacosane	0.8	D	t_R, MS
2622	Phytol	15.7	ODT	MS
2700	Heptacosane	3.4	D	t_R, MS
2781	Geranyl geraniol	4.3	OST	MS
2931	Hexadecanoic acid	2.9	D	MS
3000	Tricontane	0.8	D	MS
	OST Oxygenated sesquiterpenes	9.94		
	OMT Oxygenated monoterpenes	tr		
	DT Diterpene	32.82		
	ODT Oxygenated diterpene	17.35		
	D-Others	26.74		
	ST Sesquiterpene hydrocarbons	13.15		
	Total %	90.5		

RRI: Relative retention indices calculated against n-alkanes; %: calculated from the FID chromatograms; tr: Trace (<0.1 %). Identification method (IM): t_R, identification based on the retention times of genuine compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries and comparison with literature data.

* *m/z*: 272 [M+] (18), 188 (47), 187 (26), 159 (25), 132 (48), 133 (22), 121 (69), 119 (100), 107 (24), 105(49), 93 (43), 91 (36), 81 (22), 79 (22), 69 (67), 41 (26).

Table 2. Anticandidal effect of *Sideritis lanata* extracts (µg/mL)

	H	EtOAc	EtOH	AMP-B	Keto
Candida utilis NRRL Y-900	250	500	500	1	0.5
Candida albicans ATCC 90028	2000	2000	4000	1	0.5
Candida tropicalis ATCC 750	250	250	125	2	0.25
Candida parapsilosis ATCC 22019	2000	2000	4000	2	0.125
Candida krusei ATCC 6258	500	1000	2000	1	1

AMP-B: Amphotericin B, Keto: Ketoconazole, H: *Sideritis lanata n*-hexane extract, EtOAc: Extract with ethyl acetate, EtOH: Extract with ethanol 70%.

Table 3. Antibacterial effect of *Sideritis lanata* extracts (µg/mL)

	n-Hexane	Ethyl acetate	Ethanol %70	Ampicillin	Chloramphenicol
Staphylococcus aureus ATCC 6538*	500	2000	>8000	1	4
Pseudomonas aeruginosa ATCC 27853	4000	4000	>8000	>64	8
Serratia mar-cescens NRRL B-2544	4000	4000	>8000	>64	4
Klebsiella pne-umoniae NCTC 9633	4000	4000	8000	>16	2

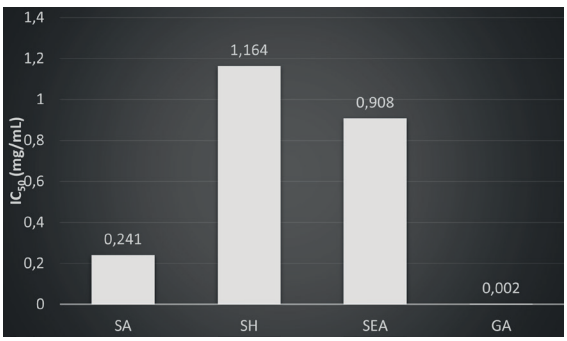

Figure 1. The ability of extract in scavenging DPPH· scavenging activity.

SA: 70% ethanol extract of *S. lanata*, SH: n-Hexane extract of *S.lanata*, SEA: Ethyl acetate extract of *S. lanata*, GA: Gallic acid.

Table 4. DPPH· scavenging activity of *S. lanata*

Extracts	DPPH· test IC_{50} (mg/mL)
Ethanol %70	0.241 ±0.115
n-Hexane	1.164 ±0.197
Ethyl acetate	0.908 ±0.165
Gallic acid	0.002 ±0.001

mg GAE/g extract: Total phenols expressed as gallic acid equivs milligrams of gallic acid per gram (dry weight) of extract

3.3. Antioxidant activity

S. lanata extracts prepared with 70% ethanol was found to have a higher DPPH· radical scavenging activity (0.241 mg/mL) than extracts prepared with other solvents (Table 4). To the best of our knowledge, there is no study on the antioxidant activity of *Sideritis lanata*. *S. syriaca, S. scardica*, and *S. montana* were extracted with different solvents in Bulgaria. Their antioxidant activities were determined by the 2,2-diphenyl 1-picrylhydrazyl (DPPH**)** radical scavenging method. The most effective extracts were found to be methanol, butanol and ethyl acetate extracts [18]. In the other study, antioxidant activity tests were carried out by lipid oxidation of liposomes using the TBA (Thiobarbituric acid test) method. TLC was used as a screening method to evaluate the antioxidant activities of freeze-dried extracts of *Sideritis* species with the DPPH· test. Antioxidant

activities of lyophilized extracts obtained from the aerial parts of 17 species (18 taxa) of which 15 taxa of *Sideritis* are endemic were compared. The highest activity in TBA method was observed in *S. brevibracteata* extract (IC_{50} mg/ml = 0.16). However, in general, the antioxidant values of the species were low. In the study, it was decided that teas made with these species did not have much antioxidant activity [19].

3.4. Folin-Ciocalteu reagent (FCR) and the total phenolic method (Total Phenol Quantification) Results

The total amount of phenol contained in the extracts obtained from the aerial parts of the plants was determined by a spectrophotometric method using the Folin-Ciocalteu reagent. The total phenol amounts of the extracts are given in Table 5 as equivalent to Gallic acid. The calibration curve and curve equation of the gallic acid used in the calculations are shown in Figure 2. According to the findings, the highest total phenol content (61.1±0.03 mg GAE/g extract) was determined in the ethanolic extract in *S. lanata* samples. Extracts with *n*-hexane were found to be weak in terms of phenolic compounds. It was determined that the total amount of phenol was also high in the ethanol extract of *S. lanata*, which has the highest antioxidant activity. These results revealed that there is a correlation between total phenol content and antioxidant activity.

Table 5. The total amount of phenol equivalent to Gallic acid contained in *S. lanata* extracts

SA	61.1±0.03mg GAE/g ekstre
SH	12.25±0.04 mg GAE/g ekstre
SEA	20.82±0.02 mg GAE/g ekstre

GAE: phenol amount equivalent to mg GAE/g extracted Gallic acid, SA: 70% ethanol extract of *S. lanata*, SH: n-hexane extract of *S.lanata*, SEA: Ethyl acetate extract of *S. lanata*.

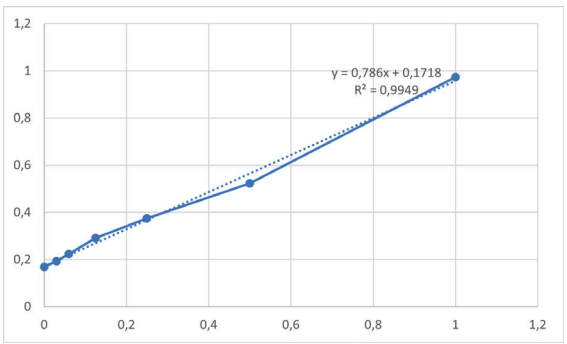

Figure 2. Gallic acid calibration curve

4. CONCLUSION

As far as we know when the literature was reviewed, the antioxidant effect of *S. lanata* has not been reported before. Our study, *S. lanata* with DPPH· scavenging activity method, can be considered the first report in terms of antioxidant activity. *S. lanata* extracts prepared with 70% ethanol were found to have higher DPPH· radical scavenging activity (0.241 mg/mL) than extracts prepared with other solvents, and according to the total phenolic method results, it was determined that the highest total phenol content was in the ethanolic (61.1±0.03 mg GAE/g) extract. The results confirmed a correlation between total phenol amount and antioxidant activity, as stated in many studies. In addition, in our study, the antibacterial effect was studied by selecting different strains from other antimicrobial activity experiments. It is very important to show the biological capacities of extracts. Therefore, in this study, *S. lanata* extracts were investigated for their advantages using various *in vitro* methods. With the successful demonstration of *in vivo* activity in future studies, a pharmaceutical product obtained from *S. lanata* will be safely functionalized.

Ethical approval

Not applicable, because this article does not contain any studies with human or animal subjects.

Author contribution

Concept: ZG, NS; Design: ZG; Supervision: YBK, Gİ; Materials: YBK; Data Collection and/or Processing: ZG, NS, Gİ, MK, YBK; Analysis and/or Interpretation: ZG, NS, Gİ, MK, YBK; Literature Search: ZG, NS; Writing: ZG, NS, Gİ, MK, YBK; Critical Reviews: Gİ, MK, YBK.

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Conflict of interest

The authors declared that there is no conflict of interest.

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