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Araştırma Makalesi - Research Article

Türkiyede Yetişen *Phedimus stoloniferus* Türünün Uçucu Kimyasal Bileşimi ve Biyoaktivite Değerlendirilmesi

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ÖZ

Bu araştırmada, Türkiye'de yetişen *Phedimus stoloniferus*'un (Crassulaceae) esansiyel yağında (EO), SPME ve n-hekzan özütünün SPME'sindeki uçucu organik bileşiklerin (VOC) GC-FID/MS ile analiz edilmiştir. *P. stoloniferus*'un EO'daki uçucu bileşiklerin ana kimyasal sınıfını karboksilik asitler oluşturur (%50.2). *P. stoloniferus*'un SPME ve heksan özütlerinin ve SPME'leri, ana bileşenlerinin aromatik bileşikler (sırasıyla %51.9 ve %65.9) olduğunu gösterdi. *P. stoloniferus*'tan elde edilen EO, SPME ve heksan özütlerinin SPME'sindeki ana bileşikler sırasıyla n-Heksadekanoik asit (%34.0), 1-metil-4-etilbenzen (%20.2) ve mesitilen (%28.8)'dir. *P. stoloniferus*'dan elde edilen esansiyel yağlarda ve çözücü (n-heksan, metanol ve su) özütlerinde antioksidan aktivite (CUPRAC yöntemi) ve tirozinaz inhibisyonları da incelenmiştir. *P. stoloniferus*'un metanol özütünde antioksidan aktivite en yüksek olarak (TEAC: 1.23± 0.04) tespit edilmişken, en yüksek tirosinaz enzimi inhibisyonu ise sulu özütte (IC_{50}: 0.168 ± 0.018) gözlendi.

Anahtar Kelimeler- Crassulaceae, Phedimus stoloniferus, SPME, GC-FID/MS, Antioksidan, Tirozinaz İnhibisyonu

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Bioactivity Evaluation and Volatile Chemical Composition of *Phedimus stoloniferus* Species Grown in Turkey

ABSTRACT

In this research, volatile organic compounds (VOCs) in the essential oil (EO), SPME and SPME of n-hexane extract of *Phedimus stoloniferus* (Crassulaceae) grown in Turkey were determined by GC-FID/MS. Major chemical class for the volatile compounds in the EO of *P. stoloniferus* is carboxylic acids (50.2%). SPME and SPME of the hexane extracts of *P. stoloniferus* revealed aromatic compounds (51.9% and 65.9%, respectively) as the main constituents. n-Hexadecanoic acid (34.0%), 1-methyl-4-ethylbenzene (20.2%), and mesitylene (28.8%) were the major compounds in the EO, SPME and SPME of hexane extract obtained from *P. stoloniferus*, respectively. Antioxidant activity (CUPRAC assay) and tyrosinase inhibitions of the essential oil and n-hexane, methanol, and water extracts obtained from *P. stoloniferus* were also studied. While the antioxidant activity was determined to be highest in methanol extract (TEAC: 1.23±0.04), tyrosinase enzyme inhibition was highest in the aqueous extract (IC_{50}: 0.168±0.018) of *P. stoloniferus*.

Keywords- Crassulaceae, Phedimus stoloniferus, SPME, GC-FID/MS, Antioxidant, Tyrosinase Inhibition

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I. INTRODUCTION

Sedum stoloniferum Gmelin (syn: *Phedimus stoloniferus* (Gmelin)'t Hart) is a species in the genus Sedum, in which 43 species are present in Turkey, belonging to the family the Crassulaceae (Stonecrop Family). *P. stoloniferum* is native to Turkey, the Caucasus and northern Iran [1-3]. Several sedum species including *Sedum album L.*, *S. pallidum* Bieb., *S. acre L.*, and *S. telephium L.*, are known for their use in Anatolian folk medicine for the treatment of diuretic problems and hemorrhoids [4-5]. Previous essential oil studies of *Sedum sediforme* (Jacq.) Pau [6], *Sedum pallidum* Bieb. var. *bithynicum* (Boiss.), *Sedum spurium* Bieb. [7] and *Sedum maximum* Farm. Vestn. [7] have been reported in the literature. The main compound in the essential oil of *S. sediforme* was reported to be α-selinene (20.4%) [6]. Thirty-eight and thirty-five components were mentioned in the essential oils of *S. pallidum* var. *bithynicum* and *S. spurium*, and the major compounds in these samples were found to be caryophyllene oxide (12.8%) and hexahydrofarnesyl acetone (15.7%), respectively [7]. In addition, strong antioxidant potential and antimicrobial activities in the essential oil and extracts of Sedum species have been mentioned [6, 9-12]. No information has been found in the literature on the essential oil composition, antioxidant activity (CUPRAC: cupric reducing antioxidant capacity), and tyrosinase inhibition activity of *P. stoloniferus*. In the first stage, VOC compositions in the EO, SPME and SPME of hexane extract of *P. stoloniferus* were determined by means of GC-FID/MS. Then, the essential oil and *n*-hexane, methanol, and water extracts of *P. stoloniferus* were evaluated for their tyrosinase inhibitory activities and antioxidant capacities.

II. MATERIAL AND METHOD

A. Plant Materials

The aerial parts of P. stoloniferus were collected from Uzungöl village of Trabzon-Turkey [UTM (European 50 DATUM) X: 615424, Y: 4490314; altitude: 1700 m] in September 2017. Prof. Dr. Salih Terzioğlu authenticated the plant [1-3], and the voucher specimen has been deposited at the herbarium of Faculty of Forestry, Karadeniz Technical University, Trabzon-Turkey. The plant material was cleaned and any accompanying compost was removed. After drying under shadow, the material was put in air-tight container and stored in the dark until use. The plant material was ground to powder form before use.

B. Essential Oil Isolation

Aerial parts of the plant were air-dried, and the powdered material (95 g) was subjected to hydrodistillation in a modified Clevenger-type system by utilizing a cooling bath system kept at -15 ºC for 3 h. The essential oil accumulated in Clevenger system was dissolved in n-hexane (0.5 mL, HPLC grade). The essential oil solution (14.5 mg w/w) was transferred into a sealed brown vial and stored at 4 ºC until analyses. One µL from the essential oil solution was introduced into GC-FID/MS injection port and analyzed for compositional profile.

C. Solvent (n-Hexane, Methanol and Water) Extracts Preparation

Air-dried and ground aerial parts of P. stoloniferus (5 g, each) were extracted with n-hexane, methanol and water which yielded the extracts of 0.057 g, 0.136 g, and 0.095 g, respectively.

D. SPME Analysis

The extractions of the volatile components were accomplished by using a manual SPME holder, polydimethylsiloxane/divinyl-benzene (PDMS/DVB) fiber (65 µm-blue hub plain), and 10 mL vials from Supelco (Bellefonte, USA). Air-dried ground plant material (1.2 g) and hexane extract (0.035 g) were placed in different vials (10 mL) which were then sealed with a silicone-rubber septum cap. The extractor fiber was inserted into an incubation chamber to achieve the incubation and extraction times and the temperature values based on the experimental plan. SPME extractions were performed at 50 ºC with a 5 min incubation time and a 10 min extraction time. The fibers with the adsorbed volatile compounds were then inserted into the GC-MS injection port, injection made with a 1:20 split ratio. The temperature program in the oven was the same as that used in GC-FID/MS method with a total run time of 62 min. Multiple analyses were done with each sample, and

the mean values for the compounds are reported. Conditioning time of 4 min was used for desorption following each extraction for subsequent assays. The temperature and times for incubation and extraction were adjusted according to the experimental protocol reported in the literature [13, 14].

E. Gas Chromatography-Mass Spectrometry (GC-FID/MS)

A Shimadzu QP2010 plus gas chromatography instrument with a Rtx-5MS capillary column (30 m x 0.25 mm, film thickness, 0.25 µm) was used for the gas chromatography-flame ionization detection (GC-FID) analyses. A flame ionization detector (FID) was used for compound quantitation, and a Shimadzu QP2010 Ultra mass selective detector (MS) was used for the qualitative analyses. The fiber with the extracted volatiles (SPME) was inserted into the GC-FID/MS injection port. Split mode with a split ratio of 1:20 was employed. The temperature program employed was as follows: initially the temperature was kept at 60 °C for 2 min, and it was increased to 240 °C with a 3 °C per min ramp, and the final temperature of 250 °C was held for 4 min, making the total analysis time as 62 min. The injector temperature was set at 280 °C, and mass transfer line temperature at 250 °C. Helium (99.999%) used as carrier gas kept at a constant flow-rate of 1 mL/min. Electron impact mode (EI) was used for the detection; ionization voltage was adjusted to 70 eV, and scan mode in 40-450 m/z range was used for mass acquisition [15-16]. The essential oil components are listed in Table 1 with retention indices and percentage data, and the compound class percentages are provided in Table 2.

F. Compound Identification

Retention indices of the volatile components were calculated by utilising Kovats method, and n-alkanes mix (C6-C32) was used as standards. The compound identification was done by comparing the retention indices and mass spectra of the compounds in samples with those of the standards previously analyzed and those kept in the mass spectral libraries (FFNSC1.2, W9N11, and NIST). The compounds were also confirmed by comparison with the literature data [13-18].

G. Antioxidant Activity (Cupric Ion Reducing Antioxidant Capacity Assay, CUPRAC)

In cupric ion reducing antioxidant capacity (CUPRAC) assay, 1 mL of 10 mM Cu (II) Cl, 1 mL of 7.5 mM neocuproine, 1 mL of 1 M NH_3COOCH_3 buffer of pH 7 and 20 µL of sample solution were mixed in test tubes. The final volume in the test tubes was adjusted to 4.1 mL by adding distilled water. After an incubation period of 45 min at room temperature, the absorbances were recorded at 450 nm against a blank containing sample solvent [19]. The CUPRAC values of samples were expressed as milligrams of Trolox per 1 mg extract. A standard curve of Trolox® (Sigma Chemical Co, USA) as the standard antioxidant compound was constructed testing it under the same conditions. The standard graph was linear in 0.125 - 8 mg/mL concentration range of Trolox (r^2 =0.998). CUPRAC activities were expressed as mg Trolox equivalent of 1 mg of the samples.

H. Tyrosinase Inhibition Activity

The tyrosinase inhibitory activities of the extracts were examined using the method described in the literature [20]. Different concentrations of the samples were prepared in 50 mM phosphate buffer of pH 7.0 from the stock sample solutions. The extracts at different concentrations, 20 µL of 250 U/mL tyrosinase and 800 µL of 50 mM pH 7.0 phosphate buffer solutions were mixed in a 96-well microplate. Following a pre-incubation period of 10 min at room temperature, 100 µL of L-DOPA solution was added onto each well, and the microplate was further incubated at room temperature for 10 min. Subsequently the absorbance caused by dopachrome at 492 nm was measured using a microplate reader. α-Kojic acid, widely used in tyrosinase inhibition studies, was tested as reference. The IC_{50} values, which are the concentrations of the inhibitors that result in 50% inhibition of the enzyme activity under the assay conditions, were calculated and used to show the extend of the inhibitory activity. Lower IC_{50} values show better tyrosinase inhibitory potential. The measurements were done in triplicates, and the results are expressed as the mean ± standard deviation (SD) (Table 3).

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III. RESULTS AND DISCUSSION

In the present work, the volatile components of the essential oil, SPME and SPME of *n*-hexane extract of the *P. stoloniferus* were determined by GC-FID-MS with using a Rxi-5MS column. Identification of the VOCs was made by a typical library search (FFNSC1.2 and W9N11) with mass data and literature comparison [13-18]. The general chemical profile of the VOCs, the percentage composition, and the constituent retention indices are given in Table 1. In total, 32, 21, and 35 compounds were identified and represented to an average of 97.0- 98.8% of the EOs, SPME and SPME of hexane extracts in *P. stoloniferus*, respectively. The data from the present study demonstrated that the component of the EOs, SPME and SPME of hexane extracts varied significantly with elevation as seen in Table 1 due to the different techniques used.

Table 1. Identified volatile organic compounds from *P. stoloniferus* growing in Turkey.

Compounds	**RI[a]**	**RI[b]**	**RT**	**A1 (%)[c]**	**A2 (%)[c]**	**A3 (%)[c]**
Toluene	782	781	7.956	-	-	1.3
Octane	800	802	8.580	-	-	0.1
n-Hexanal	803	802	8.582	5.0	10.0	-
Butyl acetate	812	814	8.986	-	-	0.1
1-Hexanol	863	861	10.588	-	3.9	-
Ethyl benzene	871	871	10.589	-	-	0.5
p-Xylene	878	877	10.770	-	5.8	3.4
2-Heptanone	889	894	11.360	-	0.7	-
Cyclohexazanone	903	903	11.685	-	8.4	-
Pimelic ketone	903	903	11.705	-	-	20.2
n-Heptanal	906	904	11.724	0.9	-	-
Butyrolactone	915	920	12.370	-	1.1	-
Methyl caproate	925	926	12.613	-	-	0.4
Cumene	929	930	12.796	-	-	0.1
4-Methyl nonane	959	954	12.952	-	-	0.1
n-Propyl benzene	960	957	13.849	-	2.1	2.1
Benzaldehyde	960	964	14.179	4.1	-	-
1-Methyl-4-ethylbenzene	963	964	14.196	-	20.2	13.5
1-Methyl-3-ethylbenzene	968	970	14.425	-	1.2	5.0
Octene-3-ol	979	981	14.838	0.6		-
1-Ethyl-2-methylbenzene	979	980	14.935	-	3.9	3.9
6-Methyl-5-hepten-3-one	981	985	15.057	-	6.6	-
2-Pentyl furan	993	991	15.281	0.9	-	-
Mesitylene	996	996	15.503	-	15.9	28.8
Octanal	1003	1001	15.701	0.4	-	-
p-Cymene	1020	1020	16.495	0.3	-	0.4
1,2,3-Trimethylbenzene	1035	1035	16.735	-	2.8	-
m-Cymene	1027	1028	16.780	-	-	5.4
Limonene	1031	1031	16.985	-	-	0.2
Indane	1041	1040	17.391	-	-	0.8
Benzene acetaldehyde	1036	1036	17.589	0.6	1.1	-
1-Ethyl-3-propylylbenzene	1053	1052	17.910	-	-	1.2
1-Ethyl-3,5-dimethylbenzene	1058	1059	18.213	-	-	0.8
4-Ethyl-1,2-dimethylbenzene	1077	1078	19.040	-	-	0.8
1-Ethyl-2,4-dimethylbenzene	1083	1080	19.117	-	-	0.7
2-Ethyl-1,4-dimethylbenzene	1085	1086	19.395	-	-	1.1
n-Nonanal	1101	1101	20.016	0.9	-	-
6-Metil-3,5-heptadien-2-one	1103	1103	20.121	-	1.0	-
1-Ethyl-2,3-dimethylbenzene	1113	1108	20.332	-	-	0.2
1,2,4,5-Tetramethylbenzene	1131	1132	20.929	-	-	0.7
1,2,3,4-Tetramethylbenzene	1159	1154	22.334	-	-	0.4
Benzyl acetate	1178	1173	22.727	-	-	1.9
(2*E*)-Nonenal	1157	1157	22.431	0.6	-	-
1-(4-Methylphenyl)ethanone	1182	1184	23.193	-	7.8	-
Naphthalene	1181	1179	23.839	-	-	1.4
α-Terpineol	1191	1192	23.955	1.9	-	-
Dodecane	1200	1203	24.019	-	1.2	0.4
Methyl nonanoate	1227	1229	25.103	-	-	0.3

Eucarvone	1223	1224	25.291	10.0	1.3	-
Carvone	1246	1246	26.220	0.5	-	-
Nonanoic acid	1267	1265	27.035	1.3	-	-
Tridecane	1300	1303	28.197	0.6	-	0.1
o-Methoxy acetophenone	1298	1304	28.670	-	1.2	-
Tetradecane	1400	1403	32.212	0.9	2.4	1.2
Geranyl acetone	1453	1449	34.378	1.3	-	-
trans-*β*-Ionone	1487	1487	35.857	0.4	-	-
Spathulenol	1577	1575	39.441	0.6	-	-
Hexadecane	1600	1601	39.661	-	0.2	0.4
Lauryl acetate	1610	1612	40.035	-	-	0.1
α-Bisabolol	1685	1685	42.944	0.6	-	-
Tetradecanoic acid	1763	1762	45.431	12.2	-	-
Hexahydrofarnesyl acetone	1837	1839	47.995	3.4	-	-
(5*E*,6*E*)-Farnesyl acetone	1913	1914	50.399	0.6	-	-
Methyl palmitate	1926	1928	50.496	1.1	-	0.3
Hexadecanoic acid	1966	1966	51.963	34.0	-	-
Ethyl palmitate	1990	1986	52.570	2.7	-	-
Methyl Linoleate	2101	2105	55.781	2.8	-	-
Phytol	2110	2111	56.235	2.6	-	-
Linoleic acid	2131	2130	56.749	0.2	-	-
Oleic acid	2152	2155	57.462	2.5	-	-
Phytol acetate	2223	2221	59.001	0.4	-	-
Tetracosane	2300	2298	61.082	2.1	-	-
		Total		97.0	98.8	98.5

[a]Retention index of references,
[b]Retention index calculated by using retention times of n-alkane (C_6-C_{32}) series,
[c]Percentages obtained by area normalization of the peaks from FID chromatograms,
A1: EO of *P. stoloniferus*,
A2: SPME of *P. stoloniferus*,
A3: SPME for the *n*-hexane extract of *P. stoloniferus*.

The major components of the essential oil obtained by hydrodistillation were hexadecanoic acid (34.0%), tetradecanoic acid (12.2%), eucarvone (10.0%), *n*-hexanal (5.0%), hexahydrofarnesyl acetone (3.4%), methyl linoleate (2.8%), ethyl palmitate (2.7%), and phytol (2.6%). 1-Methyl-4-ethylbenzene (20.2%), mesitylene (15.9%), *n*-hexanal (10.0%), cyclohexanone (8.4%), 1-(4-methylphenyl)-etanone (7.8%), and *p*-xylene (5.8%) were the major components in the SPME, and mesitylene (28.8%), pimelic ketone (20.2%), 1-methyl-4-ethylbenzene (13.5%), 1-methyl-3-ethylbenzene (5.0%), *p*-cymene (5.4%), 1-ethyl-2-methylbenzene (3.9%), and *p*-xylene (3.4%) in the SPME of *n*-hexane extract obtained from *P. stoloniferus*. The chemical classes distributions of the volatiles of *P. stoloniferus* are summarized in Table 2. The volatile compounds were classified into thirteen groups: monoterpenes, monoterpenoids, sesquiterpenoids, diterpenoids, terpene related compounds, aromatic hydrocarbons, aliphatic hydrocarbons, ketones, aldehydes, alcohols, acids, esters, and others (Table 2).

Table 2. The chemical class distribution of *P. stoloniferus* volatiles.

Classification	**A1**		**A2**		**A3**	
	%[a]	**NC[b]**	**%[a]**	**NC[b]**	**%[a]**	**NC[b]**
Monoterpene hydrocarbons	0.3	1	-	-	6	3
Monoterpenoids	12.4	3	1.3	1	-	-
Sesquterpenoids	1.2	2	-	-	-	-
Diterpenoids	2.6	1	-	-	-	-
Terpene related compounds	5.7	4	-	-	-	-
Aromatics hydrocarbons	0.9	1	51.9	7	65.9	18
Aliphatic hydrocarbons	3.6	3	5.0	4	2.3	6
Ketone	-	-	24.5	5	20.2	1
Aldeyhde	12.5	7	11.1	2	-	-
Alcohol	0.6	1	3.9	1	-	-
Acids	50.2	5	-	-		
Esters	7.0	4	1.1	1	3.1	6
Other	-	-	-	-	0.8	1
Total	97.0	32	98.8	21	98.3	35

[a] Percentage area of compounds,
[b] NC: Number of compounds,
A1: EO of *P. stoloniferus*,
A2: SPME of *P. stoloniferus*,
A3: SPME for the *n*-hexane extract of *P. stoloniferus.*

The number of the identified terpene/terpenoid type compounds in the essential oil, SPME and SPME of hexane extract from the aerial parts of *P. stoloniferus* were 7, 1, and 3, respectively. The species of this genus previously reported to have high percentage of terpene/terpenoids are *S. sediforme* [6], *S. pallidum* var. *bithynicum* and *S. spurium* [7]. Carboxylic acid in the EO (50.2%) and aromatic hydrocarbons in the SPME and SPME (51.9% and 65.9%, respectively) of *n*-hexane extract of *P. stoloniferus* were predominant.

When the present data for volatile constituents (Table 1) are compared with those reported previously in literature [6-8], different compositional profile in each Sedum species appears to exist. There are several reports about the major essential oil components in the literature. *S. sediforme* has been reported to have α-selinene (20.4%), 2,5-di-tert-octyl-p-benzoquinone (13.1%), valencene (6.3%), and carvone oxide (4.3%) [6]. The major components of *S. pallidum* var. *bithynicum* have been reported as caryophyllene oxide (12.8%), *n*-nonanal (9.4%), α-bisabolol (6.8%), β sesquiphellandrene (4.5%), and β-bisabolene (3.7%) [7]. In another study, hexahydrofarnesyl acetone (15.7%), (*Z*)-phytol (10.2%), δ-cadinene (4.7%), *allo*-aromadendrene (3.8%), and geranyl acetone (3.6%) have been identified as the major volatile components of *S. spurium* [7]. Volatile chemical variations were also observed between the results obtained with hydrodistillation, SPME and SPME of hexane extract samples, which is probably related to the nature of the method used.

The extracts were tested for antioxidant activity by utilizing widely used CUPRAC method and the results were expressed in terms of Trolox equivalence (Table 3). The methanol extract showed the highest antioxidant potential with TEAC value of 1.23, which is generally observed with most plant extracts. Essential oil did not show any antioxidant activity. In determination of antioxidant activity of *Sedum sediforme* by CUPRAC method, Ertaş et al. found the methanol and aqueous extracts to show antioxidant activities approximately 1.3 and 2.0 times that of a-tocopherol, which is close to the current findings for *S. stoloniferus* [6].

The extracts were also investigated for their tyrosinase inhibitory activity, and the results of the measurements were expressed as IC_{50}, with which the lower value shows higher activity (Table 3). Water extract showed much higher activity with 0.168 μg/mL IC_{50} value in this test. There are example studies for the members of the Crassulaceae family in which tyrosinase inhibitory activities have been investigated. Chen et al. Prepared ethanol extracts of fresh and fermented *Rhodiola rosea* and *Lonicera japonica* species and found the IC50 values in the order of 1-10 mg/mL [23], which appears to be much higher concentration around four orders of magnitude higher. The current findings shows further investigation into the determination of active antiyrosinase compounds should be done.

Table 3. Antioxidant and tyrosinase inhibitory activities of the EO and solvent extracts obtained from *P. stoloniferus.*

Extracts	**Antioxidant $TEAC_{CUPRAC}$***	**Tyrosinase Inhibition IC_{50}, μg/mL**
Water	1.19±0.10	0.168±0.018
Methanol	1.23±0.04	0.216±0.023
n-Hexane	0.75±0.03	nd
Essential oil	nd	nd
Kojic acid		3.1±0.2

n.d.: not detectable.
* Trolox was used as standard for the calculation of TEAC (Trolox equivalent antioxidant capacity, mg Trolox per mg sample) values.) values.

IV. CONCLUSIONS

All of the extracts (n-hexane, methanol and water) obtained from *P. stoloniferus* exhibited strong antioxidant activity (Table 3) except the EO. Furthermore, the methanol and water extracts of *P. stoloniferus* gave higher tyrosinase enzyme inhibitions with IC_{50} value as 0.216 ± 0.023 µg/mL, and 0.168 ± 0.018 µg/mL, respectively (Table 3). The literature data on the other *Sedum* species show that terpene/terpenoid compounds [6-7] are major components in the EOs, which is not consistent with the result we obtained. Fatty acids were found to be abundant in the essential oil of *P. stoloniferus*. The comparative evaluation of the chemical profiles of the volatiles of *Sedum* species shows that significant differences exist in their volatile chemical components, probably not only because of species differences, but also differences in growth conditions and plant collection times and localities.

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