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RESEARCH PAPER

Cytotoxicity, Radical Scavenging, Antioxidant Properties and Chemical Composition of the Essential Oil of *Satureja cilicica* P.H. Davis from Turkey

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ABSTRACT

Satureja cilicica P.H. Davis is an endemic species of Lamiaceae, distributed in Turkey. In this study, the essential oil of *S. cilicica* was obtained by hydrodistillation from the aerial parts. The essential oil was analyzed by GC/MS and the main constituents are identified. Radical scavenging capacities of oil obtained from *S. cilicica* were determined by using DPPH and ABTS radical scavenging assays. In addition, ferric-reducing power of the essential oil was detected. Cytotoxic activity of the essential oil was determined by MTT assay after 24 h treatment of MCF-7. The major components of the essential oil are *p*-cymene (17.68%), carvacrol (14.02%), γ-terpinene (11.23 %) and thymol (8.76%). Radical scavenging capacities of the essential oil were measured as 3.28±0.02 and 238.15±3.59 mg Trolox equivalent per milliliter of oil in term of DPPH and ABTS radical scavenging, respectively. Ferric-reducing power of essential oil was found 39.76±3.66 mg Trolox equivalent per milliliter of essential oil. Growth inhibition effect of essential oil against MCF-7 cancer cell is established. According to the results, essential oil of *S. cilicica* exhibit low cytotoxic activity, with IC_{50} values, with IC_{50} value of 268 μg/mL. The findings of the analyzed species are compared with the results of previous studies.

Keywords: ABTS, cytotoxicity, DPPH, essential oil composition, thyme

INTRODUCTION

Herbs may be the alternative main source of drugs and folk medicines. Many natural compounds, a wide spectrum of biological activities, were isolated from herbs. Especially, the active metabolites of essential oils extracted from herbs are useful in treating various diseases with no or less toxicity (1). Moreover, essential oils are proven to the great importance in several fields such as food pharmacology and pharmaceutics (2).

Satureja L. is an aromatic and medicinal genus of Lamiaceae, including about 38 species in the Mediterranean region, North Africa, Morocco and Libya (3). The genus is represented in Turkey by 40 species (42 taxa), of which 18 are endemic in Turkey (4).

The genus *Satureja* is being used worldwide as herbal beverages, spices, food additives and flavoring, perfume and cosmetic industries. Traditionally, in folk medicine to treat various ailments, such as cramps, muscle pains, nausea, indigestion, diarrhea and infectious diseases (5-6).

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Satureja species are usually subject of antibacterial, antifungal, antioxidant, cytotoxicity, antidiabetes, anti-HIV, anti-hyperlipidemic, reproduction stimulatory, expectorant and vasodilatory activity studies (5-8).

Herbal metabolites have been used as medicinal purposes for a long time and added in the food to prevent the undesirable oxidation products. In addition, the researchers conducted on the opportunity of antioxidant and cytotoxic agent of herbs essential oils in the last years. Therefore, nowadays, a large amount of studies, metabolites of herb and various natural products remain to be explored in relation to their biological potential.

The essential oil composition and antioxidant activity such as BHT by DPPH of *Satureja cilicica* P.H. Davis were given in previous studies (5, 9-10). It was indicate that *S. cilicica* essential oil has natural antioxidant agent for butters (5). Carvacrol and thymol, which are the main components of the essential oil of *S. cilicica*, were shown antimicrobial activities (12). Therefore, it was aimed to determine the essential oil composition, radical scavenging by Ferric-reducing power and antioxidant properties by using DPPH and ABTS radical scavenging assays and cytotoxic activity of the essential oil of *S. cilicica* in this study.

MATERIALS AND METHODS

Plant material and isolation of the oil

The aerial parts of *Satureja cilicica* P.H. Davis (Lamiaceae) (Local name: Kınalı Kekik) were collected at flowering stage, on September 2014, from Osmaniye province, in Turkey. The specimens were air-dried at room temperature. The essential oil was obtained from the aerial parts by hydrodistillation for 3 h with Clevenger-type apparatus, according to the Turkish Pharmacopeia. Voucher specimen (No: Arabacı 2947) of species is identified by the first author according to the Flora of Turkey and deposited in the herbarium of the Faculty of Pharmacy, İnönü University, Malatya, Turkey (11).

Essential oil analysis

GC/MS analyses of the essential oil were made by using an Agilent Technologies 6890N Network system. The MS transfer line temperature was set at 250°C. The oven temperature was linearly raised from 60 to 250 °C at a rate of 5 °C/min., then kept constant at 250°C for 20 min. Helium was used as the carrier gas at a flow rate of 1.7 mL/min. GC/MS analyses were carried out under the same conditions (oven, temperature, flow rate of the carrier gas) with GC by Agilent Technologies 6890N Network system gas chromatograph equipped with an Agilent Technologies 5973 inert Mass Selective Detector (Agilent G3180B Two-Ways Splitters with Makeup gas) in the electron impact mode (70eV). The mass range was between *m/z* 10 and 550. The column, HPInnowax column, 60 m x 0.25 mm i.d., 0.25 µm film thickness was used. The injection was performed as described above. Injection was carried out by automatic mode (split ratio 30:1). Nist08Wiley8L, Nist05aL and Flavor2L were used for library search. Relative indexes were calculated according to series of linear alkanes C8-C23 used as a reference. Retention index from literature were given in Table 1 (12-13).

Radical scavenging capacities of essential oil of *Satureja cilicica*

DPPH radical scavenging activity

DPPH (1,1-diphenyl-2-picrylhydrazyl) studies were based on the method given by Brand-Williams *et al.* with some modifications (14). DPPH radical was prepared in ethanol. The absorbance of this solution was adjusted to 0.700 ± 0.005 by dilution with ethanol at 520 nm. Fifty microliters of sample solutions (diluted with ethanol by 1:1000; v:v) were mixed sufficiently with 2.45 mL DPPH radical final concentration solution and 30 min. waited in a dark space. The absorbances of the ultimate solution were measured against ethanol. Trolox equivalent radical scavenging capacity of essential oil was determined. The results are expressed as mean values±standard deviations.

ABTS radical scavenging activity

$ABTS^{\bullet+}$ (2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) radical assay was determined on the base of the method followed by Re *et al.* (15). A 30 mg $ABTS^{\bullet+}$ were dissolved in 7.8 mL of 2.46 mM potassium peroxydisulfate for preparing the $ABTS^{\bullet+}$ stock solution. The stock solution incubated 12–16 h. at room temperature and then, diluted with ethanol to give 0.700 ± 0.005 absorbance at 734 nm. A 100 µL of diluted (as in DPPH method) sample was mixed with 2.4 mL of $ABTS^{\bullet+}$ solution. After incubation 5 min. at room temperature, the absorbance was measured. The results are expressed as mean values±standard deviations and Trolox equivalent/mL essential oil.

Reducing power of essential oil was given following the method of Oyaizu (16). 2.5 mL 0.2 M phosphate buffer (pH

6.6) and 2.5 mL 1% potassium ferricyanide were added to 1 mL solution of essential oil and mixed gently. The mixtures were hold on a water bath at 50 °C for 20 min. for incubation. Reaction was finished by adding 2.5 mL 10% trichloroacetic acid (TCA). Then, the mixtures were centrifuged at 6,000 rpm for 10 min. 2.5 mL of mixtures were taken from the top layer and transferred into tubes that containing 2.5 mL distilled water and 0.5 mL 0.1 % ferric chloride ($FeCl_3.6H_2O$). The resultants were mixed 5 min, then, the color intensity read at 700 nm against blank. The results are expressed as mean values±standard deviations and Trolox equivalent/mL essential oil.

Table 1. Chemical composition, retention indices (RI[a]) and compound percentage (%) of the essential oil of *Satureja cilicica*.

Compound	RI[a]	RI[b]	Relative percent %
Tricyclene	1002	1014	0.11
α-Pinene	1014	1032	2.92
Camphene	1049	1065	1.99
β-Pinene	1095	1103	0.30
Myrcene	1170	1170	1.43
α-Terpinene	1193	1180	2.46
Limonene	1218	1197	0.41
1,8-Cineole	1230	1213	1.18
γ-Terpinene	**1286**	**1248**	**11.23**
p-Cymene	**1311**	**1274**	**17.68**
Alloocimene	1406	1382	0.09
3-Octanol	1418	1393	0.11
1-Octen-3-ol	1465	1452	1.19
α-Copaene	1529	1497	0.64
Linalool	1545	1553	2.30
Thymol methyl ether	1584	1604	0.31
β-Caryophyllene	1607	1612	1.85
Aromadendrene	1615	1628	1.13
cis-Isodihydrocarvone	1647	1645	0.43
Isoborneol	1664	1664	4.51
γ-Muurolene	1674	1676	0.46
Valencene	1681	1740	0.69
β-Bisabolene	1707	1741	5.21
δ-Cadinene	1736	1772	1.13
Cuminaldehyde	1745	1802	0.64
p-Cymen-8-ol	1804	1864	0.53
Caryophyllene oxide	2074	2008	1.21
Cuminyl alcohol	2259	2113	0.50
Spathulenol	2315	2144	1.42
Aromadendrene oxide 2	2360	-	0.06
Thymol	**2374**	**2205**	**8.76**
Carvacrol	**2407**	**2246**	**14.02**
Cadalene	2417	2256	0.64
Total identified			**87.56**
		Grouped components(%)	
		Monoterpene hydrocarbons	38.62
		Oxygenated monoterpenes	32.87
		Sesquiterpene hydrocarbons	11.75
		Oxygenated sesquiterpenes	2.69
		Others	1.61

[a] retention index on a HP Innowax column
[b] retention index from literature (12-13)

MTT Assay

MCF-7 (breast cancer cells) was maintained in 25 mL plastic flasks in RPMI 1640 supplemented with 10% fetal bovine serum, 1% penicillin–streptomycin. Cells were kept incubated in CO_2 incubator at 37 °C, under a humidified atmosphere of 5% CO_2. The medium was replaced three times a week. For enumeration, 100 µL of cells concentration were stained with trypan blue (0.4%) and cells were counted using a haemocytometer.

MCF-7 ($5x10^3$ cells) was seeded in each well of a 96 well plate, using 100 µL culture media. They were allowed to attach for 24 h. in CO_2 incubator. Media was aspirated and adherent cells exposed to medium containing varying concentrations of essential oil (100, 200, 400, and 1000 µg/mL) for 24 h. After exposure, 10 µL of the solubilized MTT (12 mM MTT was prepared by adding 1 mL of sterile PBS to one 5 mg vial of MTT) was added to each well, and the cultures were incubated for an additional 4 h. Media was carefully discarded. For solubilization of formazan crystals (MTT formazan), 100 µL of DMSO (dimethylsulfoxide) was added to each well. A microplate reader was used to determine the absorbance of each well at 540 nm. The growths of the treated and untreated cells were compared and calculated using the formula: (absorbance treated wells/absorbance untreated wells) ×100.

RESULTS AND DISCUSSIONS

Chemical composition of the essential oil and compound relative percentages of *Satureja cilicica*, an endemic species of Turkey, are given in Table 1. The yield of the content of essential oil was calculated as 0.69% (v/w) on per weight of the dry plant material. The numbers of the identified compounds are 33 and representing 87.54% of the oil. *p*-cymene (17.68%), carvacrol (14.02%), γ-terpinene (11.23 %) and thymol (8.76%) are identified as the major components of the essential oil in this study. The main components of the essential oil of *S. cilicica* were found as thymol (22.76%), carvacrol (18.90%), p-cymene (19.52%) and c-terpinene (13.40%) in previous study (9). In another study made on *S. cilicica*, carvacrol (37.58%), *p*-cymene (14.38%), γ-terpinene (13.38%) and thymol (7.41%) reported as the main compounds (10). In our study, *p*-cymene (17.68%) is most abundance, while carvacrol is represented with 14.02%. These differences may be due to several reasons such as genetic diversity, climatic conditions, and ecological differences (17).

The radical scavenging capacity of plants could be correlated with antioxidant properties (18-19). The DPPH scavenging assay is a feasible method for the evaluation of the antioxidant activity of a compound (20). In this assay, Trolox was used as a standard for comparison with the DPPH scavenging activities of the essential oil. As shown in Table 2,DPPH and ABTS radical scavenging capacities of the essential oil were measured as 3.28±0.02 and 238.15±3.59 mg Trolox equivalent per milliliter of essential oil, respectively. Ferric-reducing power of essential oil was found 39.76±3.66 mg Trolox equivalent per milliliter of essential oil. The antioxidant capacity of the essential oil of *S. cilicica* from Germany was found as 101.16±3.32 µg ascorbic acid equivalent per milliliter of essential oil by phosphomolybdenum method and IC_{50}= 32.02±0.58 µg/mL by DPPH scavenging method (5). According to the results, the ABTS radical scavenging and reducing power of the essential oil appeared to be better than those of the DPPH scavenging activity. Generally, radical scavenging power of the plant essential oils was measured more high in ABTS method compare with DPPH (21). The higher activity could be attributed to the higher specificity of the assay for hydrophilic compounds.

Cytotoxic activity of essential oil of *S. cilicica* was determined by MTT assay after 24 h treatment of MCF-7. Results are shown in Figure 1 as % inhibition. In additon, IC_{50} value of essential oil was determined from obtained data (Figure 1). The essential oil of *S. intermedia* showed great potent for antimicrobial and cytotoxic activities. Thymol (34.5%), γ-terpinene (18.2%) and *ρ*-cymene (10.5%) were the main components of the essential oil (7). The essential oil of *S. montana* ssp. *pisidica* demonstrated significantly better

Table 2. Antioxidant properties of the essential oil of *Satureja cilicica.*

Antioxidant test	DPPH (mg trolox equivalent/mL EO)	ABTS (mg trolox equivalent/mL EO)	Reducing Power (mg trolox equivalent/mL EO)
EO of *Satureja cilicica*	3.28±0.02	238.15±3.59	39.76±3.66

Each value is the average of triplicate experiments with standard deviation.

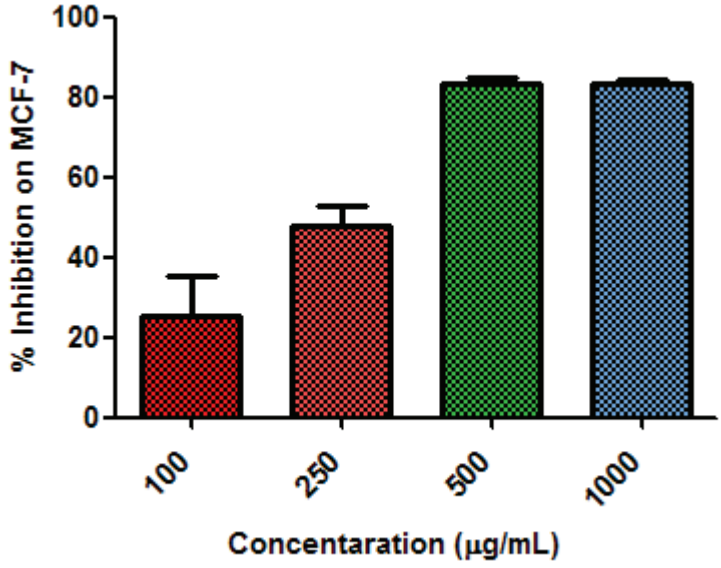

Figure 1. Growth inhibition effect of *S. cilicica* essential oil against MCF-7 cancer cell lines.

results against HeLa and MDA-MB-453 cell lines. The major compounds of this oil were carvacrol (37.6%), thymol (24.5%), carvacrol methyl ether (11.8%) and ß-linalool (15.2%) (8). Essential oil of *S. cilicica* exhibit low cytotoxic activity, with IC_{50} values, 268 µg/mL. The IC_{50} value of doxorubicin, a cancer drug in breast cancer, was measured at 0.056 µM against the MCF-7 cell line (22). As far as our knowledge, this is the first radical scavenging by Ferric-reducing power, antioxidant properties by using ABTS radical scavenging assays and cytotoxic activity studies on the essential oil of *S. cilicica*.

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Türkiye'de Yetişen *Satureja cilicica* P.H. Davis Türünün Uçucu Yağının Sitotoksisitesi, Antioksidan Aktivitesi ve Kimyasal Kompozisyonu

ÖZ

Satureja cilicica P.H. Davis Lamiaceae familyasının Türkiye'de yetişen endemik bir türüdür. Bu çalışmada, *S. cilicica*'nın uçucu yağı bitkinin toprak üstü kısımlarından su distilasyonu yöntemi ile elde edilmiştir. Uçucu yağ GC/MS ile analiz edilmiş ve ana bileşenleri belirlenmiştir. *S. cilicica*'dan elde edilen yağın radikal süpürme kapasiteleri DPPH radikali ve ABTS radikali süpürme yöntemi ile belirlenmiştir. Ayrıca uçucu yağın ferrik indirgeme gücü tespit edilmiştir. Uçucu yağın sitotoksik aktivitesi 24 saat MCF-7 ile muamele edildikten sonra MTT yöntemi ile belirlenmiştir. *p*-Simen (%17.68), karvakrol %(14.02), γ-terpinen (%11.23) ve timol (%8.76) uçucu yağın ana bileşenleri olarak saptanmıştır. Uçucu yağın radikal süpürme kapasiteleri sırasıyla DPPH ve ABTS radikal süpürme yöntemi ile 3,28±0,02 ve 238,15±3,59 mg eşdeğer trolox/mL uçucu yağ olarak ölçülmüştür. Uçucu yağın ferrik indirgeme gücü, 39,76 ± 3,66 mg eşdeğer trolox/mL uçucu yağ olarak bulunmuştur. Uçucu yağın MCF-7 kanser hücresine karşı büyüme inhibisyon etkisi araştırılmıştır. Sonuç olarak, *S. cilicica*'nın uçucu yağının 268 µg/mL IC_{50} değeri ile düşük sitotoksik aktiviteye sahip olduğu görülmüştür. Analiz edilen türden elde edilen veriler önceki çalışmalar ile karşılaştırılmıştır.

Anahtar kelimeler: ABTS, DPPH, kekik, sitotoksisite, uçucu yağ kompozisyonu

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