ORIGINAL ARTICLE

A C G publications

Rec. Agric. Food. Chem. 5:1 (2025) 34-41

records of agricultural and food chemistry

Antioxidant Activity of *Satureja aintabensis* P.H. Davis

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(Received June 17, 2025; Revised June 28, 2025; Accepted June 28, 2025)

Abstract: In this study, the antioxidant activity of the essential oil, methanol and chloroform extracts of *Satureja aintabensis* P.H. Davis, an endemic Turkish species traditionally consumed as tea and spice, was investigated for the first time using CUPRAC (cupric (Cu^{2+}) ion reduction) and DPPH (1,1-diphenyl-2-picrylhydrazyl free radical scavenging) assays. The aerial parts of the plant were extracted with methanol and chloroform and the essential oil was obtained by the hydrodistillation method using Clevenger apparatus. The methanol extract and essential oil exhibited comparable CUPRAC activity (0.55 ± 0.04 and 0.54 ± 0.03, respectively), while the methanol extract demonstrated the highest DPPH scavenging (13.0–82.0%), suggesting its dominance in neutralizing hydrophilic radicals. This result shows that S. aintabensis contains both hydrophilic and lipophilic antioxidants.
Keywords: *Satureja aintabensis P.H. Davis*; antioxidant; DPPH; CUPRAC.

1. Introduction

An essential structural component of living metabolism, oxygen is required to meet the energy requirements of biological tissues. It is utilized in the process of enzymatic bio catalysis in cells and tissues and has the ability to transfer electrons between atoms. Consequently, electron transfer can yield

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The article was published by ACG Publications
https://www.acgpubs.org/journal/records-of-agricultural-and-food-chemistry January-June 2025 EISSN:2792-0763
DOI: http://doi.org/10.25135/rfac.18.2506.3568
Available online: June 19, 2025

the formation of highly unstable and reactive free radicals, characterized by the absence of an electron. Reactive oxygen species (ROS) are highly reactive molecules produced during aerobic metabolism, mainly involving oxygen. The resulting reactive oxygen species are stabilized by antioxidant systems. The balance between antioxidants and ROS is crucial for biological systems. When there is a significant imbalance between the production of ROS and antioxidant levels, the condition known as oxidative stress (OS) arises and the excess ROS damages cells. Antioxidants are compounds that scavenge free radicals and lower oxidative stress, which can delay, slow, or even stop oxidation. [1-10]. These can be either natural or synthetic. Given the significant potential for adverse effects and toxicity associated with synthetic forms, the use of natural antioxidants has become increasingly relevant. Natural antioxidant defense systems consist primarily of phytochemical compounds that are ingested through dietary intake [11,12]. Medicinal and aromatic plants represent a distinctive source of antioxidants. Secondary metabolites, including polyphenols, terpenoids and vitamins, which are found in plants, possess significant antioxidant properties. Consequently, the antioxidant properties of medicinal and aromatic plants, attributable to their chemical constituents, are gaining prominence as a novel therapeutic modality for a variety of ailments, including cancer [12-14].

The Lamiaceae (Labiatae) family, which is represented by approximately 240 genera and 7200 species worldwide and 45 genera and 614 species in Türkiye, holds significant importance in both folk medicine and the pharmaceutical industry due to its therapeutic properties, which are attributed to the secondary metabolites it contains. It is also one of the plant families that are highly valuable as food due to their aromatic properties [15-17]. With an endemism rate of 44%, the Lamiaceae family is the third largest family in Türkiye in terms of the number of taxa and the fourth largest family in terms of the number of species. Many species of the genera, such as *Origanum* L., *Satureja* L., *Salvia* L., *Sideritis* L. and *Thymus* L., which can be collected from the environment where they grow in nature or from cultivated areas, are found on tables in Türkiye, especially among the local people, both as spices and herbal teas [15-19]. Recent studies have showed that the most of the members of the Lamiaceae family have high levels of biological activity, such as antioxidant, antifungal, anti-cholinesterase, antimicrobial, anticancer, cytotoxic, insecticidal activity [20-25]. One of the most economically important members of the Lamiaceae family is the genus *Satureja* [8,13,14].

Satureja species, like *Origanum* spp. and *Thymus* spp., are called thyme because of their essential oils with high thymol/carvacrol content [26,27]. Similar to other thyme species, they are used extensively as tea, spice and flavoring [28,29]. *Satureja aintabensis* P.H. Davis is an endemic species for Türkiye and known as “Antep kaya kekiği” [30,31]. The primary components of the species' essential oil were found to be p-cymene (33% and 59%) and thymol (32% and 17.5%) in two different studies into the oil's constituents [32,33]. The phenolic content of methanol, ethyl acetate and petroleum ether extracts analyzed by HPLC and found rosmarinic acid and hesperidin as the main components [34]. They also reported that the high content of rosmarinic acid in the extracts is the reason for the antimycobacterial activity of the plant [34].

Satureja species are renowned for their medicinal properties, but the antioxidant potential of this specific taxon has been particularly focused on *S. hortensis* and *S. cuenifolia*, which have widespread use and commercial value. Considering the increasing demand for natural antioxidants in food and pharmaceuticals, this study aimed to evaluate and compare the antioxidant capacity of the essential oil, methanol and chloroform extracts of *S. aintabensis*, which is endemic to Türkiye and has not been studied in the literature to date, using DPPH (radical scavenging) and CUPRAC (cupric ion reduction) assays.

2. Materials and Methods

2.1. Plant Material

Fresh aerial parts of *Satureja aintabensis* P.H. Davis was collected by Prof. Tuncay Dirmenci (Balikesir University) and the herbarium sample of this species was recorded and stored in Balıkesir University Necatibey Education Faculty Herbarium with the code TD 5210.

2.2. Chemicals

Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), 2,9-dimethyl-1,10-phenanthroline (neocuproine), 1,1- diphenyl-2-picryl-hydrazyl (DPPH), ascorbic acid, trichloroacetic acid (TCA) and α-tocopherol were purchased from Sigma-Aldrich Corp., St. Louis MO, USA) and (Steinheim, Germany). Methanol (≥%99.5 Tekkim) and chloroform (≥%99.9 Tekkim) were used for obtain extracts.

2.3. Extractions of Samples

Ten grams of the plant were weighed and put in a 250 mL capped Schott flask once the aerial parts of *S. aintabensis* had been allowed to dry in the shade and ground up. 100 mL of solvent was added and macerated for 4 days. The solvent was evaporated with the help of a rotary evaporator until dryness and crude extracts were obtained.

2.4. Isolation of the essential oils

A Clevenger-type device was used to hydrodistill 100 g of dried aerial parts of *S. aintabensis* for three hours. After being dried over anhydrous $CaCl_2$, the resulting essential oil was kept at 4°C until the experiment.

2.5. Antioxidant Activity

Antioxidant potential of the *Satureja aintabensis* extracts and essential oil was determined using two common methods: 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging and cupric (Cu^{2+}) ions reducing ability assay (CUPRAC). Each experiment was repeated three times and averaged and the experimental results were calculated as mean ± standard deviation. the chemicals butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and α-tocopherol were used as standards, ethanol was used as a negative control.

2.5.1. Cupric Ions (Cu^{2+}) Reducing Ability Assay (CUPRAC)

The capacity of antioxidants to convert copper ions (Cu^{2+}) to copper ions (Cu^{+}) is measured by the CUPRAC assay. The resulting colored complex has a maximum absorbance of 450 nm and is spectrophotometrically measurable. In summary, the following were combined in volume ratios of 1:1:1:0.6: 1 M NH_4Ac (pH = 7.0), 10 mM $CuCl_2$, 7.5 mM Neocuproine, 1 M NH_4CH_3COO (pH 7.0) and distilled water. An aliquot of 180 µL of the mixture was placed into each well. For half an hour, the wells were left at room temperature. At 450 nm, the absorbance was lastly measured in comparison to a blank [7,13,14,35,36].

2.5.2. 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Assay

The principle behind the widely used DPPH free radical scavenging capacity method for assessing antioxidant activity is the change in color of the purple DPPH solution that occurs when DPPH reacts with free radicals at a specific concentration. The sample and DPPH solutions was prepared in ethanol. The sample solutions (40 µL; at concentrations 10, 25, 50 and 100 µM) were mixed with DPPH solutions (160 µL of a 0.1 mM) and the mixture was incubated and subjected to a 30-minute period of darkness. At 517 nm, the absorbance of the samples was finally measured [7,13,14,37-39].

2.6. Statistical Analyses

Triplicate analyses were averaged for the experiment. Data are presented as mean ± standard deviation. Variance ANOVA including one-way analysis was realized. Using Duncan's multiple range tests, significant differences between means were noted. $p< 0.05$ was regarded as significant and $p< 0.01$ was very significant.

3. Results and Discussion

Fruits, vegetables and plants—the foundation of a balanced diet—are natural sources of these compounds. Nowadays, plants—especially medicinal plants—that can be utilized as natural sources of antioxidant compounds that can fight oxidative stress are the subject of most research. In general, it can be said that because of their wide range of therapeutic applications, essential oils and bioactive compounds derived from plants have attracted much interest in the nutraceutical, cosmetic and pharmaceutical sectors. Secondary metabolites from plants, like phenolic compounds and terpenes, are strong antioxidants that help control ROS levels and prevent related diseases, according to numerous studies conducted in both *in vitro* and *in vivo* [12,40,41]. Research on the genus *Satureja* has revealed that the essential oils and extracts of these species have antimicrobial, antimycobacterial, anti-cholesterolemic, antidiabetic and antioxidant properties [22,24,25,28,42-44].

This study determined the antioxidant activities of the essential oil and methanol and chloroform extracts of *Satureja aintabensis* using two methods: DPPH free radical scavenging activity and cupric ion reducing antioxidant capacity. In the CUPRAC method, the reducing capacity of the essential oils and extracts of the samples was determined as Trolox Equivalent Antioxidant Capacity (TEAC). Cu^{2+}-neocuproin reagent was used as an oxidizing reagent to achieve this. The results are given in Table 1.

Table 1. Cupric ions' (Cu^{2+}) reducing capacity of the extracts by CUPRAC method

	$TEAC_{CUPRAC}$ (mmol TR/g)		
Sample	**Essential oil**	**Methanol Extract**	**Chloroform Extract**
Satureja aintabensis	0.54 ± 0.03	0.55 ± 0.04	0.32 ± 0.01
Negative control	0.11 ± 0	0.11 ± 0	0.16 ± 0.01
α-TOC	0.95 ± 0.06	0.95 ± 0.14	0.95 ± 0.06

α-TOC: α-tocopherol; TEAC: Trolox® equivalent antioxidant capacity.

When the cupric ions reducing capacities of the essential oil, extracts obtained from the above-ground part of the plant and the standard compound (α-tocopherol) were compared, it was determined that the reducing capacities of the essential oil and methanol extract were almost equal (0.54 ± 0.03 mmol TR/g and 0.55 ± 0.04 mmol TR/g, respectively) while the chloroform extract showed a relatively weaker reducing capacity (0.32 ± 0.01 mmol TR/g). However, the (Cu^{2+}) reducing capacity of the extracts and oil was not as strong as α-tocopherol (0.95 ± 0. 06 mmol TR/g).

Table 2. DPPH free radical scavenging activity and lipid peroxidation of the essential oil, extracts of *S. aintabensis*, α-tocopherol, BHA and BHT (inhibition%)

Antioxidants	**10 µg/mL**	**25 µg/mL**	**50 µg/mL**	**100 µg/mL**
Essential oil	2.1	4.3	7.6	13.4
Methanol	13.0	23.9	45.9	82.0
Chloroform	0.1	4.0	7.3	15.9
Blank	0.4	0.4	0.4	0.4
α-TOC	62.3	86.2	87.8	86.5
BHA	82.1	85.8	86.3	86.7
BHT	40.8	59.3	65.1	79.0

BHT:Buthylated hydroxytoluene; BHA:Buthylated hydroxyanisole; α-TOC: α-TOC: α-tocopherol

Table 2 shows the DPPH· free radical scavenging activity results of *Satureja aintabensis* essential oil, methanol and chloroform extracts as well as positive antioxidants like BHA, BHT and α-Tocopherol. When antioxidant substances provide an electron to the DPPH· radical, the radical loses its purple color and yellow color formation is observed. The DPPH· free radical scavenging activity of the substances is determined by measuring the change in absorbance at 517 nm in the spectrophotometer by this colorimetric change [45,46]. For this purpose, samples as well as standards were prepared at four different concentrations (10, 25, 50 and 100 µg/mL). The essential oil of *S. aintabensis* (2.1%–13.4%) and chloroform extract (0.1%–14.9%) demonstrated extremely low radical scavenging activity, according to DPPH free radical scavenging activity results. The methanol extract, on the other hand,

showed 82.0% inhibition very close to the standards, especially at a concentration of 100 µg/mL (86.5%, 86.7%, 79.0% for α-TOC, BHA and BHT, respectively).

Methanolic extracts of plants are widely studied for their antioxidant properties due to methanol dissolves a wide variety of bioactive substances, such as phenolics, flavonoids, alkaloids and terpenoids. Polar solvents such as ethanol and acetone are also commonly used for this purpose. Studies showed that these extracts often exhibit strong free radical scavenging, metal chelation and reducing power [12]. For the extraction of medium- to low-polarity biocomponents from plants, low-polarity solvents like petroleum ether and chloroform are recommended. Chloroform is an organic solvent and widely used to extract. It has terpenoids, fatty acids, some alkaloids and some phenolic derivatives [47]. Phenolic substances are known to have very high antioxidant capacity. Studies on antioxidant compounds from plants have shown that there is an excellent correlation ($R^2 > 0.99$) between total phenolic content (measured by Folin-Ciocalteu reagent) and antioxidant activity (measured by Ferric reducing antioxidant power, TEAC or DPPH assays) [48-50]. For both extracts, the activity results obtained are in line with previous research.

The two methods we used to determine antioxidant capacity in our study are both electron transfer (ET)-based. The DPPH method determines the antioxidant's radical scavenging capacity, while the CUPRAC method determines its metal reduction capacity [37,46,49,50]. In their study, Apak et al. evaluated the antioxidant capacity determination methods, focusing particularly on the CUPRAC method. They concluded that the CUPRAC method is a more comprehensive antioxidant testing method because it can be applied to both hydrophilic and lipophilic antioxidants, in contrast to the DPPH method. This may facilitate comprehension of the observed fact that *Satureja ainthabensis* essential oil demonstrates a metal-reducing capacity that is at least equivalent to that of methanol extract according to the CUPRAC method, while it exhibits a lower capacity in the DPPH method [49]. A similar result was observed in our study on the *S. metastasiantha* species [26]. Research on the antioxidant capacities of essential oils and extracts derived from *Satureja* species has demonstrated that among the essential oil components, species containing high levels of thymol and carvacrol exhibit relatively higher antioxidant capacities. Among the extracts, particularly polar extracts, demonstrate high activity due to their substantial phenolic content [13,14,22,26,28,44,51-61].

Herbal extracts showing high antioxidant capacity may become a valuable natural additive in functional foods or beverages to combat oxidative stress-related diseases. For this purpose, it has become important to determine the antioxidant capacity of new antioxidant sources, especially species such as *Satureja*, which is used for spice/healing purposes in Anatolia.

Both the extract and the essential oil added from *S. aintabensis* could be incorporated into shelf-stable products (e.g., oils or herbal teas), selected as natural alternatives to synthetic antioxidants (e.g., BHA/BHT) added to extend shelf life in food or cosmetics, or investigated for their synergistic effects with conventional antioxidants (e.g. vitamin E). They could thus be new sources to meet the growing consumer demand for clean-label products.

4. Conclusion

This study investigated the antioxidant capacity of methanol, chloroform extracts and essential oil from *Satureja aintabensis* P.H. Davis using DPPH and CUPRAC methods. To our knowledge, this is the first study to directly compare the antioxidant profiles of *S. aintabensis* essential oil and extract using both hydrophilic (DPPH) and lipophilic (CUPRAC) models. The results showed that *S. aintabensis*, like other members of the genus *Satureja*, possesses antioxidant properties due to its beneficial compounds. Therefore, this species, with its potential as a natural antioxidant source for food, pharmaceutical, cosmetic and veterinary industries, warrants further investigation.

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