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ANA SAYFA › SON EKLENEN YAYINLAR › DETERMİNATİON OF THE VOLATİLE CONSTİTUEN...

Determination of the volatile constituents and total phenolic contents of some endemic Stachys taxa from Turkey

Ozkan G., Gokturk R. S. , Unal O., Celik S.

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The total phenolic contents and the essential oil compositions of the previously unknown Stachys taxa (Labiatae), including Stachys pinardii Boiss, Stachys cretica L subsp. mersinaea (Boiss.) Rech., and Stachys aleurites Boiss. & Heldr., all endemic to Turkey, were studied. Their essential oil compositions were investigated by GC-MS. It was found that the main constituents were alpha-curcumene (34.10%)for S. cretica, cedrandiol (25.26%) and caryophyllene dioxide (22.15%) for S. pinardii, and (Z)-beta-caryophyllene (31.60%) for S. aleurites. The total phenolic contents, by the Folin-Ciocalteu colorimetric method, of the S. pinardii, S. cretica subsp. mersinaea, and S. aleurites methanolic extract were found to be 600.74+/-0.23, 1200.94+/-0.11, and 900.61+/-0.06 mg gallic acid equivalent (GAE)/100 g in dried herb, respectively.

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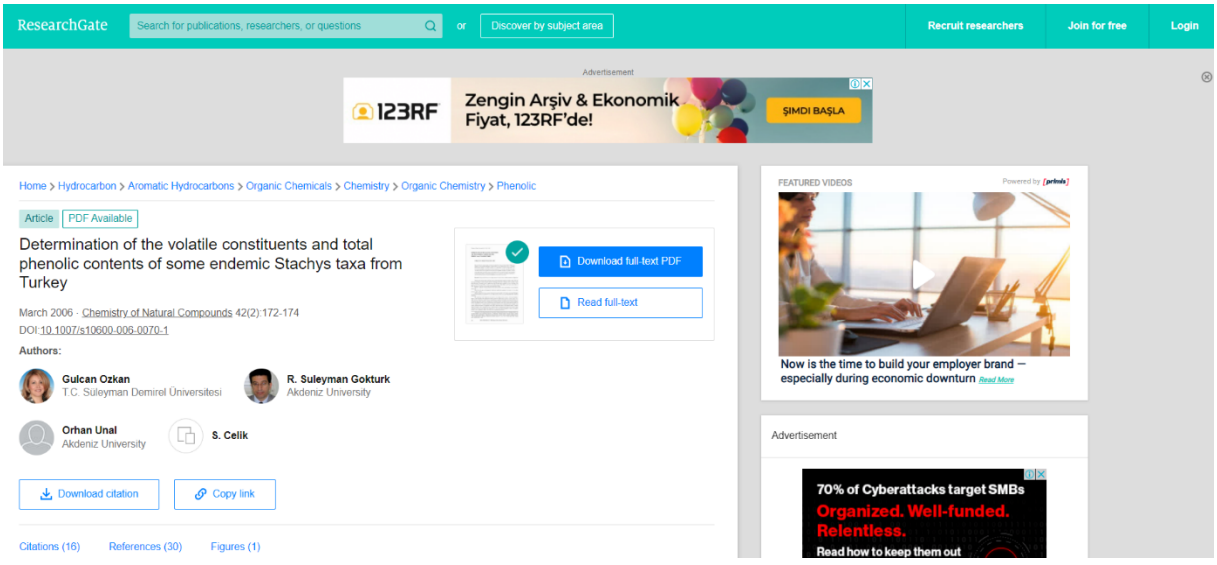
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Chemistry of Natural Compounds, Vol. 42, No. 2, 2006

DETERMINATION OF THE VOLATILE CONSTITUENTS AND TOTAL PHENOLIC CONTENTS OF SOME ENDEMIC *Stachys* TAXA FROM TURKEY

G. Ozkan,[1] R. S. Gokturk,[2] O. Unal,[2] and S. Celik[3]

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The total phenolic contents and the essential oil compositions of the previously unknown Stachys *taxa (*Labiatae*), including* Stachys pinardii *Boiss,* Stachys cretica *L. subsp.* mersinaea *(Boiss.) Rech., and* Stachys aleurites *Boiss. & Heldr., all endemic to Turkey, were studied. Their essential oil compositions were investigated by GC-MS. It was found that the main constituents were α-curcumene (34.10%) for* S. cretica*, cedrandiol (25.26%) and caryophyllene dioxide (22.15%) for* S. pinardii*, and (Z)-β-caryophyllene (31.60%) for* S. aleurites*. The total phenolic contents, by the Folin-Ciocalteu colorimetric method, of the* S. pinardii, S. cretica *subsp.* mersinaea*, and* S. aleurites *methanolic extract were found to be 600.74±0.23, 1200.94±0.11, and 900.61±0.06 mg gallic acid equivalent (GAE)/100 g in dried herb, respectively.*

Key words: *Stachys pinardii, Stachys cretica* subsp. *mersinaea, Stachys aleurites,* volatile constituents, total phenolics.

Natural products of plant origin are still a major part of traditional medical systems in developing countries. There has also been a resurgence of interest in herbal medicines in Western countries [1] as alternative sources of drugs for often intractable diseases. The genus *Stachys* L. comprises more than 270 species and is considered to be one of the largest of the *Lamiaceae* [2]. *Stachys pinardii* Boiss., *Stachys cretica* L. subsp. *mersinaea* (Boiss.)Rech., and *Stachys aleurites* Boiss. & Heldr, all endemic to Anatolia, are native plants widely distributed in Turkey.

Extracts or components of *Stachys* genus have shown various activities such as anti-inflammatory and antinephritic effects [3–5].

The present study aims at investigating the volatile and total phenolic compounds of *S. pinardii, S. cretica* subsp. *mersinaea*, and *S. aleurites.*

All the investigated *Stachys* taxa contain essential oils that range from 0.02 to 0.15% based on dry weight. The highest oil contents were found in *S. pinardii* (0.15%). *S. cretica* subsp. *mersinaea* and *S. aleurites* have 0.02 and 0.10% essential oil, respectively.

The compositions of the essential oil of *S. pinardii, S. cretica* subsp. *mersinaea*, and *S. aleurites*, all endemic to Turkey, are shown in Table 1. We identified 84.17, 56.57, and 53.73% of whole oil for *S. pinardii, S. aleurites*, and *S. cretica* subsp. *mersinaea*, respectively. In spite of the fact that their main components were different, all of them were found to be sesquiterpene hydrocarbons. The main constituents were α-curcumene (34.10%), tetradecanol (6.27%), (*Z*)-β-caryophyllene (4.84%), caryophyllene dioxide (3.97%), germacrene D (2.14%), humulene oxide (1.36%), and β-bourbonene (1.05%) for *S. cretica* subsp. *mersinaea*. Cedrandiol (25.26%), caryophyllene dioxide (22.15%), α-humulene (8.65%), humulene oxide (7.66%), germacrene D (6.04%), Caryophyllene oxide (3.26%), α-bisabolene (3.01%), alloaromadrene (2.05%), γ-cadinene (2.00%), δ-cadinene (1.37%), β-eudesmol (1.24%), α-cubebene (0.83%), and β-bourbonene (0.65%) were found to be main components of *Stachys pinardii*, and the main components of *S. aleurites* were (*Z*)-β-caryophyllene (31.60%), caryophyllene dioxide (12.12%), α-humulene (5.00%), and carvacrol (5%) and germacrene D (2.85%).

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TABLE 1. Compositions of Essential Oils of *Stachys pinardii*, *Stachys cretica* subsp. *mersinaea*, and *Stachys aleurites*, %

Constituents	RT[a]	*Stachys cretica*	*Stachys pinardii*	*Stachys aleurites*
β-Bourbonene	23.45	1.05±0.45	0.65±2.10	-
(*Z*)-β-Caryophyllene	25.83	4.84±1.38	-	31.60±1.01
Alloaromadrene	27.19	-	2.05±2.54	-
β-Himachalene	27.68	-	-	-
α-Humulene	27.83	-	8.65±4.03	5.00±3.00
α-Cubebene	28.30	-	0.83±0.87	-
Germacrene D	28.85	2.14±1.67	6.04±0.45	2.85±0.00
γ-Cadinene	29.16	-	2.00±2.21	-
δ-Cadinene	29.72	-	1.37±0.46	-
Caryophyllene oxide	34.90	-	3.26±3.21	-
α-Bisabolene	36.51	-	3.01±3.21	-
Caryophyllene dioxide	36.85	3.97±1.14	22.15±3.35	12.12±5.10
Humulene oxide	38.45	1.36±2.41	7.66±3.34	-
Carvacrol	39.13	-	-	5.00±2.26
α-Curcumene	44.18	34.10±0.99	-	-
Cedrandiol	44.31	-	25.26±0.98	-
β-Eudesmol	45.37	-	1.24±0.02	-
Tetradecanol	68.05	6.27±1.20	-	-

[a]Retention time.

"-" not detected.

Flamini *et al*. [6] found sesquiterpene hydrocarbons such as β-caryophyllene (33.7%), bicylogermacrene (14.50%), and germacrene D (9.6%) to be the main components of *S. aleurites* essential oil; all the plants were obtained without woody parts. It was also reported that *S. cretica* ssp. *cretica* produced sesquiterpene hydrocarbons [7]. The main components of *S. menthifolia*, *S. germanica* ssp. *heldreichii*, *S. euboica*, *S. scardica* [7], *Stachys* subsect. swainsonianeae [8], and *S. lavandufolia* [9, 10] were also reported to be sesquiterpene hydrocarbons. In addition, it was found that some *Stachys* species contain similar amounts of sesquiterpene hydrocarbons and oxygenated mono- and sesquiterpenes, such as *S. byzantina*, *S. grandiflora* and *S. sylvatica* [11], *S. balansae* [12], *S. alopecuros, S. spinulosa* [7], and *S. oblique* [13]. Our findings were generally similar to the literature results for the major components, with minor differences in the research parameters. These variations may be due to the different subspecies that can influence the oil composition. As a result, the data show that *S. pinardii, S. cretica* subsp. *mersinaea* and *S. aleurites* oil were characterized by a relatively high concentration of sesquiterpene hydrocarbons.

The total phenolic contents, by the Folin-Ciocalteu colorimetric method, of the *S. pinardii, S. cretica*, and *S. aleurites* aqueous methanolic extract were found to be 600.74±0.23, 1200.94±0.11, and 900.61±0.06 mg gallic acid equivalent (GAE)/100 g in dried herb, respectively. To our knowledge, there were no conclusive data. However, it was reported that medicinal plants and herbs, especially the lamiaceae family, have an extremely high total phenolic content and a relationship exists between the antioxidant-antimicrobial properties and the total phenolic content of the extract [14–16].

As a result, the essential oils of these plants with good aromatic properties and their aqueous extracts with high phenolic content could be considered a natural food additive because of their antioxidant and antimicrobial properties or for other medicinal purposes.

EXPERIMENTAL

Samples. The flowering aerial parts of *S. pinardii* (37 00 193 N, 30 49 126 E), *S. cretica* subsp. *Mersinaea* (36 31 675 N, 29 48 941 E), and *S. aleurites* (36 53 068 N, 30 40 697 E) were collected in Turkey.

Analysis of Volatile Compounds. Two hundred grams of air-dried plant material, including flowers, leaves, and stems, from each population were cut in small pieces, and the essential oils were obtained by hydrodistillation in 3000 mL H_2O

for 3 h by a Clevenger apparatus. The oils, dried over anhydrous sodium sulfate, were subsequently analyzed by GC-MS and stored at –20°C. The composition of the volatile constituents was established by GC-MS/quadrupole detector analyses using a Shimadzu QP 5050 system, fitted with an FFAP (50 m × 0.32 mm (i.d.), film thickness: 0.25 μm) capillary column. Detector and injector temperature were set at 230°C. The temperature program for the FFAP column was from 120°C (1 min) to 230°C at a rate of 6°C/min and than held at 200°C for 35 min. Helium was used as a carrier gas at a flow 14 psi (split 1:10) and injection volume of each sample was 1 μL. The percentage composition was computed from the GC peak areas according to the 100% method without using any correction factors. The identification of the components was based on comparison of their mass spectra with those of Wiley and Nist, Tutore Libraries. The ionization energy was set at 70 eV.

Determination of Total Phenolics. The dry plant material was milled using a laboratory-scale mill (Retsch, Germany); 0.5 g of the milled sample was placed in an Erlenmeyer flask, 10 mL methanol–water (5:5) was added, and the Erlenmeyer flask was ultrasonicated (Super RK 255 H, Bandelin electronic, Berlin, Germany) for 10 min, and then a 5 mL sample in a test tube was centrifuged at 4500 rpm for 5 min. The supernatant was collected and filtered before the analysis [17]. The total phenolic content of the extract was determined by the Folin-Ciocalteu colorimetric method [18]. The estimation of phenolic compounds in the extracts was carried out in triplicate and calculated by a calibration curve obtained with gallic acid. Total phenolic content was expressed as gallic acid equivalents (mg GAE/100 g dried herb).

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