Akdeniz Üniversitesi
Akademik Veri Yönetim Sistemi

Araştırmacı Girişi English

Araştırmacı, Yayın, Proje, Bilimsel Hakemlik, Araştırma Grubu, Faaliyet Ara

Tümü

Detaylı Arama

ANA SAYFA > SON EKLENEN YAYINLAR > ESSENTİAL OİL COMPOSİTİONS AND BİOACTİVİ...

Essential Oil Compositions and Bioactivities of Thymus revolutus and Cyclotrichium origanifolium

GÖKTÜRK R. S. , SAĞDIÇ O., ÖZKAN G., ÜNAL O. , AKSOY A. , ALBAYRAK S., ...Daha Fazla

JOURNAL OF ESSENTIAL OIL BEARING PLANTS, cilt.16, sa.6, ss.795-805, 2013 (SCI İndekslerine Giren Dergi)

Atıflar
Web of Science: 8
Scopus: 8

Yayın Türü: Makale / Tam Makale

Cilt numarası: 16 **Sayı:** 6

Basım Tarihi: 2013

Doi Numarası: 10.1080/0972060x.2013.813208

Dergi Adı: JOURNAL OF ESSENTIAL OIL BEARING PLANTS

Sayfa Sayıları: ss.795-805

Anahtar Kelimeler: Thymus revolutus, Cyclotrichium origanifolium, essential oil composition, antimicrobial activity, antioxidant activity, ANTIOXIDANT ACTIVITIES, CHEMICAL-COMPOSITION, METHANOL EXTRACTS, VULGARIS L., ANTIBACTERIAL, MANDEN., SCHENG, NIVEUM, LEAVES, TURKEY

Citations
CrossRef - Citation Indexes: 2
Scopus - Citation Indexes: 8

Usage
EBSCO - Abstract Views: 5

Captures
Mendeley - Readers: 16

PLUMX - see details

Özet

Thymus revolutus Celak (Lamiaceae) is an endemic species in Turkey. Cyclotrichium origanifolium (Labill.) Manden & Scheng (Lamiaceae) has been widely used as a flavoring agent in soups and salads, and herbal tea in Turkey. The essential oil (EO) components, antioxidant and antimicrobial activities of the extracts and EOs of T. revolutus and C. origanifolium were examined. The EO compositions were analyzed by GC-MS. Total phenolic contents, flavanols, flavonols and antioxidant activities of the extracts were determined by spectrophotometer. Antimicrobial activity was investigated against 15 microorganisms by agar diffusion method. Carvacrol, thymol, p-cimene and borneol were predominantly found in T. revolutus EO. The C. origanifolium EO contained bicyclo[3.1.1]hepten-3-one, 2,6,6-trimethyl-,(1.alpha, 2.beta, 5.alpha), pulegone and 2-cylohexen-1-ol, 1-methyl-4-(1-methylethyl),-cis as main constituents. The levels of total phenolic contents, flavanols, and flavonols of T. revolutus and C. origanifolium extracts were 38.68 +/- 2.5 and 78.57 +/- 0.6 mg gallic acid equivalent/g; 2250.06 +/- 328.0 and 610.84 +/- 13.3 mg catechin equivalent/100 g; and 1287.35 +/- 91.1 and 2712.36 +/- 20.9 mg rutin equivalent/100 g, respectively. Antioxidant activities of T. revolutus and C. origanifolium were 48.91 +/- 0.2 % and 74.86 +/- 0.3 % at 0.1 mg/mL concentration, respectively in the DPPH assay. C. origanifolium EO sat 2 % and 1 % concentrations showed antibacterial activity against tested Gram (+) bacteria but not Mycobacterium smegmatis. Both of the EOs had no inhibitory effects at 0.2 and 0.5 % concentrationson on the 15 tested microorganisms. Gram (+) bacteria found to be more sensitive to the EOs and extracts than Gram (-) bacteria in this study. The EOs and the extracts of T. revolutus and C. origanifolium could be used as natural products in many areas such as food, pharmaceutical, alternative medicine and natural therapy.



Journal of Essential Oil Bearing Plants

ISSN: 0972-060X (Print) 0976-5026 (Online) Journal homepage: https://www.tandfonline.com/loi/teop20

Essential Oil Compositions and Bioactivities of *Thymus revolutus* and *Cyclotrichium origanifolium*

R. Suleyman Gokturk, Osman Sagdic, Gulcan Ozkan, Orhan Unal, Ahmet Aksoy, Sevil Albayrak, Muhammet Arici & M. Zeki Durak

To cite this article: R. Suleyman Gokturk, Osman Sagdic, Gulcan Ozkan, Orhan Unal, Ahmet Aksoy, Sevil Albayrak, Muhammet Arici & M. Zeki Durak (2013) Essential Oil Compositions and Bioactivities of *Thymus revolutus* and *Cyclotrichium origanifolium*, Journal of Essential Oil Bearing Plants, 16:6, 795-805, DOI: 10.1080/0972060X.2013.813208

To link to this article: https://doi.org/10.1080/0972060X.2013.813208

Published online: 24 Dec 2013.

Submit your article to this journal

Article views: 139

View related articles

Citing articles: 2 View citing articles

Full Terms & Conditions of access and use can be found at
https://www.tandfonline.com/action/journalInformation?journalCode=teop20

Taylor & Francis
Taylor & Francis Group

ISSN Print: 0972-060X
ISSN Online: 0976-5026

Essential Oil Compositions and Bioactivities of *Thymus revolutus* and *Cyclotrichium origanifolium*

R. Suleyman Gokturk [1], Osman Sagdic [2]*, Gulcan Ozkan [3], OrhanUnal [1], Ahmet Aksoy [1], Sevil Albayrak [4], Muhammet Arici [2] and M. Zeki Durak [2]

[1] Akdeniz University, Faculty of Science, Department of Biology, 07058 Antalya-Turkey
[2] Yildiz Technical University, Chemical and Metallurgical Engineering Faculty, Department of Food Engineering, 34210 Istanbul-Turkey
[3] Suleyman Demirel University, Faculty of Engineering, Department of Food Engineering, 32260 Isparta-Turkey
[4] Erciyes University, Faculty of Science, Department of Biology, 38039 Kayseri-Turkey

Received 24 August 2012; accepted in revised form 29 December 2012

Abstract: *Thymus revolutus* Celak (Lamiaceae) is an endemic species in Turkey. *Cyclotrichium origanifolium* (Labill.) Manden & Scheng (Lamiaceae) has been widely used as a flavoring agent in soups and salads, and herbal tea in Turkey. The essential oil (EO) components, antioxidant and antimicrobial activities of the extracts and EOs of *T. revolutus* and *C. origanifolium* were examined. The EO compositions were analyzed by GC-MS. Total phenolic contents, flavanols, flavonols and antioxidant activities of the extracts were determined by spectrophotometer. Antimicrobial activity was investigated against 15 microorganisms by agar diffusion method. Carvacrol, thymol, *p*-cimene and borneol were predominantly found in *T. revolutus* EO. The *C. origanifolium* EO contained bicyclo[3.1.l]hepten-3-one, 2,6,6-trimethyl-,(l.α, 2.β, 5.α), pulegone and 2-cylohexen-1-ol, 1-methyl-4-(1-methylethyl),-*cis* as main constituents. The levels of total phenolic contents, flavanols, and flavonols of *T. revolutus* and *C. origanifolium* extracts were 38.68±2.5 and 78.57±0.6 mg gallic acid equivalent/g; 2250.06±328.0 and 610.84±13.3 mg catechin equivalent/100 g; and 1287.35±91.1 and 2712.36±20.9 mg rutin equivalent/100 g, respectively. Antioxidant activities of *T. revolutus* and *C. origanifolium* were 48.91±0.2 % and 74.86±0.3 % at 0.1 mg/mL concentration, respectively in the DPPH assay. *C. origanifolium* EO sat 2 % and 1 % concentrations showed antibacterial activity against tested Gram (+) bacteria but not *Mycobacterium smegmatis*. Both of the EOs had no inhibitory effects at 0.2 and 0.5 % concentrationson on the 15 tested microorganisms. Gram (+) bacteria found to be more sensitive to the EOs and extracts than Gram (-) bacteria in this study. The EOs and the extracts of *T. revolutus* and *C. origanifolium* could be used as natural products in many areas such as food, pharmaceutical, alternative medicine and natural therapy.

Key words: *Thymus revolutus*; *Cyclotrichium origanifolium*; essential oil composition; antimicrobial activity; antioxidant activity.

Introduction

Free radicals are often generated by oxidation product of biological reactions or exogenous factors. It is well known that reactive oxygen species induce some oxidative damage to biomolecules like lipids, nucleic acids, proteins, amines, deoxyribonucleic acid, and carbohydrates. Its damage causes ageing, cancer, and many other diseases [22]. There is an increasing interest in antioxidants, particularly in those

*Corresponding author (Osman Sagdic)
E-mail: < osagdic@yildiz.edu.tr, sagdic@gmail.com >

© 2013, Har Krishan Bhalla & Sons

intended to prevent the presumed deleterious effects of free radicals in the human body, and prevent the deterioration of fats and other constituents of food stuffs. Consumers prefer antioxidants from natural sources rather than synthetic sources [7]. Because, the commonly used synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxy toluene (BHT) are suspected to have some toxic and carcinogenic effects in living organisms [29].

Plant phenolics are considered to be multifunctional antioxidants and they might act as singlet oxygen quencher, reducing agents, and hydrogen donors. They also have transition metal chelating properties. These multiple potential mechanisms of antioxidant action make the diverse group of phenolic compounds an interesting target in the search of health beneficial phytochemicals, and also offer a possibility to use phenolic compounds or extracts in lipid rich foods in order to extend their shelf life [13].

Thymus revolutus Celak (Lamiaceae) is an endemic species in Turkey that grows on open rocky and gravelly ground. The plant is a dwarf shrub with leaves that are lanceolate-falcate with revolute margins, and the inflorescence is a compact conical head with purple corolla [10]. The oil of the plant was reported to have antimicrobial, antioxidant, spasmolytic, carminative, and expectorant activities. In addition to their numerous traditional uses, the plant (herb) and its EO have found diverse applications in pharmaceutical and medicine [15].

Even though antioxidant and antimicrobial effects of some *Thymus* species have been thoroughly studied by some researchers [16,1,19,24,29], the biological activities of *T. revolutus* extract do not seem to have been reported before.

The *Cyclotrichium* genus is represented in Turkish flora by 5 species of which two are endemic [10]. *Cyclotrichium origanifolium* (Labill.) Manden & Scheng (Lamiaceae) has been widely used as flavoring agent in soups, salads and herbal tea in Turkey [6]. To the best of our knowledge, there is a limited number of biological reports on *Cyclotrichium* species [3,4,5,12]. Apart from these studies, the antimicrobial and antioxidant activities of EO and various extracts of *C. origanifolium* have recently been reported [30].

Therefore, the aim of this study was to determine total phenolics, flavanols, flavonols, antioxidant and antimicrobial activities, and EO compositions of *T. revolutus* and *C. origanifolium* in an attempt to contribute the use of these alternative ingredients and folk medicine in oxidative and microbial control for food preservation and human health.

Materials and methods

Plant materials

The flowering aerial parts of *Thymus revolutus* Celak (Lamiaceae) were collected from Turkey, C3 Antalya, Akdeniz University Campus, disturbed macchie, about 32 m above sea level at the end of May 2005. A voucher specimen has been deposited at AKDU (Herbarium of the Biology Department of Akdeniz University) as Unal 1564 by Dr. Orhan Unal. *T. revolutus* is an endemic plant of Turkey.

Cyclotrichium origanifolium (Labill.) Manden & Scheng (Lamiaceae) was collected from Nigde, Ulukisla, Turkey in July 2006 (1850-1900 m). Plants were collected during their flowering season. They were identified by senior taxonomist Dr. Ahmet Aksoy from the Department of Biology, Erciyes University, Turkey. A voucher specimen has been deposited at the Herbarium of the Department of Biology, Erciyes University, Kayseri, Turkey (Voucher no.: A. Aksoy 2019).

Isolation of essential oil

Air dried *T. revolutus* and *C. origanifolium* samples (200 g) were subjected to steam distillation for 3 h using Clevenger-type apparatus. The essential oils were dried over anhydrous sodium sulphate to remove any traces of water and, after filtration, stored at -20°C until tested and analyzed. The extraction yields were found as 0.6 % and 1.7 % (v/w) in *T. revolutus* and *C. origanifolium*, respectively.

Preparation of the extracts

Herb leaves were dried at room temperature and grounded to a fine powder with a grinder. Then, the powdered samples (15 g) of *T. revolutus* were extracted in a Soxhlet extractor with 200

mL mixture of methanol:acetone:water:acetic acid (90:90:18:2) at 60°C for 6 h. The powdered samples (15 g) of *C. origanifolium* were extracted in a Soxhlet extractor with methanol at 60°C for 6 h. The extracts were concentrated by a rotary evaporator (Rotary Evaporator R 200 (Buchi, Switzerland), T<40°C) under vacuum and lyophilizers (Benchtop 2K Series Freeze Dryers; *T* = -60°C) (SP Industries, Gardiner, NY, USA) in order to obtain crude extracts. Dried extracts were stored in a desiccator until use [25]. The extraction yields were found as 24.53 % and 15.4 % (w/w) for *T. revolutus* and *C. origanifolium*, respectively.

GC-MS analysis of essential oil

The composition of the volatile constituents was established by GC-MS/Quadrupole detector analyses, using a Shimadzu QP 5050 system, fitted with an FFAP polar capillary column (50 m × 0.32 mm (i.d.), film thickness: 0.25 μm). Detector and injector temperature were set at 230°C. The temperature program for FFAP column was from 120°C (1 min) to 230°C at a rate of 6ºC/min and then held at 230°C for 35 min. Helium was used as a carrier gas at a flow 14 psi. (Split 1:10 mL/min) and injection volume of each sample was 1 μL. The ionization energy was set at 70 eV. Qualitative analysis was based on the comparison of retention times and the computer mass spectra libraries using Wiley GC-MS Library and Nist, Tutore Libraries. The percentage composition was computed from the GC peak areas according to the 100 % method without using any correction factors.

Determination of total phenolics, flavonols and flavanols

The content of total phenolic compounds in the extracts was determined by Folin-Ciocalteu method [27]. Methanol extract (40 μL) (1 mg/mL) was transferred into a test tube, 2.4 mL distilled water and 200 μLFolin-Ciocalteu reagent were added and test tube was shaken thoroughly. After this prepared solution was mixed with 600 μL sodium carbonate (20 % Na_2CO_3), 760 μL distilled water was added and mixed. Methanol was used as a blank in the study. The absorbance of reaction mixture was measured at 765 nm (Shimadzu UV-Vis 1240, Japan) after incubation at room temperature for 2 h in dark. The total phenolic was expressed as mg of gallic acid (GAE) equivalents/g extract.

Total flavonols were determined with Neu's reagent solution by the method of Dai *et al.* [9]. The absorbance of the samples and the standard were measured at 410 nm and the flavonols were expressed as mg rutin equivalent (RE)/g herb extract.

Total flavanols were assayed colorimetrically by the vanillin method using catechin as a standard [8]. The absorbances of samples were measured at 500 nm and the content of total flavanol in the extract was expressed as catechin equivalents (CE)/g herb extract. All determinations were carried out in triplicate and the results were averaged.

Determination of antioxidant activity

The ability of the extracts to scavenge DPPH (2,2-diphenyl-1-picrylhydrazyl) was determined by the method of Lee *et al.*[20]. The methanol extract (50 μL) (0.1 mg/mL) was added to 450 μLTris-HCL buffer (pH = 7.4) and 1 mL of the methanol DPPH solution (0.1 mM). Methanol was used as a control instead of extract and EO. After a 30 min incubation period at room temperature in the dark, the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I %) was calculated in the following way:

I (%) = 100 x (1-Absorbance of sample/ Absorbance of control)

Synthetic antioxidant reagent butylated hydroxytoluene (BHT) was used as positive control. All tests were carried out in triplicate and the results were averaged.

Determination of antimicrobial activity

The 15 microorganisms containing thirteen bacteria and two yeasts were used as test organisms: *Aeromonashydrophila* ATCC 7965, *Bacillus brevis* FMC3, *B. cereus* FMC 19, *B. subtilis* ATCC 6630, *B. subtilis* var. *niger* ATCC

10, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* FMC 5, *Morganella morganii*, *Mycobacterium smegmatis* RUT, *Proteus mirabilis* BC 3624, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 28213, *Yersinia enterocolitica* ATCC 1501, *Candida albicans* ATCC 1223, *Saccharomyces cerevisiae* BC 5461. These microorganisms were supplied by the Department of Food Engineering, Erciyes University, Kayseri, Turkey.

Test yeasts namely *C. albicans* and *S. cerevisiae* and *Y. enterocolitica* were grown in malt extract and nutrient broths at 25°C for 18 h, respectively. The other microorganisms were grown in nutrient broth at 35°C for 18 h. All test microorganisms in nutrient broth or malt extract broth were enumerated by using the serial dilution method. Their final cell concentrations were 10^6-10^7 cfu/mL. The agar diffusion method was used to detect antimicrobial activity [25]. Each microorganism (250 µL) was added into a flask containing 25 mL sterile nutrient agar or malt extract agar at 45°C and poured into Petri dishes (9 cmin diameter). Then the agars were allowed to solidify at 4°C for 1 h. Four equidistant holes were made in the agar using sterile cork borers (Ø = 4 mm). EO (50 µL) at 0.2 %, 0.5 %, 1 %, and 2 % concentrations in dimethylsulfoxide (DMSO, Merck, Germany) were filled to the holes using a pipettor and DMSO without the EO was used as a control. Again, the extracts (50 µL) were prepared at 1 %, 2.5 %, 5 %, and 10 % concentrations in same solvent and were applied to the holes using a pipettor and extraction solvent (absolute methanol) without herb extract was used as a control. *Y. enterocolitica*, *C. albicans* and *S. cerevisiae* was incubated at 25°C for 14-24 h in the inverted position. The other microorganisms were incubated at 35°C for 18-24 h. At the end of the period, inhibition zones which formed on the medium were measured in millimeters (mm).

Results and discussion

In this study, total yields (%), total phenolics, flavonols, and, flavanols and antioxidant activities of *T. revolutus* and *C. origanifolium* extracts were examined. Also, chemical compositions of EOs obtained from these plants were determined. About 19 compounds of *T. revolutus* EO were determined (Table 1). GC-MS analysis demonstrated that major constituents of *T. revolutus* EO were carvacrol (24.53 %), thymol (15.39 %), p-cimene(15.50 %) and borneol (14.66 %), as listed in Table 1. The EO composition of the various *Thymus* species has been previously studied [2,7,19,26,29]. Our results are in accordance with the findings of Karaman *et al*. [16], who demonstrated that major components of *T. revolutus* were carvacrol (43.13 %), δ-terpinene (20.86 %), p-cymene (13.94 %), β-caryophyllene (5.40 %), and thymol (4.62 %).

Thirty-two components were characterized from *C. origanifolium* EO and are shown in Table 2. The *C. origanifolium* EO contained bicyclo [3.1.1]hepten-3-one, 2,6,6-trimethyl-,(l.α, 2.β, 5.α) (21.28 %), pulegone (16.15 %) and 2-cylohexen-1-ol, 1-methyl-4-(1-methylethyl),-*cis* (15.42 %) as main constituents. Previously, the EO compositions of *C. leucitrichum*, *C. staminеum* [3], *C. niveum* [5,12,23] and *C. origanifolium* [4,30] were analyzed by many researchers. The EOs of *C. origanifolium* collected from four different locations in Turkey by Baser *et al.*[4] were analyzed by GC-MS and *cis*-isopulegone (3.52-52.17 %), pulegone (6.85-37.15 %), isomenthone (1.98-30.67 %), and isomenthol (0.28-11.22 %) were reported as the main components of the EO. Also, EO compositions of *C. origanifolium* were analyzed by Tepe *et al.* [30] and pulegone (49.8 %), menthone (32.5 %), and limonene (6.0 %) were determined as main components of EO. According to GC-MS results, the percentages of EO components are particularly in agreement with our results. Except for some negligible differences between the EOs tested, *T. revolutus* and *C. origanifolium* have highly similar chemical compositions. Generally, a great variability and diversity are observed concerning the chemical composition of the EOs of *Thymus* and *Cyclotrichium* species due to climatic, seasonal, geographical and geological variation etc. [29,30].

The literature survey indicates that the plant phenolics constitute one of the major groups of compounds acting as primary antioxidants or free radical terminators. Therefore, it is worthwhile

Table 1. Chemical composition of *T. revolutus* essential oil

Compounds	RT[b]	%
α-Pinene[a]	8.937	1.86[c]
Camphene	10.713	0.80
γ-Terpinene	19.972	0.70
p-Cimene	21.570	15.50
7-Octen-4-ol	32.377	0.67
α-Thujone	33.630	0.65
trans-Thujon-4-ol	36.182	0.90
Camphor	39.980	2.55
Linalool	38.732	2.95
4-Terpineol	42.880	1.64
trans-Caryophyllene	43.038	3.59
Sabineol	45.902	1.47
Pulegone	46.157	4.05
LinalylPropioanate	48.618	2.11
Borneol	49.063	14.66
p-Cymen-8-ol	57.578	0.79
Caryophyllene oxide	66.110	5.18
Thymol	75.397	15.39
Carvacrol	76.961	24.53

[a]: Compounds listed in order of elution from a FFAP MS column
[b]: Retention time (as minutes)
[c]: The percentage composition was computed from the GC peak areas

Table 2. Chemical composition of *C. origanifolium* essential oil

Compounds	RT[b]	%
α-Pinene[a]	8.940	0.81[c]
3-Pinene	12.592	6.70
Sabinen	13.105	1.15
Limonene	17.269	1.16
Eucolyptol	17.820	5.96
p-Mentha-3,8-diene	21.242	0.20
p-Cymol (p-cimene)	21.603	0.13
3-Octanol,acetate	25.168	0.22
3-Octanol	28.633	0.31
Terpineol	33.673	0.08
Isomentahone	3 6.229	2.08
Isocitronellen	38.029	1.77
Linalool	38.785	0.21
Bicyclo[3.1.l]hepten-3-one, 2,6,6-trimethyl-,(l.α, 2.β, 5.α)	39.968	21.28
Methyl acetate	40.281	0.84

table 2. (continued).

Compounds	RT[b]	%
3-Undycene	41.560	0.97
Neoisomenthol	42.3 59	2.15
2-Cylohexen-1-ol, 1-methyl-4-(1-methylethyl),-*cis*	43.031	15.42
Neoiso (iso) pelegol	44.555	1.99
Pulegone	42.221	16.15
D-Neoisomenthol	46.788	7.73
α-Terpineol	47.141	0.28
Myrtenyl propionate	48.434	1.84
Linalyl acetate	48.660	0.49
Borneol	49.110	0.94
2-Isopropyl4-methylhex-2-enol	50.403	1.50
trans-Pinocaruylacetate	52.581	1.34
Myrtenol	54.511	0.73
Piperitenone	62.635	0.14
Caryopinyllene oxide	66.318	0.60
Thymol	75.450	0.99
Carvacrol	76.948	3.73

[a]: Compounds listed in order of elution from a FFAP MS column

[b]: Retention time (as minutes)

[c]: The percentage composition was computed from the GC peak areas

to determine their total amount in the plants. Flavonoids as one of the most diverse and widespread group of natural compounds, are likely to be the most important natural phenolics. Polyphenols, including vitamins, pigments and flavonoids, possess a broad spectrum of chemical and biological activities including radical scavenging properties. Such properties are especially distinct for flavonols [18]. Therefore, the content of flavonol, flavanol, and total phenolics are determined in the extracts (Table 3).

The total phenolic contents of the extracts were determined by Folin-Ciocalteau method and expressed as gallic acid equivalents. The total phenolic contents were determined as 38.68±2.5 and 78.57±0.6 mg GAE/g extract for *T. revolutus*

Table 3. Total phenolic, flavanol, flavonol contents and % DPPH inhibition rates of *T. revolutus* and *C. origanifolium* extracts

Plants	Total phenolics (mg GAE/g)	Flavanol (mg CE/100g)	Flavonol (mg RE/100g)	% DPPH
T. revolutus	38.68±2.5*	2250.06±328.0	1287.35±91.1	48.91±0.2
C. origanifolium	78.57±0.6	610.84±13.3	2712.36±20.9	74.86±3.0

*: Values expressed are mean ± standard deviation of three experiments. Total phenolic content expressed as gallic acid equivalent (GAE), total flavanols expressed as catechin equivalent (CE), total flavonol expressed as rutin equivalent (RE), DPPH: 2,2-diphenyl-1-picrylhydrazyl. % Inhibition value of BHT as positive control was found to be 55.8±0.7 % in the DPPH assay.

and *C. origanifolium*, respectively.

The flavonol contents of the extracts were determined by vanillin colorimetric methods and expressed as rutin equivalents. The flavonol contents of the extracts were determined as 1287.35±91.1 and 2712.36±20.9 mg RE/100 g extract for *T. revolutus* and *C. origanifolium* respectively.

The flavanol contents of the extracts were determined by Neu's reagent solution and expressed as catechin equivalents. The flavanol contents were determined as 2250.06±328.0 and 610.84±13.3mg CE/100 g extract for *T. revolutus* and *C. origanifolium*, respectively.

According to our knowledge, there was no previous literature on total phenolic, flavonol and flavanol contents of *T. revolutus* extract. Sokmen *et al*. [28] showed that gallic acid equivalent total phenolic constituent of the polar and non-polar subfractions of *T. spathulifolius* methanol extract was 141±0.90 µg/mg and 102±0.55 µg/mg, respectively. In other study, the total phenolic content of *T. argaeus* was found to be 83.31±0.59 mg GAE/g which is higher than that of *T. revolutus* [25]. The total phenolic contents of the various extracts prepared by using solvents of varying polarity from *C. origanifolium* were previously determined by Tepe *et al*. [30]. However, it is the first time that the flavonol and flavanol contents of *C. origanifolium* methanolic extract were described in this study.

The antioxidant activities of the extracts were measured by DPPH assay (Table 3). *T. revolutus* and *C. origanifolium* extracts were able to reduce the stable free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH). At 0.1 mg/mL concentration, the antioxidant activities of the *T. revolutus* and *C. origanifolium* extracts were 48.91±0.2 % and 74.86±3.0 %, respectively. At this concentration, the antioxidant activity of BHT was found to be 55.8±0.7 %. The antioxidant activity of *C. origanifolium* is greater than that of BHT and *T. revolutus*. Similarly, it is previously stated that the methanol extract of *C. origanifolium* showed strong free radical scavenging activity [30]. As far as our literature survey could ascertain, we could not find any reports on the antioxidant activity of *T. revolutus* in the literature. However, there are many reports on the antioxidant activities of the various *Thymus* species [17,19,21,28,29].

T. revolutus and *C. origanifolium* showed radical scavenging activities. The total phenolic, flavonol contents and DPPH radical scavenging capacity of *C. origanifolium* were significantly higher than that of *T. revolutus*.

Antimicrobial activities of the EOs of *T. revolutus* and *C. origanifolium* at 0.2, 0.5, 1, and 2 % concentrations in DMSO were examined against 15 microorganisms containing 13 bacteria and 2 yeasts (Table 4). DMSO used as control had no inhibitory effects on the 15 microorganisms tested. *C. origanifolium* EO at 2 % and 1 % concentrations showed antibacterial activity against Gram (+) bacteria tested, but not against *M. smegmatis*. *S. aureus* was the most sensitive microorganism to *C. origanifolium* EO. Additionally, the EO had antibacterial activity against Gram (-) bacteria tested, but not against *E. coli*, *P. aeruginosa* and *Y. enterocolitica*. *C. origanifolium* EO at 2 % concentration was active against *S. cerevisiae* while it was inactive against *C. albicans*. *T. revolutus* EO inhibited the growth of Gram (+) bacteria tested. Also, *T. revolutus* EO possessed antibacterial activity against Gram (-) bacteria tested but not against *Y. enterocolitica*. The most sensitive microorganism to EO was *B. cereus*. While *T. revolutus* EO at 2 % concentration had inhibitory effect against both of the yeasts tested, *C. origanifolium* EO at 2 % concentration was active against only *S. cerevisiae*. Both of the EOs had no inhibitory effects on the fifteen microorganism tested at 0.2 and 0.5 % concentration.

At a concentration of 1, 2.5, 5, and 10 %, the antimicrobial activities of *T. revolutus* and *C. origanifolium* extracts are shown in Table 4. Extraction solvent (absolute methanol) used as control had no inhibitory effects on the 15 microorganisms tested. Antimicrobial activities of extracts and EOs varied related to the test organisms. *C. origanifolium* extract had antibacterial activity against all Gram (+) bacteria tested except *S. aureus*. *C. origanifolium* extract was only active against *A. hydrophila*, *K. pneumoniae* and *P. aeruginosa*from Gram (-) bacteria tested. *C. origanifolium* extract was only

Table 4. Antimicrobial activities of *C. origanifolium* and *T. revolutus* essential oils and methanol extracts at different concentrations (inhibition zone diameter, mm[a]).

Microorganisms	*C. origanifolium*								*T. revolutus*							
	Essential oil (%)				Extract (%)				Essential oil (%)				Extract (%)			
	2	1	0.5	0.2	10	5	2.5	1	2	1	0.5	0.2	10	5	2.5	1
Gram (+)																
B. brevis	11.0±1	9.0±0	-	-	9.0±1	-	-	-	9.0±1	8.0±0	-	-	11.0±1	10.0±0	8.0±0	-
B. cereus	11.0±0	9.0±1	-	-	12.0±1	9.0±0	-	-	13.0±1	10.0±0	-	-	-	-	-	-
B. subtilis	10.0±1	9.0±1	-	-	16.0±1	10.0±1	-	-	10.0±2	9.0±1	-	-	12.0±2	10.0±1	8.0±0	-
*B. subtilis*var.*niger*	10.0±1	9.0±0	-	-	10.0±1	9.0±1	-	-	10.0±0	-	-	-	16.0±2	13.0±2	10.0±1	-
M. smegmatis	-	-	-	-	13.0±2	11.0±2	9.0±0	-	12.0±1	10.0±0	-	-	17.0±1	12.0±1	-	-
S. aureus	16.0±2	11.0±1	-	-	-	-	-	-	9.0±1	-	-	-	10.0±0	8.0±1	-	-
Gram (-)																
A. hydrophila	11.0±1	9.0±0	-	-	12.0±1	8.0±0	-	-	10.0±1	-	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-	-	10.0±1	-	-	-	-	-	-	-
K. pneumoniae	10.0±0	-	-	-	11.0±0	9.0±0	-	-	9.0±0	-	-	-	9.0±1	8.0±0	-	-
M. morganii	10.0±0	-	-	-	-	-	-	-	9.0±0	-	-	-	-	-	-	-
P. mirabilis	9.0±1	-	-	-	-	-	-	-	9.0±1	8.0±1	-	-	-	-	-	-
P. aeruginosa	-	-	-	-	14.0±1	11.0±2	9.0±1	-	12.0±2	10.0±1	-	-	18.0±2	15.0±2	-	-
Y. enterocolitica	-	-	-	-	-	-	-	-	-	-	-	-	11.0±1	8.0±1	-	-
Yeasts																
C. albicans	-	-	-	-	-	-	-	-	8.0±0	-	-	-	-	-	-	-
S. cerevisiae	9.0±0	-	-	-	9.0±1	-	-	-	11.0±0	-	-	-	-	-	-	-

-: ineffective

[a]: Includes diameter of hole (4 mm)

effective against *S. cerevisiae* from yeasts tested at 10 % concentration. *T. revolutus* extract showed antibacterial activity against all Gram (+) bacteria tested except *B. cereus*. Also, extract was only active against *K. pneumoniae, P. aeruginosa* and *Y. enterocolitica* from Gram (-) bacteria tested. *T. revolutus* extract was inactive against the yeasts including *C. albicans* and *S. cerevisiae* at all concentrations. Both of the extracts were inactive against microorganisms tested at 1 % concentrations. Generally, Gram (+) bacteria are more sensitive to the EOs and extracts tested than Gram (-) bacteria.

As far as our literature survey could ascertain, we could find two reports on the antimicrobial activity of *Cyclotrichium* in the literature [1,30]. Tepe *et al.* [30] previously stated that *C. origanifolium* EO was particularly possessed strong antimicrobial activity while other non-polar extracts and subfractions showed moderate activity, and polar extracts remained almost inactive. Alim *et al.* [1] reported that *C. niveum* EO showed remarkable antibacterial activity against *K. pneumoniae* and *S. aureus.*

Several studies have been conducted for antimicrobial activities of EOs and extracts from various *Thymus* species [11,14,16,25]. However, antimicrobial properties of *T. revolutus* extract do not seem to have been reported before. It was previously determined that *T. revolutus* EO exhibited a significant antibacterial and antifungal activity [16]. Recently, Dob *et al.* [11] found that *T. algeriensis* EO exhibited a significant *in vitro* antimicrobial activity against *B. subtilis*, as well as against two yeasts and two filamentous fungi tested. Hazzit *et al*. [14] showed that the EOs of *T. algeriensis, T. pallescens* and *T. dreatensis* possessed antimicrobial activity against *B. cereus, L. monocytogenes, Salmonella* sp., *S. aureus, Helicobacter pylori* and *C. albicans*. It was determined that the microorganisms most sensitive to *T. argaeus* EO were *A. hydrophila* and *P. aeruginosa*, while the microorganism most sensitive to the extract was *P. aeruginosa* [25]. Also, it was reported that the non-polar and polar subfractions of the methanol extract and EO of *T. spathulifolius* had great potential of antimicrobial activities [28]. The antibacterial activity of the extracts depended on the plant chemotype, extract preparation, solvent used and finally the sensitivity of bacteria.

Conclusions

Total phenolics, flavonols, flavanols, antioxidant, and antimicrobial activities of *T. revolutus* and *C. origanifolium* EOs and extracts constitutes enough evidence that these two herbs could be used as natural additives in various food products instead of artificial preservatives. In addition, they could potentially find a usage in pharmaceuticals, alternative medicine, and natural therapy. Further studies are needed to evaluate the *in vivo* potential of these EOs and extracts in animal models.

References

1. **Alim, A., Goze, I., Cetin, A., Atas, A.D., Vural, N. and Donmez, E. (2009).**Antimicrobial activity of the essential oil of *Cyclotrichium niveum*(Boiss.)Manden. Et Scheng. Afr. J. Microbiol. Res. 3: 422-425.
2. **Azaz, A.D., Irtema, H.A., Kurkcuoglu, M. and Baser, K.H.C. (2004).**Composition and the *in vitro* antimicrobial activities of the essential oils of some *Thymus* species. Z. Naturforsch. 59c: 75-80.
3. **Baser, K.H.C., Demirci, B., Demirci, F., Kirimer, N. and Hedge, I.C. (2001).** Microdistillation as a useful tool for the analysis of minute amounts of aromatic plant materials. Chem. Nat. Compd. 37: 336-338.
4. **Baser, K.H.C., Kirimer, N., Kurkcuoglu, M., Ozek, T. and Tumen, G. (1996).** Essential oil of *Cyclotrichium origanifolium* (Labill.) Manden. et Scheng. from Turkey. J. Essent. Oil Res. 8: 569-570.
5. **Baser, K.H.C., Sarikardasoglu, S. and Tumen, G. (1994).** The essential oil of *Cyclotrichium niveum* (Boiss.) Manden. et Scheng. J. Essent. Oil Res. 6: 9-12.

6. **Baytop, T. (1997).** *Turkcebitkiadlarisozlugu* (A dictionary of vernacular names of wild plants of Turkey), Ankara. Publication of the Turk Dil Kurumu (The Turkish Language Society) No: 578, Ankara-Turkey.
7. **Bounatirou, S., Smiti, S., Miguel, M.G., Faleiro, L., Rejeb, M.N., Neffati, M., Costa, M.M., Figueiredo, A.C., Barroso, J.G. and Pedro, L.G. (2007).** Chemical composition, antioxidant and antibacterial activities of the essential oils isolated from Tunisian *Thymus capitatus* Hoff. et Link. Food Chem. 105: 146-155.
8. **Butler, L.G., Price, M.C. and Brotherton, J.E. (1982).** Vanillin assay for proanthocyanidins (condensed tannins): modification of solvent estimation of the degree of polymerization. J. Agric. Food Chem.30: 1087-1089.
9. **Dai, G.H., Andary, C., Mondolot-Cosson, L. and Boubals, D. (1995).** Histochemical responses of leaves of *in vitro* plantlets of *Vitis* spp. to infection with *Plasmopara viticola*. Phytopathology. 85: 149-154.
10. **Davis, P.H. (1982).** Flora of Turkey and The East Aegean Islands, Edinburgh University Press, 7: 347-354, Edinburgh, UK.
11. **Dob, T., Dahmane, D., Benabdelkader, T. and Chelghoum, C. (2006).** Studies on the essential oil composition and antimicrobial activity of *Thymus algeriensis* Boiss.et Reut. Int. J. Aromatherapy. 16(2): 95-100.
12. **Doganca, S., Ulubelen, A. and Tuzlaci, E. (1989).** Flavanones from *Cyclotrichium niveum*. Phytochemistry. 28: 3561-3562.
13. **Dorman, H.J.D., Peltoketo, A., Hiltunen, R. and Tikkanen, M.J. (2003).** Characterisation of the antioxidant properties of de-odourised aqueous extracts from selected Lamiaceae herbs. Food Chem. 83: 255-262.
14. **Hazzit, M., Baaliouamer, A., Veríssimo, A.R., Faleiro, M.L. and Miguel, M.G. (2009).** Chemical composition and biological activities of Algerian *Thymus* oils. Food Chem. 116: 714-721.
15. **Hudaib, M., Speroni, E., Pietra, A.M.D. and Cavrini, V. (2002).** GC/MS evaluation of thyme (*Thymus* v*ulgaris* L.) oil composition and variations during the vegetative cycle. J. Pharmaceut. Biomed. 29: 691-700.
16. **Karaman, S., Digrak, M., Ravid, U. and Ilcim, A. (2001).** Antibacterial and antifungal activity of the essential oils of *Thymus revolutus* Celak from Turkey. J. Ethnopharmacol. 76: 183-186.
17. **Kosar, M., Dorman, H.J.D. and Hiltunen, R. (2005).** Effect of an acid treatment on the phytochemical and antioxidant characteristics of extracts from selected Lamiaceae species. Food Chem. 91: 525-533.
18. **Kumaran, A. and Karunakaran, R.J. (2007).** In vitro antioxidant activities of methanol extracts of five *Phyllanthus* species from India. LWT Food Sci. Technol. 40: 344-352.
19. **Lee, S.J., Umano, K., Shibamoto, T. and Lee, K.G. (2005).** Identification of volatile components in basil (*Ocimumbasilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties.Food Chem. 91: 131-137.
20. **Lee, S.K., Mbwambo, Z.H., Chung, H.S., Luyengi, L., Games, E.J.C., Metha, R.G., Kinghorn, A.D. and Pezzuto, J.M. (1998).** Evaluation of the antioxidant potential of natural products. Comb. Chem. High T. Scr. 1: 35-46.
21. **Miguel, G., Simoes, M., Figueiredo, A.C., Barroso, J.G., Pedro, L.G. and Carvalho, L. (2004).** Composition and antioxidant activities of the essential oils of *Thymus caespititius*, *Thymus camphoratus* and *Thymus mastichina*. Food Chem. 86: 183-188.
22. **Oktay, M., Gulcin, I. and Kufrevioglu, O.I. (2003).** Determination of *in vitro* antioxidant activity of fennel (*Foeniculum vulgare*) seed extracts. LWT Food Sci. Technol. 36: 263-271.
23. **Orhan, I., Senol, F.S., Gulpinar, A.R., Kartal, M., Sekeroglu, N., Deveci, M., Kan, Y. and Sener, B. (2009).** Acetylcholinesterase inhibitory and antioxidant properties of *Cyclotrichium*

niveum, Thymus praecox subsp. *caucasicus* var. *caucasicus*, *Echinacea purpurea* and *E. pallida*. Food Chem. Toxicol. 47: 1304-1310.

24. **Rasooli, I., Rezaei, M.B. and Allameh, A. (2006).** Growth inhibition and morphological alterations of *Aspergillus niger* by essential oils from *Thymus eriocalyx* and *Thymus x-porlock.* Food Control 17: 359-364.
25. **Sagdic, O., Ozkan, G., Aksoy, A. and Yetim, H. (2009).** Bioactivities of essential oil and extract of *Thymus argaeus*, Turkish endemic wild thyme. J. Sci. Food Agr. 89: 791-795.
26. **Schulz, H., Ozkan, G., Baranska M., Kruger, H. and Ozcan, M. (2005).** Characterisation of essential oil plants from Turkey by IR and Raman spectroscopy. Vib. Spectrosc. 39: 249-256.
27. **Singleton, V.L. and Rossi, J.A. Jr. (1965).** Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Viticult.16: 144-158.
28. **Sokmen, A., Gulluce, M., Akpulat, H.A., Daferera, D., Tepe, B., Polissiou, M., Sokmen, M. and Sahin, F. (2004).** The *in vitro* antimicrobial and antioxidant activities of the essential oils and methanol extracts of endemic *Thymus spathulifolius*. Food Control 15: 627-634.
29. **Tepe, B., Sokmen, M., Akpulat, H.A., Daferera, D., Polissiou, M. and Sokmen, A. (2005a).** Antioxidative activity of the essential oils of *Thymus sipyleus* subsp. *sipyleus*var. *sipyleus* and *Thymus sipyleus* subsp. *sipyleus* var. *rosulans*.J. Food Eng. 66: 447-454.
30. **Tepe, B., Sokmen, M., Sokmen, A., Daferera, D. and Polissiou, M. (2005b).** Antimicrobial and antioxidative activity of the essential oil and various extracts of *Cyclotrichium origanifolium* (Labill.) Manden. & Scheng. J. Food Eng. 69: 335-342.