Analysis of Essential Oil of *Coridothymus capitatus* (L.) and Its Antibacterial and Antifungal Activity

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The water-distilled essential oil the leaves of *Coridothymus capitatus* were analyzed by GC/MS and also analyzed by direct thermal desorption GC/MS. Comparison was made between two analyses techniques. The essential oil consisted mainly of monoterpenes 98.9 %, while oxygenated hydrocarbons were identified as 55.6 % and non-oxygenated hydrocarbons as 43.6 %. As major components were found carvacrol (35.6 %), *p*-cymene (21.0 %), thymol (18.6 %), γ-terpinene (12.3 %), α-terpinene (3.2 %), β-myrcene (3.0 %) and α-thujene (1.3 %) by hydrodistillation and by the GC/MS method. The direct thermal desorption GC/MS analysis also showed the same major components, namely carvacrol (51.6 %), thymol (21.7 %), *p*-cymene (9.7 %) γ-terpinene (8.2 %), α-terpinene (1.64 %). The essential oil of *C. capitatus* showed strong activity against *S. aureus*, *P. vulgaris*, *P. aeruginosa*, *E. coli*, *K. pneumonia*, *B. subtilis*, *E. faecalis*, *S. epidermidis* and *C. albicans*.

Key words: Coridothymus capitatus, Antimicrobial Activity of Essential Oil, Thermal Desorber GC/MS

Introduction

The aromatic plant of *Coridothymus capitatus* (L.) is very common member of Labiatae family and it is distributed in the Mediterranean area, especially Turkey, Greece and Spain (Davis, 1982; Kokkini and Vokou, 1989) and it's known in trade as "Spanish Origanum". Oregano species, *Coridothymus, Satureja, Origanum, Thymus* etc., have commercial importance in Turkey as well as in the world (8000 ton plants/year). These species are collected, their leaves are used as thyme and also essential oil of species exported (Satıl *et al.*, 2002). Their essential oils are also important for the perfume, cosmetic, flavoring and pharmaceutical industries. They are used in folk medicine against cold, influenza and throat infection in Turkey (Baytop, 1962; Tabata *et al.*, 1988).

Insecticidal (Karpouhtsis *et al.*, 1998), antioxidant (Demo *et al.*, 1998; Lagouri *et al.*, 1993), fungitoxic (Mullerriebau *et al.*, 1995), nematicidal (Oka *et al.*, 2000) activity studies and antibacterial effect of *C. capitatus* essential oil on potato storage (Vokou *et al.*, 1993), were reported along with their chemical composition (Karpouhtsis *et al.*, 1998; Fleisher and Fleisher, 2002; Ozek *et al.*, 1995). The essential oil of the Turkish *C. capitatus* was reported for three different localities which are Marmara island, Marmaris and Eceabat region of Turkey (Ozek *et al.*, 1995). In this study we report the analysis of the essential oil of *C. capitatus*, collected from Çeşme-İzmir, by hydrodistillation and thermal desorber GC-MS techniques and compare the results.

Materials and Methods

Plant material

Plant material of *C. capitatus* (L.) Reichb. fil. was collected from Çeşme-İzmir in July 2002. The plant was identified by Dr. Fatih Satıl of Balıkesir University, Turkey. A voucher specimen was deposited in the Herbarium of Department of Biology, Faculty of Arts and Science, Balıkesir University (F. S. 1037)

Isolation of essential oil

The essential oil of leaves of *C. capitatus* was obtained by hydrodistillation for 3 h in a Clev-

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enger type apparatus. The yield of oil was 1.1 % (v/w). The oil was dried over anhydrous Na_2SO_4, filtered by cotton and the oil stored at + 4 °C until analysis.

Thermal desorber analysis conditions

Perkin Elmer Turbomatrix ATD was used for thermal desorption analysis. The program and conditions are as follows: The tube temperature was 150 °C in analysis conditions. Transfer line and valve temperature was 150 °C. Tenax-TA (a porous polymer resin based on 2,6-diphenyleneoxide) was used for trapping, trap low temperature – 30 °C, trap high temperature 280 °C. Pneumatic program; inlet split 30 ml/min, outlet split 20 ml/min, desorb flow 30 ml/min, tube desorb time 10 min, trap hold time 5 min.

Gas chromatography/mass spectrometry

The essential oil composition were analyzed using a Fisons model GC8000 series gas chromatography and Fisons model MD800 mass spectrometry. DB5 fused silica column (60 m × 0.25 mm, ∅ with 0.5 μm film thickness) was used with helium at 1 ml/min (0.14 MPa) as carrier gas, GC oven temperature was kept at 40 °C for 5 min and programmed to 280 °C at rate of 5 °C/min and kept constant at 280 °C for 20 min. The split ratio was adjusted to 1:20 the injection volume was 0.1 μl. EI/MS were taken at 70 eV ionization energy. Mass range was from *m/z* 35–450 amu. Scan time 0.5 s with 0.1 s interscan delay. The library search carried out using NIST and Wiley GC-MS library and TÜBITAK-MRC library of essential oil. The relative percentage amount of separated compounds were calculated from total ion chromatogram by a computerized integrator.

Antimicrobial activity test

The essential oil of *C. capitatus* was tested against standard bacterial strains which are *S. aureus* ATCC 6538P, *P. vulgaris* ATCC 6897, *P. aeruginosa* ATCC 27853, *E. coli* ATCC 29995, *K. pneumonia* CCM 2318, *B. subtilis* ATCC 6633, *E. coli* ATCC 11230, *E. faecalis* ATCC 29212, *S. epidermidis* ATCC 12228 and the yeast *C. albicans* ATCC 10239. The microdilution procedure outlined by National Committee for Clinical Laboratory Standards was used for the determination of antimicrobial activities of essential oil of *C. capitatus*. The oil was dissolved in 10 % ethanol water solution to give a final concentration of 8.8 μg/ml. This solution was diluted within the range of 8.8–0.004 μg/ml using the 10 % ethanol water solution and were inoculated 20 μl onto each microplate containing 160 μl of Mueller Hinton Broth (Sabouraud dextrose both for the yeast *C. albicans*). Then 20 μl of 10^6 colony forming units (cfu/ml) (according to McFarland turbidity standards) of standardized microorganism suspensions were inoculated onto microplates. The same test was carried out with 10 % ethanol water solution as a control. End-point was determined after incubation at 37 °C for 24 h. The complete absence of growth was considered the minimum inhibitory concentration (MIC). 10 % ethanol water solution had no effect on the organisms. Gentamycin and fluconazole were used as reference compounds for antibacterial and antifungal activities (NNCLS, 1990).

Results and Discussion

Water-distilled essential oil from aerial parts of *C. capitatus* collected from Çesme-İzmir in Turkey and has been analyzed by GC and GC/MS. Furthermore, the aerial parts of *C. capitatus* were analyzed by thermal desorber GC/MS technique. The results are given in Table I.

The oil was shown to contain a mixture of components mainly monoterpenes together with small amount of sesquiterpenoids. 28 components were identified which represented 99.8 % of the total oils.

The analysis of essential oil composition of *C. capitatus* by hydrodistillation and GC/MS techniques was characterized by a high percentage of the monoterpene fraction amounting to 99.8 %, dominated by oxygenated monoterpenes 55.6 % and non-oxygenated components 43.6 %. The monoterpene fraction showed carvacrol (35.6 %), thymol (18.6 %), *p*-cymene (21.0 %), γ-terpinene (12.3 %), α-terpinene (3.2 %), β-myrcene (3.0 %) and other minor compounds as shown in Table I. Comparing the literature data of the analysis of essential oil of Greek *C. capitatus* (Karpouhtsis *et al.*, 1998), the essential oil has 81.5 % of carvacrol while the Turkish species has only 35.6 %. Other compounds in the Greek species were re-

Table I. Chemical Composition of the essential oil of *C. capitatus* by hydrodistillation and thermal desorber GC/MS techniques.

RT	Compounds	By hydrodistillation and GC-MS (%)	By thermal desorber GC-MS (%)
15.53	methyl isovalerate	t	t
15.58	methly 2-methyl butyrate	t	t
22.43	α-thujene	1.3	0.6
22.87	α-pinene	1.0	0.4
23.61	camphene	0.4	0.1
24.19	1-octen-3-ol	0.5	0.1
24.75	β-myrcene	3.0	1.6
24.82	unidentified	t	t
25.65	α-phellandrene	0.4	t
25.93	β-phellandrene	t	t
26.13	α-terpinene	3.2	1.6
26.49	*p*-cymene	21.0	9.6
26.63	limonene	0.5	0.2
26.70	α-terpinolone	t	t
26.75	3-octanol	0.2	0.1
26.93	*cis*-β-ocimene	t	t
27.74	γ-terpinene	12.3	8.2
28.91	linalool	0.5	0.5
30.16	*trans*-sabinene hydrate	t	0.3
32.03	isoborneol	0.3	0.7
32.24	4-terpineol	0.5	0.3
32.51	*cis*-sabinene hydrate	t	t
32.73	α-terpineol	t	t
32.90	pulegone	–	t
33.02	spathulenol	t	t
35.58	thymol	18.6	21.7
36.09	carvacrol	35.6	51.6
40.46	*trans*-caryophyllene	0.2	1.5
45.21	caryophyllene oxide	t	t
	total	99.8	99.5

RT: Retention time, t: < 0.1 %,

ported as *p*-cymene (6.4 %), γ-terpinene (2.2 %), and thymol (1.5 %). This difference is very important, because both species are grown on the two side of Aegean Sea, but the percentage of chemical composition of the plants are very different.

In this study we analyzed the essential oil composition of *C. capitatus* by thermal desorber GC/MS analysis technique. There is no study about analysis of essential oil composition of plants using direct thermal desorber GC/MS technique in the literature. Our objective was to compare the experimental results and to find the main compounds of the oil of plants without using the hydrodistillation technique and using a very small amount of the plant. According to thermal desorber analyses the main compounds were similar to the hydrodistillation technique. But the percentage of compounds were different from the essential oil which was obtained by hydrodistillation as carvacrol (51.6 %), thymol (21.7 %), *p*-cymen (9.6 %), γ-terpinene (8.2 %) and α-terpinene 1.6 %) (Table I) but, we can find the main composition of the plants by the direct thermal desorber analysis with only 30 mg of sample. This method gave very fast result for the prediction of the essential oil composition. If we want to analyze the essential oil composition of the 50 different plants, we can analyze them only at maximum 50 h and using 30 mg of sample for the each plants, 1.5 g of plant material is enough to find the essential oil composition of the 50 different plants.

The essential oil of *C. capitatus* leaves was tested against standard bacterial strains (see experimental part). The essential oil showed strong activity against all of them. MIC values were determined (Table II)as 2.2 μg/ml against *S. aureus* ATCC

Table II. Antimicrobial activity (MIC)[a] of essential oil of *C. capitatus*.

Microorganisms	Essential oil of *C. capitatus*	Gentamycin
S. aureus ATCC 6538 P	2.2	0.48
P. vulgaris ATCC 6897	1.1	0.24
P. aeruginosa ATCC 27853	8.8	0.97
E. coli ATCC 29995	2.2	3.9
K. pneumoniae CCM 2318	4.4	0.48
B. subtilis ATCC 6633	1.1	0.97
E. coli ATCC 11230	1.1	3.9
E. faecalis ATCC 29212	1.1	3.1
S. epidermidis ATCC 12228	2.2	7.8
C. albicans ATCC 10239	2.2	–

[a] Minimal inhibitory concentrations of the compounds are given as μg/ml.

6538 P, *E. coli* ATCC 29995, *S. epidermidis* ATCC 12228, 1.1 μg/ml against *P. vulgaris* ATCC 6897, *B. subtilis* ATCC 6633, *E. coli* ATCC 11230, *E. faecalis* ATCC 29212, 4.4 μg/ml against *K. pneumoniae* CCM 2318 and 8.8 μg/ml against *P. aeruginosa* ATCC 27853. Also the essential oil of *C. capitatus* showed highly strong activity against *C. albicans* ATCC 10239 as 2.2 μg/ml MIC value while MIC value of fluconazole, positive control, was only 15.6 μg/ml against the yeast. As a conclusion, the essential oil of the *C. capitatus* is a potential antibacterial and antifungal agent.

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