African Journal of Microbiology Research Vol. 3(8) pp. 422-425, August, 2009
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 ©2009 Academic Journals

Full Length Research Paper

Antimicrobial activity of the essential oil of *Cyclotrichium niveum* (Boiss.) Manden. Et Scheng

Ahmet Alim[1]*, Ismihan Goze[2], Ali Cetin[3], Ahmet D. Atas[4], Nilufer Vural[5] and Erol Donmez[6]

[1]Public Health Laboratory, Sivas Directorate of Health, Sivas-Turkey.
[2]Vocational School of Health Services, Cumhuriyet University, Sivas-Turkey.
[3]Department of Obstetrics and Gynecology, Cumhuriyet University School of Medicine, Sivas-Turkey.
[4]Public Health Laboratory, Tokat Directorate of Health, Tokat-Turkey.
[5]Science and Technology Research and Application Center, Bitaum, Ankara University, Ankara-Turkey.
[6]Department of Biology, Faculty of Science and Literature, Cumhuriyet University, Sivas-Turkey.

Accepted 7 July, 2009

The present study was designed to examine *in vitro* antimicrobial and antifungal activities of the essential oil of *Cyclotrichium niveum*. The major constituents of the essential oil were determined as pulegone (50.46%) and iso-menthone (34.53%). The antimicrobial activity of the oil was also tested against gram-positive and -negative bacteria and fungus using a disc-diffusion method and the minimal inhibitory concentration (MIC) values. The oil showed remarkable antibacterial activity against *Klebsiella pneumoniae* and *Staphylococcus aureus.* The essential oil exhibited also, strong antifungal activity against *Candida albicans*.

Key words: *Cyclotrichium niveum*, essential oil, antimicrobial activity.

INTRODUCTION

The essential oils and extracts of many plant species have become popular in recent years and attempts to characterize their bioactive principles have recently gained momentum in many pharmaceutical foods processing application (Sokmen et al., 1999; Reynolds, 1998). Many plants have been used for different purposes, such as food, drug and perfumery. The essential oils of the plants have been of great interest for their potential uses as alternative remedies for the treatment of many infectious diseases and pharmaceutical alternative medicine and natural therapies (Reynolds, 1998).

The development of bacterial resistance to presently available antibiotics has necessitated the search for new antibacterial agents (Bhattacharjee et al., 2005). Numerous researchers showed interest for biologically active components isolated from plants and for their influence on the elimination of pathogenic microorganisms (Tepe et al., 2005). The resistance which certain microorganisms have developed against antibiotics initiated antimicrobial investigations and different applications of essentials oils or plants against a wide range of bacteria (Gram-negative and Gram-positive) including antibiotic resistant species, fungal species and yeast (Jimanez -Arellanes et al., 2003; Hammer et al., 1999; Hammer et al., 1998; Nelson, 1997).

Cyclotrichium a member of the family labiatae, is a perennial plant endemic to Turkey. The *Cyclotrichium* genus is presented in Turkish flora by 5 species of which 2 are endemic and these endemic species growing in eastern Anatolia (Tepe et al., 2005a; Baser et al., 1994; Davis, 1988, 1982). One members of this genus *C. niveum* have been widely used as herbal tea in addition to its medicinal uses since ancient times. *C. niveum* is an annual herb used in the traditional medicine of Sivas (Turkey) for treating influenza, nausea and muscle pain disorders. The local name of this plant is “Dag Nanesi” in Turkish. The other endemic species *C. origanofolium* is widely used as flavoring agents in soup and salats in Turkey (Baytop, 1997). There is limited number of reports on the genus *Cyclotrichium spp* (Baser et al., 1994; Davis, 1988; Baser et al., 1996, 2001; Doganca et al., 1989). The chemical composition and antioxidant activity of *C. niveum* have

*Corresponding author. E-mail: alim58@gmail.com. Tel.: 90 346 2253514. Fax: 90 346 2245125.

previously been reported (Baser et al., 1994; Cetinus et al., 2007). But no information is available about the antimicrobial and antifungal nature of this plants essential oil. The aim of the present study was to asses the *in vitro* antimicrobial and antifungal activities of essential oil *C. niveum* from the Turkish flora.

MATERIALS AND METHODS

Plant material

C. niveum plants were collected from Divrigi (1200 m), Sivas-Turkey when flowering (late July, 2006). The taxonomic identification was made during flowering season 19/07/2006. The voucher specimen was identified by Dr. Erol Donmez at the department of Biology, Cumhuriyet University, Sivas-Turkey and has been deposited at the herbarium of the department of biology, Cumhuriyet University, Sivas-Turkey (CUFH-Voucher No: ED 9912).

Isolation of the essential oil

The air-dried and finely ground aerial parts of *C. niveum* were subjected for 3 h to water distillation using a Clevenger-type apparatus (yield 2.1% v/w). The essential oil obtained was dried over anhydrous sodium sulphate and after filtration, stored at +4°C until tested and analyzed. The amount of distilled material is obtained 9.3 g.

Gas chromatography (GC)/EIMS analysis

GC/EIMS analyses were performed with a varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 µm) and a varian saturn 2000 ion trap mass detector. Analytical conditions were: injector and transfer line temperatures 220 and 240°C, respectively, oven temperature programmed from 60 to 240°C at 3°C /min; carrier gas helium at 1 ml/min, injection of 0.2 µl (10% hexane solution), split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of n-hydrocarbons and on computer matching against commercial (NIST 98 and ADAMS) and homemade library mass spectra built up from pure substances and components of known oils and MS literature data (Adams, 1995; Davies, 1990; Jennings et al., 1980; Massada, 1976; Stenhagen et al., 1974; Swigar and Silverstein, 1981). Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI^- ionizing.

Antimicrobial activity

Microbial strains

Antimicrobial and antifungal activities of the essential oil was evaluated against 3 Gram-positive and 5 Gram-negative bacteria, 1 fungus by the disk diffusion method. The microorganisms were used *Staphylococcus aureus* ATCC-25923**,** *Escherichia coli* ATCC-35218, *Pseudomonas aeruginosa* ATCC-27853, *Salmonella thyphi* NCTC-9394, *Klebsiella pneunomonia* NCTC-5046, *Proteus vulgaris* RSHM-96022, *Bacillus subtilis* ATCC-6633, *Corynebacterium diphteriae* RSHM-633 and *Candida albicans* ATCC-10231. Cultures were obtained from the culture collections of the department of Health of Refik Saydam Hygiene Center Contagious Diseases Research Department (Ankara-Turkey). Bacterial strains were cultured overnight at 37°C in Mueller Hinton Agar (MHA-Oxoid-CM337). The yeast was cultured overnight at 30°C in Sabouraud Dextrose Agar (SDA-Oxoid-CM41). All the experiments were carried out in triplicate and average and standard deviation (SD) were calculated for the inhibition zone diameters.

Disc diffusion method

Agar disc diffusion method was employed for the determination on antimicrobial activities of the essential oil in question (NCCLS, 1997; NCCLS, 1999). Briefly, a suspension of the tested micro-organism (0.1 ml 10^8 cells per ml) was spread on the solid media plates. Filter paper discs (6 mm in diameter) were impregnated with 10 µl of the oil and placed on the inoculated plates. These plates, after staying at 4°C for 2 h, were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for the yeast. The diameters of the inhibition zones were measured in milimeters.

Determination of minimum inhibitory concentration

A broth microdilution susceptibility assay was used, as recommended by NCCLS, for the determination of MIC (NCCLS, 1997; NCCLS, 1999). All tests were performed in Mueller Hinton Broth (MHB; OXOID-CM405) with the exception of the yeasts (sabouraud dextrose broth-SDB; DIFCO). Bacterial strains were cultured overnight at 37°C in Mueller Hinton Agar (MHA) and the yeasts were cultured overnight at 30°C in Sabouraud Dextrose Agar (SDA). Test strains were suspended in MHB to give a final density of 5 × 105 cfu/ml and these were confirmed by viable counts. Geometric dilutions ranging from ½ µg/ml to 1/6, 400 µg/ml of the extract were prepared in a 96 well microtiter plate, including one growth control and one sterility control. Plates were incubated under normal atmospheric conditions at 37°C for 24 h for bacteria and at 30°C for 48 h for the yeasts. The MIC of amikacin, clindamycine and ciprofloxacine was individually determined in parallel experiments in order to control the sensitivity of the test organisms. Bacterial growth was indicated by the presence of a white “pellet” on the well bottom.

RESULTS AND DISCUSSION

Chemical composition of the essential oil

According to gas chromatography (GC)/EIMS analysis, 29 (96.98%) compounds were identified in *C. niveum* essential oil (Table 1). Among the constituents identified, pulegone (50.46%) and isomenthone (34.53%) were the major ones. These are followed by limonene, 1,8-cineole, menthone, Y-elemene and α-pinene in small quantities.

To the best of our knowledge, essential oil composition of *C. niveum* has previously been reported elsewhere (Orhan et al., 2009; Cetinus et al., 2007; Baser et al., 1994). According to these reports, isomenthone and pulegone are the main compounds of the oils. From this point of view, results obtained from this part are highly in agreement with the previous studies.

Antimicrobial activity

There is a need for the discovery of new substances from natural sources, including plants. Although several studies are available on the chemical composition, antifungal, antiradical, antioxidant and antibacterial activities of different *Cyclotricium* species (Kaya et al., 2000;

Table 1. Chemical composition of the essential oil of *C. niveum.*

No	LRI[a]	Compounds	Composition (%)
1	802	hexane [b]	0.02
2	939	α-pentene [c]	1.46
3	954	camphene [c]	0.04
4	975	Sabinene [b]	0.12
5	978	*β* -pentene [c]	0.34
6	991	Myrcene [c]	0.04
7	1017	α-terpinene [c]	0.05
8	1025	*p*-cymene [c]	0.10
9	1029	Limonene [b]	2.11
10	1031	1,8-cineole [c]	1.91
11	1036	(E)-β-Ocimene [b]	0.03
12	1037	(Z)-β -Ocimene [b]	0.10
13	1070	*cis*-sabinene hydrate [b]	0.03
14	1097	Linalool [c]	0.09
15	1101	Nonanal [b]	0.05
16	1102	*Cis*-thujone [b]	0.07
17	1153	Menthone [c]	1.34
18	1162	Isoborneol [b]	0.08
19	1189	α-terpineol [c]	0.04
20	1237	Pulegone [c]	50.46
21	1253	Piperitenone [b]	1.42
22	1290	thymol [c]	0.06
23	1295	Isomenthone [c]	34.53
24	1299	carvacrol [c]	0.05
25	1359	Eugenol [c]	0.02
26	1388	*β*-Bourbonene [b]	0.09
27	1437	γ-elemene [b]	1.53
28	1485	Germacrene D [c]	0.35
29	1578	Spathulenol [c]	0.45
		Total	**96.98**

[a] LRI, Linear Retention Indices (HP-5 column); [b] Tentative identification; [c] Identification of components based on standard compounds.

Table 2. Antimicrobial and antifungal activity of the essential oil of *C niveum* using agar disc diffusion method and minimal inhibition consentration (MIC).

Micro-organisms	DD [a]	Gen [b]	Nys [c]	MIC [d]
S. aureus	47.00 ± 1.25	23 ± 0.76	-	3.12
E. coli	12.00 ± 0.76	16 ± 0.96	-	25.00
P. aeruginosa	6.00 ± 0.00	20 ±1.06	-	125.00
S. thyphi	14.00 ± 1.26	10 ± 0,45	-	16.66
K. pneumonia	48.00 ± 1.16	20 ± 0.70	-	2.94
P. vulgaris	12.00 ± 1.08	22 ± 1.40	-	10.25
B. subtilis	17.00 ± 1.01	29 ± 1.15	-	12.50
C. diphteriae	16.00 ± 0.85	23 ± 1.10	-	12.50
C. albicans	45.00 ± 1.85	-	25 ± 0.90	3.12

[a] DD, agar disc diffusion method. Diameter of inhibition zone (mm); including disk diameter of 6 mm; [b] Gentamycin (antibacterial); [c] Nystatin (antifungal); [d] MIC: Minimal Inhibitory Concentrations as (µg/ml).

Sanon et al., 2007; Aslan et al., 2007; Masoudi et al., 2006). However, only a few studies have been carried out with *C. niveum* (Orhan et al., 2009; Cetinus et al., 2007; Baser et al., 1994).

As far as our literature survey could ascertain, we could reach no reports on antimicrobial activity of *C. niveum* essential oil except a study on antibacterial and antifungal effect of *C. niveum* extract (Gulcin et al., 2008).

The *in vitro* antimicrobial activity carried out by the agar disc diffusion method and minimum inhibitory concentration (MIC) values of the essential oil resulted in a range of growth inhibition pattern against pathogenic microorganisms Table 2.

In the present study the result showed that 10 µl *C. niveum* essential oil inhibited the growth of 2 pathogenic bacteria and pathogenic yeast.

Gulcin et al. (2008) examined the antimicrobial activities of ethanol and water extract of *C. niveum*. According to this study, no remarkable activity profile had been observed with ethanol and water extracts. Additionally in this study, extract showed weak antioxidant activity capacity. On the other hand, according to a study carried out by Ozbay et al. (2009), *C. niveum* extracts having several polarities showed weak antimicrobial activities.

To the best of our knowledge, some reports were found regarding pulegone which is the main compound of the oil sample examined here. According to these studies, pulegone have been reported as a more active antimicrobial compound than limonene and metnhone (Oumzil et al., 2002; Flamini et al., 1999).

We could also reach another report in the literature about the antimicrobial activity of *C. origanifolium* which is another endemic species (Tepe et al., 2005a). According to this study, essential oil of *C. origanofolium* has weak antimicrobial activity. That is characterized by sensitivity of only a few species such as *C. albicans, C. krusei* and *Mycobacterium smegmatis*.

In the case of minimal inhibitory concentrations (MIC), essential oil of *C. niveum* showed remarkable antimicrobial activity against two pathogenic bacteria and a pathogenic yeast. Sensitive microorganisms were *K. pneunomonia, S. aureus* and *Candida albicans* in decreasing sensitivity, respectively. The weakest activity was observed against *Escherichia coli*. Among the test microorganisms, the most resistant was *P. aeruginosa*.

This study could be assumed as the first report on the antimicrobial activity of the essential oil of *C. niveum*. Due

to the respectable antimicrobial activity results, this plant may be regarded as a natural source that can be freely used in the food industry as a culinary herb. We hope that our results will provide a starting point for investigations designed new natural antimicrobial essential oil of this plant species.

ACKNOWLEDGEMENT

The authors would like to thank Prof. Dr. Osman Cakmak for his contribution to this work.

REFERENCES

Adams RP (1995). Identification of essential oil components by gas chromatography/mass spectroscopy. Carol Stream: Allured.

Aslan S, Firat M, Konuklugil B (2007). Essential oil of *Cyclotrichium longiflorum* leblebici. Chem. Nat. Compd. 43(6): 727-725.

Baser KHC, Demirci B, Demirci F, Kirimer N, Hedge IC (2001). Micro-distilasyon as a Useful Tool for the Analysis of Minute Amounts of Aromatic Plant Materials. Chem. Nat. Comp. 37: 336-338.

Baser KHC, Kirimer, N, Kurkcuoglu M, Ozek T, Tumen G (1996). The Essential Oil of *Cyclotrichium origanifolium* (Labill.) Manden. et Scheng. from Turkey. J. Essent. Oil Res. 8: 569-570.

Baser KHC, Sarikardasoglu S, Tumen G (1994). The Essential Oil of *Cyclotrichium niveum* (Boiss.) Manden et Scheng. J. Essent. Oil Res. 6(1): 9-12.

Baytop T (1997). Turkce bitki adlari sozlugu (A dictionary ofvernacular names of wild plants of Turkey), Ankara. Publication of the Turk Dil Kurumu (The Turkish Language Society) p. 578.

Bhattacharjee I, Ghosh A, Chandra G (2005). Antimicrobial activity of the essential oil of *Cestrum diurnum* (L.) (Solanales: Solanaceae). Afr. J. Biotechnol. 4: 371-374.

Cetinus AS, Goze I, Sarac B, Vural N (2007). Scavenging effect and antispasmodic activity of the essential oil of *Cyclotrichium niveum*. Fitoterapia 78: 129-133.

Davies NW (1990). Gas chromatographic retention indexes of monoterpenes and sesquiterpenes on methyl silicone and carbowax 20 M phases. J. Chromatogr. 503: 1–24.

Davis PH (1988). Flora of Turkey and the East Aegean Islands, (supplement-I). Edinburgh University Press: Edinburgh. 10: 538.

Davis PH (1982). Flora of Turkey and the East Aegean Islands. Edinburgh University Press: Edinburgh 7: 349.

Doganca S, Ulubelen A, Tuzlacı E (1989). Flavanones from *Cyclotrichium niveum*. Phytochem. 28(12): 3561-3562.

Flamini G, Cioni PL, Puleio R, Morelli I, Panizzi L (1999). Antimicrobial activity of the essential oil of *Calamintha nepeta* and its constituent pulegone against bacteria and fungi. Phytother. Res. 13: 349-351.

Gulcin I, Tel AZ, Kirecci E (2008). Antioxidant, Antimicrobial, Antifungal, and Antiradical Activities Of *Cyclotrichium niveum* (Boiss.) Manden And Schengi. Int. J. Food Properties 11: 450-471.

Hammer KA, Carson CF, Riley TV (1999). Antimicrobial activity of essential oils and other plant extracts. J. Appl. Microbiol. 86: 985-990.

Hammer KA, Carson CF, Riley TV (1998). *In vitro* activity of essential oils, in particular *Melaleuca alternifolia* (tea tree) oil and tea tree oil products, against *Candida spp*. J. Antimicrob. Chemother. 42: 591-595.

Jennings W, Shibamoto T (1980). Qualitative analysis of .avor and fragrance volatiles by glass capillary chromatography. New York: Academic Pres.

Jimenez-Arellanes A, Meckes M, Ramirez R, Torres J, Luna-Herrera J (2003). Activity against multidrug-resistant *Mycobacterium tuberculosis* in Mexican plants used to treat respiratory diseases. Phytother. Res. 17: 903-908.

Kaya A, Başer KHC (2000). Morphological and Anatomical Studies on *Cyclotrichium origanifolium* (Labill.) Manden. & Scheng. (Labiatae). Turk J. Bot. 24: 273-278.

Masoudi S, Rustaiyan A, Azar PA, Larijani K (2006). Composition of the Essential Oils of *Cyclotrichium straussii* (Bornm.) Rech. f. and Phlomis pungens Willd. from Iran. J. Essent. Oil Res. Jan-Feb. 18(1): 16-18.

Massada Y (1976). Analysis of essential oils by gas chromatography and mass spectrometry. New York: John Wiley & Sons.

NCCLS (National Committee for Clinical Laboratory Standards) (1999). Performance standards for antimicrobial susceptibility testing. 9th International Supplement. M100-S9, Wayne Pa.

NCCLS (National Committee for Clinical Laboratory Standards) (1997). Performance standards for antimicrobial disk susceptibility test. 6th ed. Approved Standard. M2-A6, Wayne Pa.

Nelson RRS (1997). *In-vitro* activities of five plant essential oils against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium*. J. Antimicrob. Chemother. 40: 305-306.

Orhan I, Senol FS, Gulpinar AR, Kartal M, Sekeroglu N, Deveci M, Kan Y, Sener B (2009). Anticholinesterase inhibitory and antioxidant properties of Cyclotr*ichium niveum, Thymus praecox* subsp. *caucasicus* var. *caucasicus*, *Echinacea purpurea* and *E. pallida*. Food Chem. Toxicol. 47: 1304-1310.

Oumzil H, Ghoulami S, Rhajaoui M, Hidrissi A, Fkih-Tetouani S, Faid M, Benjouad A (2002). Antibacterial and antifungal activity of essential oils of *Mentha suaveolens*. Phytother. Res. 16: 727-731.

Ozbay H, Alim A (2009). Antimicrobial Activity of Some Water Plants from the Northeastern Anatolian Region of Turkey. Molecules 14: 321-328.

Reynolds JEF (1998). Martindale: The Extra Pharmacopeia (31st ed.). London: Royal Pharmaceutical Society of Great Britain. pp. 592-601.

Sanon B, Azaz DA, Dirmenci T (2007). Identified Saprophytic Microfungi on the *Cyclotrichium* (Boiss.) Manden.&Scheng. species Distributed in Turkey. J. Appl. Biol. Sci. 1(3): 57-59.

Sokmen A, Jones BM, Erturk M (1999). The *in vitro* antibacterial activity of Turkish medicinal plants. J. Ethnopharm. 67: 79-86.

Stenhagen E, Abrahamsson S, McLafferty FW (1974). Registry of mass spectral data. New York: John Wiley & Sons.

Swigar AA, Silverstein RM (1981). Monoterpenes. Milwaukee: Aldrich Chem. Compd. pp 3, 5, 13, 59, 72-78.

Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M (2005). Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). Food Chem. 90: 333-340

Tepe B, Sokmen M, Sokmen A, Daferera D, Polissiou M (2005a). Antimicrobial and antioxidant activities of the essential oil and various extracts of *Cyclotrichium origanifolium* (Labill.) Manden. and Scheng. J. Food Eng. 69: 335-342.