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

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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
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 were evaluated against 3 Gram-positive and 5 Gram-negative bacteria, 1 fungus and 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 pneumonia nCTC-5046, Proteus vulgaris RSH-M 6022, Bacillus subtilis ATCC-6633, Corynebacterium diphtheriae RSH-M 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 microorganism (0.1 ml 10^6 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 millimeters.

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 x 10^5 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 ciprofloxacin 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.
growth inhibition pattern against pathogenic microorganisms. Disk diffusion method and minimum inhibitory concentration (MIC) values of the essential oil resulted in a range of effect of the essential oil except a study on antibacterial and antifungal activity of C. niveum (Baser et al., 1994).

However, only a few studies have been carried out in vitro antimicrobial activity carried out by the agar disc diffusion method and minimum inhibitory concentration (MIC) of the essential oil sample examined here. According to these studies, extract showed weak antioxidant activity capability. On the other hand, according to a study carried out by Ozbay et al. (2009), the essential oil of C. niveum has been reported as a more active antimicrobial compound than limonene and menthone (Oumzil et al., 2008) regarding pulegone which is the main compound of the oil sample examined here. According to these studies, pulegone has been reported as a more active antimicrobial compound than limonene and metnhome.

As far as our literature survey could ascertain, we could 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, no remarkable activity profile had been observed with ethanol and water extracts. Additionally in this study, extract showed weak antioxidant activity capability. 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 has been reported as a more active antimicrobial compound than limonene and metnhome (Oumzil et al., 2002; Flamini et al., 1999).

We could also reach another report in the literature about the antimicrobial activity of C. origanofolium which is another endemic species (Tepe et al., 2005a). According to this study, the 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), the essential oil of C. niveum showed remarkable antimicrobial activity against two pathogenic bacteria and a pathogenic yeast. Sensitive microorganisms were K. pneumonia, 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.

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 capability. 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.

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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.

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REFERENCES


