Asian Journal of Chemistry Vol. 20, No. 8 (2008), 6518-6522

Antibacterial Activity of Three Endemic *Micromeria* Species

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The antibacterial activity of *Micromeria cilicica* Hausskn. ex P.H.Davis, *Micromeria dolichodontha* P.H. Davis and *Micromeria cremnophila* subsp. *amana* (Rech. fil.) P.H. Davis was studied using the disk diffusion method and determination of minimum inhibitory concentration (MIC) values against *Staylococcus aureus* ATCC 6538P, *Streptococcus sanguis* PTCC 1449, *Escherichia coli* ATCC 11230, *Pseudomonas aeruginosa* ATCC 27583 and *Klebsiella pneumoniae* UC57. The methanol and chloroform extracts of the aerial parts of *M. cilicica*, *M. dolichodontha* and *M. cremnophila* subsp. *amana* exhibited concentration-dependent antibacterial activity against all tested bacteria.

Key Words: Antibacterial activity, *Micromeria cilicica*, *Micromeria dolichodontha*, *Micromeria cremnophila* subsp. *amana*.

INTRODUCTION

The genus *Micromeria* is a member of Labiatae family. This genus has 14 species and 22 taxa in Turkey and 12 of them are endemic[1]. Also these species are grown naturally in Turkey. Micromeria species are used against heart disorders, headache, wounds and skin infections and the most usage of this genus are in colds[2,3]. The leaves from some *Micromeria* species are utilized a seasoning for food preparation in Turkey[2,4-6].

Pulegone, isomenthone, *p*-menthone, limonene, linalol, α-pinene, β-pinene, *p*-cymene, α-terpinene, γ-terpinene, α-terpineol, camphene, β-bourbonene and borneol are the most encountered essential components in *Micromeria* species[7].

Micromeria cilicica Hausskn. ex P.H. Davis, *M. dolichodontha* P.H. Davis and *M. cremnophila* subsp. *amana* (Rech. fil.) P.H. Davis are endemic to Turkey[1]. The present aim is to determine the antimicrobial effects of plant extracts obtained from these endemic species against microorganisms.

EXPERIMENTAL

The aerial parts of *Micromeria cilicica* Hausskn. ex P.H. Davis, *M. dolichodontha* P.H. Davis and *M. cremnophila* subsp. *amana* (Rech. fil.) P.H. Davis were collected from different localities of Turkey during the months of May-July 2007. Voucher specimens were deposited in the Herbarium of Biology Department in Canakkale Onsekiz Mart University, Canakkale, Turkey.

Plant extraction: Dried plant materials were ground to fine powder. 100 g of the powders were separetely extracted twice with methanol and chloroform. The extract 40 °C and stored at -20 °C.

Detection of antibacterial activity: *in vitro* Antibacterial studies were carried out by the disk diffusion method and minimum inhibitory concentration (MIC) values were determined against test microorganisms[8,9]. In the disk diffusion method, extracts were dissolved in methanol and applied to 6 mm diameter paper disk. The extracts were tested at 10, 50, 100, 250, 500, 750 and 1000 µg/disk. Inhibition zone diameter were measured after 24 h. Gentamycin (50 µm/disk) and amikacin (3 µg/disk) (both obtained from Sigma, USA) were used as positive method at concentrations of 10, 50, 100, 250, 500 and 750 µg/mL and 1, 10 and 25 mg/mL of culture medium. Gentamycin (2 mg/mL) was used as positive control.

Microorganims: *Staphylococcus aureus* ATCC 6538P, *Streptococcus sanguis* PTCC 1449, *Escherichia coli* ATCC 11230, *Pseudomonas aeruginosa* ATCC 27583 and *Klebsiella pneumoniae* UC57 were used for testing the antibacterial activity.

RESULTS AND DISCUSSION

The yield of methanol extacts of *Micromeria cilicica, M. dolichodontha* and *M. cremnophila* subsp. *amana* was 32.4, 22.6 and 24.8 %, respectively. The yield of chloroform extracts of *Micromeria cilicica, M. dolichodontha* and *M. cremnophila* subsp. *amana* was 18.2, 12.5 and 13.7 %, respectively.

Tables 1-3 give a summary of the *Micromeria* species investigated and the results of the antibacterial screening. The methanol and chloroform extracts of the aerial parts of *M. cilicica, M. dolichodontha* and *M. cremnophila* subsp. *amana* exhibited concentration-dependent antibacterial activity against all bacteria tested. The methanol and chloroform extracts were more active against Gram negative bacteria. The chloroform extracts were found to be effective against all tested strains.

As can be seen from literature data, the constituents of essential oil and *in vitro* antimicrobial activity of *Micromeria cilicica* were have previously been reported[7]. As a result of that study, the major component characterized in the essential oils was pulegone and other main components were determined as *cis-p*-menthone, *trans-p*-menthone, nerol and 3-octonol, respectively.

TABLE-1
ANTIBACTERIAL ACTIVITY OF THE METHANOL EXTRACTS OF *Micromeria cilicica*, *Micromeria dolichodontha* AND *Micromeria cremnophila* subsp. *Amana* [a]

Microorganisms	Diameter of zone of inhibition (mm)																						
	Micromeria cilicica (µg/disk)							*Micromeria dolichodontha* (µg/disk)							*Micromeria cremnophila* subsp. *amana* (µg/disk)								
	10	50	100	250	500	750	1000	10	50	100	250	500	750	1000	10	50	100	250	500	750	1000	Gn	Am
S. aureus (G+)	8.7	11.2	11.6	12.2	12.6	13.2	13.6	-	8.1	8.6	9.2	9.6	10.2	12.2	9.2	9.6	10.8	12.4	13.2	14.0	15.8	28.8	26.2
S. sanguis (G+)	10.4	11.7	12.2	12.8	13.6	14.2	15.8	-	-	8.4	8.8	9.6	10.4	11.8	10.2	10.8	11.6	12.4	13.8	14.4	15.6	23.4	20.8
E. coli (G-)	8.1	8.3	8.6	9.2	9.4	10.2	11.0	-	-	-	8.0	8.8	9.2	10.0	11.2	12.4	13.8	14.6	15.2	15.8	16.7	25.6	19.2
P.aeruginosa (G-)	9.6	10.2	11.4	11.8	12.2	12.8	13.4	8.2	8.6	9.0	9.6	10.2	10.4	11.0	8.8	11.6	13.0	13.6	14.7	15.3	16.2	24.2	18.4
K. pneumoniae (G-)	10.2	11.0	11.8	12.6	13.4	14.2	15.6	-	8.0	8.6	9.2	9.6	10.2	10.6	9.6	10.8	12.4	13.8	14.6	15.6	16.6	36.4	30.6

[a]Zone of inhibition, including the diameter of the filter paper disk (6 mm); mean value of eight independent experiments; Gn (Gentamycin) (50 µg/disk) and Am (amikacin) (3 µg/disk) were used as positive controls; – no inhibition.

TABLE-2
ANTIBACTERIAL ACTIVITY OF THE CHLOROFORM EXTRACTS OF *Micromeria cilicica*, *Micromeria dolichodontha* AND *Micromeria cremnophila* subsp. *amana* [a]

Microorganisms	Diameter of zone of inhibition (mm)																						
	Micromeria cilicica (µg/disk)							*Micromeria dolichodontha* (µg/disk)							*Micromeria cremnophila* subsp. *amana* (µg/disk)								
	10	50	100	250	500	750	1000	10	50	100	250	500	750	1000	10	50	100	250	500	750	1000	Gn	Am
S. aureus (G+)	9.2	9.6	10.0	10.8	11.4	12.8	14.6	-	8.0	8.4	8.8	9.4	10.2	12.6	10.8	11.4	12.2	13.0	14.2	15.8	17.2	28.8	26.2
S. sanguis (G+)	10.8	11.6	12.8	14.0	15.2	16.4	18.8	-	8.2	8.6	9.2	10.0	10.2	12.8	9.8	10.8	11.4	12.8	13.2	15.2	17.8	23.4	20.8
E. coli (G-)	10.6	11.4	11.8	12.6	13.2	14.8	16.2	-	-	8.2	9.4	9.8	10.6	12.0	10.4	11.2	12.8	13.2	14.8	15.6	16.8	25.6	19.2
P.aeruginosa (G-)	8.4	8.8	9.6	10.2	10.8	11.4	11.8	8.8	9.4	9.8	10.6	11.2	12.4	13.8	11.8	12.8	13.6	14.4	15.0	17.2	18.4	24.2	18.4
K. pneumoniae (G-)	10.0	10.8	11.4	11.8	12.6	13.2	14.6	8.4	9.0	9.6	10.2	11.4	12.0	12.8	10.2	11.4	12.6	13.2	14.4	15.4	17.0	36.4	30.6

[a]Zone of inhibition, including the diameter of the filter paper disk (6 mm); mean value of eight independent experiments; Gn (Gentamycin) (50 µg/disk) and Am (amikacin) (3 µg/disk) were used as positive controls; – no inhibition.

TABLE-3
MINIMUM INHIBITORY CONCENTRATION (MIC) OF *Micromeria cilicica*, *Micromeria dolichodontha* AND *Micromeria cremnophila* subsp. *amana* [a]

Microorganisms	MIC (mg/mL)					
	Micromeria cilicica (µg/disk)		*Micromeria dolichodontha* (µg/disk)		*Micromeria cremnophila* subsp. *amana* (µg/disk)	
	Methanol extract	Chloroform extract	Methanol extract	Chloroform extract	Methanol extract	Chloroform extract
S. aureus (G+)	40	25	40	40	10	1
S. sanguis (G+)	25	10	40	40	10	1
E. coli (G-)	25	10	40	40	25	10
P.aeruginosa (G-)	40	40	40	25	10	1
K. pneumoniae (G-)	40	25	40	40	10	1

[a]All determination were done in triplicate; Gn (gentamycin) (2 mg/mL) was used as positive control.

Essential oils obtained by hydro and steam distillation and organic solvent extracts of the aerial parts of the plant were investigated for antimicrobial activities on several microorganisms including bacteria and yeast. The extracts and pulegone exhibited a significant antibacterial and antifungal activity. The activities were increased depend on the amount of extracts and pulegone. The results in this study are similar to those reported in the mentioned studies.

Although chemical composition of *M. dolichodontha* and *M. cremnophila* subsp. *amana* has been reported, their antimicrobial activities have not been investigated. Composition of the essential oil of *M. dolichodontha* was previously determined[10]. 58 Components were characterized, representing 98.9 % of the total oil. Isomenthone (23.5 %) and pulegone (14.9 %) were the major constituents of the oil. In another study, composition of the essential oil of *M. cremnophila* subsp. *amana* was reported[11]. Water-distilled oil from the aerial parts of this plant was analyzed and seventy components were characterized, representing 91.5 % of the total components detected, with germacrene D (24 %) and β-caryophyllene (23 %) as major constituents.

The screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of new antiinfective agents[12-14]. The results obtained in this study provided evidence that the *Micromeria* species are potentially a rich source of antimicrobial agents against bacteria. Hence, the extracts of *Micromeria* species used in this study may be useful as an alternative antimicrobial agent in natural medicine for the treatment of many infectious diseases. More pharmacological investigations are necessary.

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(*Received*: 17 March 2008; *Accepted*: 17 July 2008) AJC-6714