Asian Journal of Chemistry; Vol. 23, No. 1 (2011), 416-418

ASIAN JOURNAL OF CHEMISTRY

www.asianjournalofchemistry.co.in

Antibacterial Activity of *Ballota acetabulosa* Against Methicillin-Resistant *Stapylococcus aureus*

BASARAN DULGER[1,*] and MEHMET ALI KILCIK[2]

[1]Department of Biology, Faculty of Science and Arts Canakkale Onsekiz Mart University, 17100 Canakkale, Turkey
[2]Canakkale Anadolu Hospital, 17100 Canakkale, Turkey

*Corresponding author: E-mail: basarandulger@yahoo.com

(*Received*: 7 May 2010; *Accepted*: 30 August 2010) AJC-9070

Aqueous and ethanolic extracts obtained from *Ballota acetabulosa* (L.) Benth. (Lamiaceae) used as traditional medicine in Turkey have been investigated for their ability to inhibit 35 hospital isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA). Both aqueous and ethanolic extracts were shown antimicrobial activity against MRSA isolates. The minimum inhibitory concentration (MIC) values of the ethanolic extract with the greatest antibacterial effect were those of the plant MIC 0.4-1.6 µg/mL and MBC 3.2-12.5 µg/mL, respectively.

Key Words: Antibacterial activity, *Ballota acetabulosa*, Methicillin-resistant *Staphylococcus aureus*.

INTRODUCTION

Herbal medicine has been improved in developing countries as an alternative solution to health problems and costs of pharmaceutical products. The development of drug resistance in human pathogens against antibiotics has necessitated a search for new antimicrobial substances from other sources, including plants. Plants used for traditional medicine contain a wide range of substances that are used to treat chronic as well as infectious diseases[1].

The genus *Ballota* L. (Lamiaceae) consist of about 33 species growing mainly in the Mediterranean region. In Turkey, the genus *Ballota* is reprensented by 11 species, 6 subspecies, 10 of which are endemic[2]. Plants of this genus have been used traditionally for neusea, vomiting, nervous dyspepsia, specifically for vomiting of central origin and are used for antiemetic, sedative, antibacterial and mild astringent properties[3,4].

Ballota acetabulosa (L.) Benth. is a herbaceous plant growing in rocks and rough ground in dry hills up to 900 m in Greece and Western Anatolia[5]. During our field excursions, it was determined that these plants have been used externally in the treatment of wounds and burns. Aerial parts of the plant are used internally to treat inflammation, to suppress cough and against gastrointestinal disorders. So, the aim of this works is to evaluate the antimicrobial activity of the plant as wild-growing in Turkey.

EXPERIMENTAL

Aerial parts of the plant were collected from Gokceada, Canakkale, Turkey in September, 2009. Voucher specimens of the plant were deposited in the Biology Department at Canakkale Onsekiz Mart University (Canakkale, Turkey).

Preparation of extracts: Aerial parts of the plants were air-dried. Each dry powdered plant material (20 g) was extracted with 150 mL of 80 % ethanol (Merck, Darmstadt, Germany) and 150 mL of distilled water for 24 h by using Soxhlet equipment, separately[6]. The extract was filtered using Whatman filter paper No. 1 and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55 ºC. Dried extracts were stored in labeled sterile screw capped bottles at -20 ºC.

Microorganisms: Thirty-five clinical isolates of methicillin-resistant *Staphylococcus aureus* (MRSA) (BD 01-BD 35) were kindly provided by Research Hospital of Medical Faculty from Trakya University, Edirne, Turkey and from Canakkale Anadolu Hospital, Canakkale, Turkey. *S. aureus* ATCC 25923 was used as a reference strain.

Screening for antimicrobial activities: The dried plant extracts were observed in 10 % aqueous dimethyl sulphoxide to a final concentration of 200 mg/mL and sterilized by filtration through a 0.45 µm membrane filter. Empty sterilized antibiotic discs having a diameter of 6 mm (Schleicher and Schull No. 2668, Dassel, Germany) were soaked with 10 µL of extract residue and diluted in the corresponding solvent to a concen-

tration of 250 mg/mL; thus each disc contained 2.5 mg of residue. Discs dried at 37 ºC over night were placed on the surface of Mueller Hinton Agar (MHA; Difco, Le Pont-De-Claix, France) plates seeded with c. 10^8 CFU of the tested isolates grown overnight in Trypticase Soy Broth (Oxoid, Basingstoke, UK). Antibacterial activity was evaluated by measuring the diameter of inhibition zone. Each experiment was performed in triplicate[7,8].

Determination of minimum inhibition concentration: A modified agar dilution method with millipore filters was used to determine the MICs of aqueous and ethanolic extracts of the plant extracts that produced inhibition zones. For each bacterial strain, 1 µL of culture (containing c. 10^4 CFU) was applied to a millipore filter on MHA supplemented with the plant extracts at concentrations ranging from 0.12-250 mg/mL. The plates were incubated at 35 ºC for 18 h. Experiments performed in triplicate and the results were expressed as the lowest concentration of colony growth. MIC's were determined with the extracts that gave significant inhibition by removing the millipore filter and placing it on a fresh MHA plate[9].

RESULTS AND DISCUSSION

The extracts of *Ballato acetabulosa* has activity against the 35 MRSA isolates tested (Table-1). The inhibition zones ranged from 15.8-18.4 mm, but these were significant differences in the inhibition zones produced by aqueous and ethanolic extracts of the plant. Significant antibacterial effects, expressed as MICs and MBCs of crude extracts against the 35 isolates of MRSA are listed in Table-2. The ethanolic extract of *B. acetabulosa* was stronger antibacterial effect than that of the other extract against the MRSA isolates, with inhibition zone of 16.4 mm and with MICs and MBCs of 0.4-1.6 and 3.2-12.5 µg/mL, respectively. In previous study, ethanol was observed as the best solvent for extracting antimicrobial substances[10]. The results in this study with ethanol are similar to those reported in the mentioned study. It is important to bear in mind that the concentration of extract used in the test may be correlated with a high activity of its chemical components.

TABLE-1
ANTIBACTERIAL ACTIVITY OF CRUDE PLANT EXTRACT AGAINST ISOLATES OF METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA)

Plant extracts	Inhibition zones (mm)			
	Aqueous extract		Ethanolic extract	
	MRSA	*S. aureus* ATCC 25923	MRSA	*S. aureus* ATCC 25923
Ballota acetabulosa	15.8 ± 0.42	17.4	16.4 ± 0.24	18.4

TABLE-2
MICS AND MBCS OF CRUDE PLANT EXTRACT OF METHICILLIN-RESISTANT *Staphylococcus aureus* (MRSA)

Plant extracts	Inhibition zones (mm)			
	Aqueous extract		Ethanolic extract	
	MRSA	*S. aureus* ATCC 25923	MRSA	*S. aureus* ATCC 25923
Ballota acetabulosa	0.8-3.2/6.3-12.5	0.8/6.3	0.4-1.6/3.2-12.5	0.4/6.3

The main components of the *Ballota* species are flavonoids, labdane diterpenoids and phenyl propanoids[11]. In our previous studies, three diterpenoids (hispanolone, ballonigrine, dehydrohispanolone) and ten flavonoids (kumatakenin, pakipodol, 5-hydroxy-7,-3',4'-trimethoxyflavone, velutin, corymbosine, 5-hydroxy-3,7,4'-trimethoxy flavone, retusin, 5-hydroxy-7,4'-dimethoxy flavone, flindulatine, ladanein) were isolated, chemically characterized and analysed by HPLC in different species of Ballota[2,12,13]. Flavonoids may be responsible for their antibacterial activity[14]. The result indicated that Ballota acetabulosa possessed significant activity against the MRSA cultures. This activity may be indicative of the presence of metabolic toxins or the mentioned plant compounds. So, this plant extracts should be analyzed further, as it might provide a new compound effective against pathogens.

Investigations of antimicrobial activity on the other *Ballota* species are limited. In previously studies, diterpenoids and flavonoids isolated from *Ballota inaequidens* are investigated for their activities against *Bacillus subtilis*, *Stapylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Candida albicans* and *Candida krusei*[15]. In that study, the compounds tested have no important inhibitory activity against bacteria but showed good activities against *C. albicans* and *C. krusei.* In addition, it is reported that three diterpenoid obtained from the aerial parts of *Ballota saxatilis* subsp. *saxatilis* and their effects against gram-positive (*S. aureus*, *S. faecalis*) and gram-negative (*P. aeruginosa*, *E. coli*, *K. pneumonia*) microorganisms and *C. albicans* in a previous study[4]. In another study, essential oil of *Ballota pseudodictamnus* has been investigated for their antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeroginosa*, *Candida albicans*, *Candida tropicalis* and *Candida glabrata* using the dilution technique[16]. Essential oil of the plant exhibited strong to moderate activity against all tested bacteria (MIC values 0.45-10.15 mg/mL), while it appeared inactive against the tested fungi. The antimicrobial activities of some *Ballota* species growing in Turkey was reported by Citoglu *et al.*[17]. The antimicrobial activities of ethanol extracts of 16 *Ballota* species growing in Turkey were studied. The ethanolic extracts were tested *in vitro* against gram-negative strains (*Escherichia coli*, *Pseudomonas aeruginosa*) and the gram-positive strains (*Staphylococcus aureus*, *Bacillus subtilis*) and the yeast cultures (*Candida albicans*, *Candida glabrata*, *Candida krusei*) by the agar diffusion method. Among *Ballota* species studied, *Ballota acetabulosa* has a strong antibacterial activity against bacterial strains. In addition, the extracts have antifungal activity against *C. albicans*, *C. glabrata* and *C. krusei*, with inhibition zones varied from 12, 13 and 12 mm, respectively. Besides, ethanol extracts of some species were tested against four diffferent *Listeria* isolates (*Listeria monocytogenes*, *L. ivanovii*, *L. innocua*, *L. murrayi*) by the agar diffusion method. Among *Ballota* species, *Ballota acetabulosa* have a strong antilisterial effects against all *Listeria* species except for *L. innocua*[1]. Equally, in this study all the extracts of *Ballota acetabulosa* were presented antimicrobial activity to the MRSA cultures. The differences between present result and others may be due to several factors, for example the infra-specific variability in the production of

secondary metabolites. In addition, there may be differences in the extraction protocols to recover the active metabolites and differences in the assay methods.

The findings in this study may indicate that especially *Ballota acetabulosa* can be used as natural preservatives in food against methicillin-resistant *Stapylococcus aureus*. According to the latest report from the National Nosocomial Infection Surveillance System, approximately 60 % of all *S. aureus* nosocomial infections in intensive care units were methicillin resistant in 2003, representing an 11 % increase in resistance compared to the preceding 5-year period[18]. The results of study also indicate that the extracts have the potential to generate novel antibiotics. The most active extract can be subjected to isolation of the therapeutic antibacterial and carry out further pharmacological evaluation.

REFERENCES

1. B.S. Yilmaz, N. Altanlar and G.S. Citoglu, *J. Fac. Pharm. Ankara*, **34**, 155 (2005).
2. P.H. Davis, Flora of Turkey and the East Aegean Islands, Edinburgh University Press, Edinburgh, Vol. 7, pp. 156-160 (1982).
3. C.A. Newall, L.A. Anderson and J.D. Philipson, Herbal Medicines, a Guide for Health-Care Professionals, The Pharmaceutical Press, London, p. 164 (1996).
4. G. Citoglu, M. Tanker, B. Sever, J. Englert, R. Anton and N. Altanlar, *Planta Med*., **64**, 484 (1998).
5. S. Sahpaz, A.L. Skaltsounis and F. Bailleul, *Biochem. Syst. Ecol*., **30**, 601 (2002).
6. N.H. Khan, M.S.A. Nur-E Kamal and M. Rahman, *Indian J. Res*., **87**, 395 (1988).
7. C.H. Collins, P.M. Lyre and J.M. Grange, Microbiological Methods, Butterworth Co. Ltd., London, edn. 6, p. 410 (1989).
8. M.S. Ali-Stayeh, R.M. Yaghmour, Y.R. Faidi, K. Salem and M.A. Al-Nur, *J. Ethnopharmacol*., **60**, 265 (1998).
9. V. Lorian, Antibiotics in Laboratory Medicines, Baltimore: Williams & Wilkins, edn. 4 (1998).
10. S.G. Jonathan and I.O. Fasidi, *Afr. J. Biomed. Res*., **6**, 85 (2003).
11. B. Sever, The Investigation of Diterpenoid and Flavonoid Contents of *Ballota* species growing in Turkey, Ph.D. Thesis Ankara (2000).
12. G.S. Citoglu, B. Sever, S. Antus, E. Baitz-Gacs and N. Altanlar, *Pharm. Biol*., **41**, 483 (2003).
13. G. Citoglu, M. Tanker and B. Sever, *Pharm. Biol*., **37**, 158 (1999).
14. M. Saeedi, K. Morteza-Semnani, M.R. Mahdavi and F. Rahimi, *Indian J. Pharm. Sci*., **70**, 403 (2008).
15. G.S. Citoglu, B. Sever, S. Antus, E. Baitz-Gac and N. Altanlar, *Pharm. Biol*., **42**, 659 (2004).
16. M. Couladis, I.B. Chinou, O. Tzakou and A. Loukis, *Phytother. Res*., **16**, 723 (2002).
17. G. Citoglu, B.S. Yilmaz and N. Altanlar, *J. Fac. Pharm. Ankara*, **32**, 93 (2003).
18. National Nosocomial Infections Surveillance System,National Nosocomial Infections Surveillance (NNIS) System Report, Data Summary from January 1992 through June 2004, issued October 2004, *Am. J. Infect. Control*, **32**, 470 (2004).