Asian Journal of Chemistry Vol. 21, No. 6 (2009), 4749-4754

Chemical Composition and Antibacterial Activity of *Bunium persicum* From West of Iran

GHOLAM REZA TALEI* and ZAHRA MOSAVI†

Rezi Herbal Medicine Research Center, Department of Microbiology, Medical School Lorestan University (Medical Sciences), Razi Street, Khoramabad 68198, Iran
Fax: (98)(661)3204005; Tel: (98)(917)3115961; E-mail: taleireza@yahoo.com

Volatile constituents of *Bunium persicum* seed was obtained by hydro-distillation and analyzed by gas chromatography-mass spectrometry (GC-MS). A total of 19 compounds accounting for 95.16 % of the black cumin (B.l) extracted by HD were identified as: γ-terpinene (5.6 %), isopulegol acetate (24.64 %), α-pinene (5.16 %), anethole (20.36 %) and camphor (10.43 %), were the major compounds identified. The oils were examined for the first time, for antibacterial activities against some standared bacteria by broth microdilution method. The extracts showed significant antibacterial activities against *Staphylococcus aureus, Escherichia coli* and *Staphylocuccus epidermidis*. The results suggest application of the *Bunium persicum* essential oils as food flavour and preservative.

Key Words: Essential oil, *Bunium persicum*, Antibacterial, Flavour, Preservative.

INTRODUCTION

Black cumin is an economically important umbellifer growing wild in the dry temperature regions of India in Jammu-Kashmir, Himachal Pradesh, Afghanistan, Baluchestan and Iran[1,2]. The seed, namely Zira in Farsi language, has been used to flavour rice in Persian cooking for centuries. It has also been consumed worldwide as condiment. In the old Persian medicines, seeds are regarded as stimulants and carminatives and found to be useful in diarrhea and dyspepsia[2,3]. This plant is also used for culinary purposes *i.e.*, for flavouring foods and beverages. It should be noted that the name of black cumin is sometimes given to entirely unrelated spice *Nigella sativa* L[4,5]. The hydro-distillation of black cumin seeds has been reported previously[1,6]. According to the reports, black cumin seeds contain essential oils rich in monoterpinene aldehydes. The main components are cuminaldehyde, *p*-mentha-1,3-dien-7-al and *p*-mentha-1,4-dien-7-al; terpene hydrocarbons are γ-terpinene, *p*-cymene and β-pinene. The latter compounds are thought to reduce the quality of the spice[1,6]. There has been no report of antibacterial activities of *Bunium periscum*

†Microbiology Laboratory, Tamin Edjtemaei Hospital, Goldasht, Khoramabad, Iran.

essential oils so far but oils from *Carum carvi* has been examined and showed to be effective against plant bacterial pathogens, namely, *Clavibacter, Curtobacterium, Rhodococcus, Erwinia, Xanthomonas, Ralstonia* and *Agrobacterium genera*[7]. So the effects of *Bunium persicum* oils on human pathogens and food bacteria remained unclear and worth investigation. The objective of this study was to prepare essential oils of *Bunium persicum* obtained from wild plant grew in heartland of Persian and analyze it by gas chromatography-mass spectrometry (GC-MS). In literature, no report is available on antibacterial activity and thus such activity has been investigated against some food contaminants and human pathogens.

EXPERIMENTAL

About 100 g of fresh aerial part at maturity were collected from wasteland around Agriculture College garden of Lorestan University, Khoramabad, Iran, in June 2006. The plant was identified and authenticated by Dr. H. Amiri, Department of Biology, University of Lorestan, Iran. The dried aerial parts were stored in a dark place at 4 °C.

Hydro-distillation: The sample (100 g of dried seeds was charged with a particle size of about 500 µm) was submitted to hydro-distillation for 1.5 h, using a Clevenger-type apparatus, according to the European Pharmacopoeia[8]. The volatile distillate was collected over anhydrous sodium sulphate and refrigerated until time of analysis. The yield of the oil was 3.1 % v/w based on dry plant weight.

Gas chromatography-mass spectrometry: GC analyses were carried out on a Shimutzu 17A gas chromatograph and a BP-5 (non-polar and 95 % dimethyl polysiloxane) capillary column (30 m × 0.25 mm; 0.25 µm film thickness). The oven temperature was maintained at 60 °C for 3 min then programmed at 5 °C /min to 300 °C. Other operating conditions were as follows: carrier gas helium, with a flow rate of 5 mL/min; injector temperature 230 °C; detector temperature 300 °C; split ratio, 1:8. GC/MS analyses were performed on a Shimutsu 17A GC coupled with Shimutsu QGD5050 Mass system. The operating conditions were the same conditions as described above but the carrier gas was helium Mass spectra were taken at 70 eV. Mass range was from m/z 50-450 amu. Quantitative data were obtained from the electronic integration of the peak areas. Retention indices were calculated using co-chromatographic standard hydrocarbons.

The constituents of the oil were identified by calculation of their retention indices under temperature-programmed conditions for *n*-alkenes (C_6-C_{24}) and the oil on BP-5 column under the same conditions. Identification of individual compounds was made by comparison of their mass spectra with those of the internal reference mass spectra library (Wiley 5.0) or with authentic compounds and confirmed by comparison of their retention indices with authentic compounds and with those of reported in the literature[9-11]. Quantitative data was obtained from FID area percentages without the use of correction factors.

Microorganisms: Antibacterial evaluations were carried out against standard bacteria in the Microbiology Research Laboratory of The Lorestan University of Medical Sciences as described before[12]. The tested bacteria were *Staphylococcus aureus* American Tissue and Culture Collections (ATCC) 25923, *Staphylococcus epidermidis* ATCC 12228, *Enterococcus faecalis* 29212, *Escherichia coli* ATCC 25922 and *Pseudomonas aeroginosa* ATCC 27853. The bacteria were obtained from the Microbiology Reference Laboratory (BoAli Hospital, Tehran). A *Bacillus cereus* strain originally isolated from rice in the Foodstuff Laboratory of the Department of Health (Khoramabad). The bacteria were grown on the Muller Hinton broth or agar (Merck, Germany).

Antibacterial Testing: The plant samples were filter-sterilized and used for disc diffusion and broth microdilution technique[13]. Paper discs (θ 6.5 mm) were impregnated with 40 μL of the samples and the solvent was evaporated under a safety cabinet at room temperature. Bacterial suspension's turbidity were compared and equalized with the Mac Farland 0.5 standard. The suspension then spread over a Muller Hinton agar plate by sterile swab gentamicin and ciprfloxacine were used as positive controls. The plates were incubated at 35 ºC overnight and the inhibition zone was measured. Minimum inhibition concentration (MIC) was determined in a 96 well flat-bottom sterile plates (Nunc, Denmark).

The bacterial inoculums were grown in Muller-Hinton broth to the lag phase and then adjusted to the turbidity of Mc Farland 0.5 Standard. The plant materials were serially diluted with medium in the wells and then 100 μL of bacterial suspensions was added to obtain a final concentration of 5×10^5 cfu/mL[13]. A growth control well, uninoculated and antibiotic controls, were included on each plate. The plates were incubated at 35 ºC and the turbidity was observed on a tray-reading stand. Samples from clear wells were cultured on nutrient agar (Merck, Germany) for determination of the MBC. The MIC is defined as the lowest concentration of the test which inhibits bacterial growth and the lowest concentration that did not grow on nutrient agar plate was taken as the MBC. All experiments were repeated three times and average values were presented as the results.

RESULTS AND DISCUSSION

A total of 19 compounds representing 95.16 % of the total components were identified in the oil (Table-1). γ-Terpinene (5.6 %), isopulegol acetate (24.64 %), α-pinene (5.16 %), anethole (20.36 %) and camphor (10.43 %), were the major compounds identified in the seed. The oil of *Bunium persicum* consisted mainly of monoterpene hydrocarbons (14.53 %), oxygenated monoterpenes (32.44 %), oxygenated sesquiterpenes (31.7 %). Isopulegol acetate was the dominant compound in the essential oil. On comparing the present results with that of reported from Indian and Tajikistan[1,6]. In all the reports, γ-terpinene and cuminaldehyde were the major constituents, but present oil was higher in isopulegol acetate (24.64 %), camphor (10.43 %) and anethole (20.36 %). On the other hand, in the Indian or Tajikistani

oils, *p*-mentha-1,4-dien-7-al is the major constituent, whereas, this component was not found in present oil. It is worthy to mention that the Indian and Tajikistani oils were obtained by hydro-distillation method too. These differences might have been derived both from harvest time and local, climatic and seasonal factors[14] or we may hypothesize that the Iranian sample belongs to a different chemotype. However, further investigations are needed to elucidate this hypothesis.

TABLE-1
CHEMICAL COMPOSITIONS OF *Bunium persicum* OILS,
THE COMPOUNDS WERE LISTED IN ORDER OF ELUTION TIME

No.	Compounds	Retention time	Kovats constant	%
1	α-Pinene	5.5	932	5.16
2	Camphene	5.8	946	0.51
3	Sabinene	6.4	960	0.22
4	β-Pinene	6.5	972	0.14
5	Myrcene	7.0	989	0.79
6	Limonene	8.0	1026	0.74
7	*trans*-Ocimene	8.7	1050	1.97
8	γ-Terpinene	9.0	1060	5.00
9	Limonene oxide	11.1	1131	0.09
10	Camphor	11.5	1142	10.43
11	Anethole	13.2	1203	20.36
12	α-Fenchyla acetate	13.8	1217	1.60
13	*cis*-Carveol	14.0	1224	0.57
14	Isopulegol acetate	14.3	1234	24.64
15	(+)-Pulegone	14.4	1241	0.62
16	Carvone	14.6	1244	3.43
17	α-Copaene	18.4	1378	0.17
18	*trans*-Caryophyllene	19.5	1418	1.04
19	Germacrene D	21.2	1480	0.19

There was a strong antibacterial activity against *Staphylocuccus aureus* since the dilution of 10 μL/mL inhibited bacterial growth (MIC) and dilution of 15 μL/mL was bacteriocidal (MBC) (Table-2). *Staphylocuccus aureus* as an important human pathogen and major causative bacteria of common food poisning was inhibited and killed at such a low dilution by *Bunium persicum* essential oil which is significant and interesting. There was a moderate antibacterial activity against *E. coli* although the starting concentration was generally low.

The antibacterial activities observed in the essential oils of *Bunium persicum* may be attributed to the presence of monoterpene such as carvon and limonene and α-pinene[15] (Table-1). Both optical isomers of carvon have been reported to be effective against a wide variety of food and human pathogenic bacteria and fungi as recently reviewed by De Carvallho *et al.*[16]. It has been significantly active against *Listeria monocytogenes, Campylobater jejuni*[17], *Escerichia coli, Aspergillus niger*[18] and

Candida albicans and *Helicobacter pylori*[18], but showed less activity against *Salmonella typhimurium* and *Photobacterium leiognathia* when compared to carvacrol[19]. In medicine, carvone and limonene have been reported to increase total number of leucocytes and antibody production in mice[20]. The role that terpens including carvone may play in medicine *i.e.* prevention of oxidative stress, carcinogenesis and cardiovascular diseases have recently been reviewed[21]. Although antioxidant activity of the oils has not been investigated, the compound found in the oils showed presence of some known antioxidants and radical scavenger such as α-pinene, β-pinene, limonene and terpinene suggestiong possible antioxidant activity in the oils.

TABLE-2
MIC AND MBCs OF IRANIAN *Bunium persicum* OILS AGAINST SOME STANDARD BACTERIA

Bacteria	Essential oil (μL/mL		Ciprofloxacine (μg/mL)	
	MIC	MBC	MIC	MBC
E. coli	15	15	1.0	5.0
P. aeroginosa	>15	>15	2.0*	2.0*
B. cerus	>15	>15	1.0	1.0
S. aureous	10	15	0.5	2.5
S. epidermidis	4	15	4.0	12.0
E. fecalis	15	>15	5.0	2.0

*Gentamycin was used.

Conclusions

The significant antibacterial activities found in the *Bunium persicum* may suggested a promising effective and safe natural preservative against food contaminant bacteria which has centuries records of consumption by people of Iran.

ACKNOWLEDGEMENTS

This research was supported by the research grants from The Lorestan University of Medical Sciences. Help and technical assistant of The Lorestan University in the GC/MS analysis are greatly appreciated. Special thanks to Dr. Mohammad Hadi Meshkatalsadat for preparation of essential oils and GC/MS analysis. The authors are also grateful to Dr. H. Amiri for identification of the plants and Enir Pharmaceutical Co. for providing some antibiotics.

REFERENCES

1. B.E. Abduganiew, U.A. Abdullaev, K.N. Aripov, K.H.C. Baser and T. Oezek, *J. Essen. Oil Res.*, **9**, 597 (1997).
2. A. Zargari, Medicinal Plants, Tehran, Tehran University Press, Vol. 3, pp. 106-108 (1990).
3. V. Mozaffarian, A Dictionary of Iranian Plants Names, Tehran, Farhang Moaser Publishers (1998).
4. G. Anitescu and C. Doneanu, *J. Supercritical Fluids*, **12**, 59 (1998).
5. G. Anitescu, C. Doneanu and V. Radulescu, *Flav. Fragrance J.*, **12**, 173 (1997).

6. R.K. Thappa, S. Ghosh, S.G. Agarwal, A.K. Raina and P.S. Jamwal, *Food Chem.*, **41**, 129 (1991).
7. I.S. Iacobellis, P.L. Cantore, F. Capasso and F.A. Senatore, *J. Agric. Food Chem.*, **53**, 57 (2004).
8. European Pharmacopoeia, Vol 3, Maisonneune S.A., Saint-Ruffine (1975).
9. R.P. Adams, Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy, USA, Allured Publ. Corp., Carol Stream. (1995).
10. C. Bicchi and P. Sandra, Capillary Gas Chromatography in Essential Oil Analysis. Heidelberg, Basel, New York, Alfred Huethig Verlag (1987).
11. W.K. Modey, D.A. Mulholland and M.W. Raynor, *Phytochem. Anal.*, **7**, 1 (1996).
12. G.R. Talei and M.H. Meshkatalsadat, *Pak. J. Biol. Sci.*, **10**, 3923 (2007).
13. C.R. Mahon and G. Manoselis, Textbook of Diagnostic Microbiology, W.B Saunders Company, pp. 13/319-13/360 (2007).
14. H. Toxopeus and H.J. Lubberts, *Ind. Corps Prod.*, **3**, 37 (2003).
15. S. Burt, *Int. J. Food Microbiol.*, **94**, 223 (2003) and the references cited therein.
16. C.C.C. De Carvalho and M.R. De Fonseca, *Food Chem.*, **95**, 413 (2006).
17. M. Friedman, P.R. Henika and R.E. Mandrell, *J. Food Protec.*, **65**, 1545 (2002).
18. R. Naigre, P. Kalck, C. Rogues, I. Roux and G. Michel, *Planta Medica*, **62**, 275 (1996).
19. I.M. Helander, H.L. Alakomi, K. Latva-Kala, T. Mattila-Sandholm, I. Pol, E.J. Smid, L.G.M. Gorris and A. von Wright, *J. Agric. Food Chem.*, **46**, 3590 (1998).
20. T.J. Raphael and G. Kuttan, *Immunopharmacol. Immunotoxicol.*, **25**, 283 (2003).
21. K.H. Wagnerm and I. Elmadfa *Ann. Nutr. Metabol.*, **47**, 95 (2003).

(*Received*: 20 September 2008; *Accepted*: 31 March 2009) AJC-7390