African Journal of Microbiology Research Vol. 4 (8), pp. 608-612, 18 April, 2010
Available online http://www.academicjournals.org/ajmr
ISSN 1996-0808 © 2010 Academic Journals

Full Length Research Paper

Antimicrobial activity of various extracts of *Centaurea cankiriense* A. Duran and H. Duman

Arzu Cansaran[1], Nazime Mercan Doğan[2]*, Mehtap Öztekin[3] and Gülümser Acar[2]

[1]Department of Biology, Faculty of Education, Amasya University, Amasya, Turkey.
[2]Department of Biology, Faculty of Science and Arts, Pamukkale University, Denizli, Turkey.
[3]Ministry of Environment and Forestry, Central Anatolia Forestry Research Institute, Herbarium ANKO, Post Box: 24, Bahcelievler, 06501, Ankara, Turkey.

Accepted 10 March, 2010

The antimicrobial activity was determined using the single disc diffusion method. The hexane, methanol and ethyl acetate extracts were assessed for antimicrobial activity against 13 bacteria and a yeast-like fungus, *Candida albicans*. While flower extracts of *Centaurea cankiriense* showed significant antibacterial activity against tested strains, the susceptibility of the test microorganisms was less pronounced in the cases of the stem extracts. Hexane extracts from both flower and stem did not show any antibacterial activity against gram-negative bacteria at test concentration, whereas ethyl acetate and methanol extract of *C. cankiriense* demonstrated the growth of both the gram-positive and the gram-negative bacteria. But, methanol extract inhibited the bacteria with the exception of two gram-negative bacteria namely *Escherichia coli* and *Klebsiella pneumoniae*. Minimum inhibition concentration (MIC) was determined on ethyl acetate extracts of flower and stem that showed high activity against the test bacteria. The MIC values for bacterial strains were in the range of 7.8 - 250 µg/ml. The results confirmed that *E. coli* (MIC = 250 µg/ml) and *Morganelle morganii* (MIC = 125 µg/ml) was the most resistant organisms to plant extracts. The flower extract of *C. cankiriense* was found to possess the strongest effect on *Bacillus cereus* with 7.8 µg/ml concentration.

Key words: *Centaurea cankiriense*, antimicrobial activity, minimum inhibition concentration, disc diffusion.

INTRODUCTION

The genus *Centaurea* is a member of Asteraceae family and Turkey is one of the main centres of diversity for the genus *Centaurea* (Wagenitz, 1986). Genus *Centaurea* is the third richest genus after *Astragalus* and *Verbascum*, both number of species and endemism whose endemism rate is up to 65%. It is known as “Peygamber Çiçeği” or “Gökbaş” by popular public (Environment waqf of Turkey). Many members of this genus, such as *Centaurea cyanus*, *Centaurea behen*, *Centaurea calcitrapa*, are used in Anatolian folk medicine (Baytop, 1995; Yeşilada et al., 1999). Biological activity of *Centaurea* species such as *Centaurea ornate*, *Centaurea nicolai*, *Centaurea solstitialis* ssp. *solstitialis*, *Centaurea thessala*, *Centaurea attica*, *Centaurea raphanina* ssp. *mixta*, *Centaurea nigra*, *Centaurea napifolia*, *Centaurea cineraria* subsp. *Umbrosa*, *Centaurea sessilis* and *Centaurea armena* were studied (Yeşilada et al., 1999; Yayli et al., 2005).

Centaurea cankiriense was introduced to the world of science in 2002 for the first time when it was collected from Çankiri (A4/ Çankiri, Atkaracalar, Dumanli Mount, Taşlik-Harmanyeri, 1500 m, steppe, 21.VII.1994) (Duran and Duman, 2002). In the flora of Turkey, 172 species plus six imperfectly known species *Centaurea* were accepted (Davis, 1975). *C. cankiriense* as a new species, the number of its species reached to 187 known in Turkey after publishing (Duran and Duman, 2002). With the advance of the exploration of this vast territory, the number of *Centaurea* species is still increasing: thirteen

*Corresponding author. E-mail: nmercan@pau.edu.tr. Tel: +90 258 296 36 72.

new taxa have been described since the completion of Flora of Turkey (Duran and Duman, 2002; Uysal et al., 2007).

C. cankiriense is an endemic species in transition territory of Central and North Anatolia. It is an Irano-Turanian element and it occurs in step on stony slopes at 1400 - 1600 m, flowers and fruits are seen in June, July and August (Duran and Duman, 2002). The range of *C. cankiriense* is restricted to single location and an area of less than 5 km^2 (Criterion B of IUCN 1994). So, it was suggested that, it should be placed under the IUCN category "Critically Endangered" by Duran and Duman (2002). Although *C. cankiriense* was collected from a different locality by Cansaran (Cansaran 3452-B), it is thought that the IUCN category of this plant which is a spreader in the second area limitedly should remain the same.

In the literature, there are several studies on antimicrobial activity of *C. thessala* and *C. attica* (Skaltsa et al., 2000), *Centaurea diffuses* (Skliar et al., 2005), *Centaurea nicolai* (Vaajs et al., 1999), *Centaurea sessilis* and *Centaurea armena* (Yayli et al., 2005), *Centaurea solstitialis* ssp. *solstitialis* (Yeşilada et al., 1999), whereas the antimicrobial activity of *C. cankiriense* which is endemic in Turkey has never been studied. Therefore, the aim of the present work is to evaluate the antimicrobial potential of the various extracts of the flowers and stems of *C. cankiriense* on several gram-positive and gram-negative bacteria and a yeast-like fungus, *Candida albicans*.

MATERIALS AND METHODS

Plant material

Specimens of *C. cankiriense* which were the sample materials of our study were collected by Arzu CANSARAN around Güllüce village in Gümüşhaciköy on Mount Eğerli (A5/ Amasya, located Middle Black-Sea Region), at 1400 m height, from stony areas on 25th July, 2005 (Cansaran 3452-B). Amasya is a transitional zone between Central and North Anatolia like Çankiri where the holotype of the specimen was collected in a nearby city. Specimens of plant were preserved in the herbarium at Amasya Education Faculty.

Preparation of the crude extract

Extracts of plant materials were prepared by using solvents of varying polarity. About 200 g of dry powdered plant material was extracted with hexane (HE), followed by ethyl acetate (ETA) and methanol (MeOH) in a soxhlet apparatus (6 h for each solvent). All solvents were purchased from Merck. The extracts were evaporated under reduced pressure and dried using rotary evaporator. Dried extracts were stored in labelled sterile screw capped bottles at -20°C (Kivrak et al., 2009).

Microorganisms

Pathogenic bacterial strains and a yeast-like fungus were used as test microorganisms. Gram negative bacteria *Pseudomonas aeruginosa* NRRL B-23, *Salmonella enteritidis* RSKK 171, *Escherichia coli* ATCC 35218, *Morganella morganii* (isolated from human urine), *Yersinia enterecolitica* RSKK 1501, *Klebsiella pneumoniae* ATCC 27736, *Proteus vulgaris* RSKK 96026, gram positive bacteria *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 12598, *Micrococcus luteus* NRRL B-4375, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* RSKK 863 and *Listeria monocytogenes* Li6 were used for the determination of antibacterial activity. *C. albicans* was used for the determination of anticandidal activity. Mueller Hinton Broth (MHB, Difco) and Mueller Hinton Agar (MHA, Oxoid) were applied for growing of the bacterial strains. Also, *C. albicans* was cultured on Sabouraud Dextrose Broth (SDB, Oxoid) and Sabouraud Dextrose Agar (SDA, Oxoid). The microorganisms suspensions used for inoculation were prepared at 10^5 cfu/ml by diluting fresh cultures at McFarland 0.5 density.

Inhibitory effect by the disc diffusion method

The antimicrobial activity of the various extracts of *C. cankiriense* was assayed by the standard disc diffusion method (Mercan et al., 2006). The microorganisms were sub-cultured into 5 ml of MHB, followed by incubation at 37 ± 0.1°C for 24 h, *C. albicans* was sub-cultured into 5 ml of SDB, followed by incubation at 28 ± 0.1°C for 48 h. MHA and SDA (15 ml) were poured into each sterile Petri dish (10 x 100 mm diameter) after injecting cultures (0.1 ml) of bacteria and yeast and distributing medium in Petri dishes homogeneously. Sterile discs (6 mm; Schleicher and Schuell, No. 2668, Germany) were impregnated with 20 µl of a solution prepared with 100 mg of extract in 1 ml dimethylsulfoxide (DMSO), and allowed to dry at room temperature. The discs injected with extracts were placed on the inoculated agar by pressing slightly. Plates injected with the yeast cultures were incubated at 28°C for 48 h, and the bacteria were incubated at 37°C for 24 h. At the end of the period, inhibition zones formed on the medium were evaluated in mm. DMSO which was used as a negative control did not show any antimicrobial activity. Ampicillin, Gentamicin and Streptomycin (6 mg/disc) were used as positive control for bacteria and Nystatin (100 U) was used as a positive control for *C. albicans*.

Minimum inhibition concentration (MIC) method

The MIC method was applied on extracts that proved their high efficacy against microorganisms by the disc diffusion method. A stock solution of each selected plant extract was prepared in 10% dimethylsulfoxide (DMSO) and then serial dilutions of extracts were made in a concentration range from 7.8 to 250 µg/ml. The 96-well plates were prepared by dispensing, into each well, 95 µl of MHB, 100 µl of plant extract and 5 µl of the inoculants (Aslim and Yucel, 2008). The minimal inhibitory concentration (MIC) was defined as the lowest concentrations of the plant extracts at which no bacterial growth was observed after incubation.

Statistical analysis

All experiments were done in triplicate. Statistical analysis was performed on the data SPSS 11.0 with statistical significance determined at $P < 0.01$. The Pearson rank order correlation test was used for comparisons of both MIC and disc diffusion methods, of the antimicrobial activity of the plant extracts.

Table 1. Antimicrobial activities of hexane, methanol and ethyl acetate extracts of *C. cankiriense* A. Duran and H. Duman[a].

	L. m.	S.a.(1)	S.a(2)	M. l.	B. s.	B. c.	S. ent.	P. v.	E. c.	K. p.	M.m.	Y. e.	P. a.	C.a.
Control (DMSO)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hexane extract														
Flower	11.5 ± 0.5	25 ± 0	26 ± 0	23 ± 0	11 ± 1	14 ± 0	-	-	-	-	-	-	-	-
Stem	7.5 ± 0.5	8 ± 0	9 ± 1	21 ± 0	8 ± 0	15 ± 0	-	-	-	-	-	-	-	-
Methanol extract														
Flower	12 ± 2	21 ± 0	22 ± 0	22 ± 0	12 ± 2	11 ± 0	6 ± 0	8 ± 0	-	-	6 ± 1	8 ± 2	9 ± 0	-
Stem	11 ± 0	6 ± 0	5 ± 0	19.5 ± 0.5	8 ± 0	11 ± 0	5 ± 0	7 ± 0	-	-	5 ± 0	6 ± 0	7 ± 0	-
Ethyl acetate extract														
Flower	30 ± 1	26 ± 0	28 ± 0	34.5 ± 0.5	28 ± 2	29 ± 0	16 ± 2	19 ± 0	23 ± 0	21 ± 0	22 ± 0	14 ± 0	16 ± 2	12± 2
Stem	19 ± 0	20 ± 0	20 ± 0	32 ± 0	19 ± 0	23 ± 0	12 ± 2	17 ± 1	11 ± 0	10 ± 1	10 ± 0	12 ± 2	9 ± 0	14± 2
Reference antibiotics														
Ampicillin	ND	ND	ND	ND	ND	ND	19	ND	20	ND	ND	12	ND	ND
Gentamicin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	10	ND	21	ND
Streptomycin	19	17	19	20	17	14	ND	18	ND	15	ND	ND	ND	ND
Nystatin	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	19

[a] = Diameter in mm of the zone of inhibition, (-) = negative, ND = Not determined, L.m. = *L. monocytogenes* Li6, S.a. (1) = *S. aureus* ATCC 12598, S.a. (2) = *S. aureus* ATCC 25923, M. l. = *M. luteus* NRRL B-4375, B. s. = *B. subtilis* ATCC 6633, B. c. = *B. cereus* RSKK 863, S. ent. = *S. enteritidis* RSKK 171, P. a. = *P. aeruginosa* NRRL B-23, E. c. = *E. coli* ATCC 35218, K. p. = *K. pneumoniae* ATCC 27736, M. m. = *M. morganii*, Y. e. = *Y. enterocolitica* RSKK 1501, P. v. = *P. vulgaris* RSKK 96026, C. a. = *C. albicans*.

RESULTS AND DISCUSSION

Results of inhibition zones in the disc diffusion method were shown in Table 1. In general, the flower extracts of *C. cankiriense* showed strong antibacterial activity against tested strains, the susceptibility of the test microorganisms was less pronounced in the cases of the stem extracts.

As seen in Table 1, while hexane extracts from both flower and stem did not show any antibacterial activity against gram-negative bacteria at test concentration, gram-positive bacteria was inhibited by this extract. The diameters of the zone of inhibition of hexane extract against gram-positive bacteria were almost similar to that of methanol extract. Ethyl acetate and methanol extract of *C. cankiriense* demon-strated the growth of both the gram-positive and the gram-negative bacteria. But, methanol extract inhibited the bacteria with the exception of two gram-negative bacteria namely, *E. coli* and *K. pneumoniae*. Similar result was also reported by Masika and Afolayan (2002). As known, *S. aureus*, *L. monocytogenes* and *Bacillus* species especially *B. cereus* are agents of food poisoning. The most interesting area of application for plant extracts is the inhibition of growth and reduction in numbers of the more serious foodborne pathogens such as *Salmonella* spp., *Escherichia coli* O157:H7 and *L. monocytogenes* (Burt, 2004). The highest level of antibacterial activity was found in the ethyl acetate fraction of *C. cankiriense*. The diameters of growth inhibition zones in this extract ranged from 9 to 34.5 mm with the highest inhibition zone values observed against the medically important pathogens such

Table 2. MIC values of ethyl acetate extract of *C. cankiriense.*

Plant part	Microorganisms	MIC (µg/ml)
Flower	*S. aureus* ATCC 25923	62.5
Flower	*S. aureus* ATCC 12598	62.5
Flower	*M. luteus* NRRL B-4375	12.5
Stem	*M. luteus* NRRL B-4375	31.2
Flower	*B. cereus* RSKK 863	7.8
Stem	*B. cereus* RSKK 863	12.5
Flower	*L. monocytogenes* Li6	62.5
Flower	*E. coli* ATCC 35218	250
Flower	*M. morganii*	125

as *S. aureus* ATCC 12598 (26 mm), *S. aureus* ATCC 25923 (28 mm), *E. coli* (23 mm), *M. morganii* (22 mm), *K. pneumoniae* (21 mm), *P. vulgaris* (19 mm) and *P. aeruginosa* (16 mm). In the present study, ethyl acetate extract of flower showed significant inhibition of *L. monocytogenes*, *M. luteus*, *B. cereus* and *B. subtilis* (diameter inhibition zones: 30, 34.5, 29 and 28 mm, respectively).

The results in Table 1 revealed that gram-positive bacteria are more sensitive to the plant extracts than gram-negative bacteria, especially the Enterobacteriaceae (*E. coli, S. enteritidis, P. vulgaris, Y. enterocolitica*) and this result was in agreement with that of Yayli et al. (2005). The extracts of hexane, methanol and ethyl acetate of flower was found active on *S. aureus* strains. *S. aureus* is a pathogen which is known to cause infectious disorders of the skin (Jones et al., 2003; Rennie et al., 2003). Thus, *C. cankiriense* showing activity against *S. aureus* may be used as agent for the treatment of skin disorders. Our results indicated that the activity of *C. cankiriense* was greater or similar to the conventional antibiotics.

Many papers about the antifungal activity of *Centaurea* genus could be found in the literature (Vaajs et al., 1999; Panagouleas et al., 2003; Berrero et al., 2000; Skliar et al., 2005), but in our study, the hexane and methanol extracts of *C. cankiriense* extracts did not show anticandidal activity against *C. albicans*. The ethyl acetate extract of *C. cankiriense* showed weak activity profile in *C. albicans.* Yayli et al. (2005) reported that antifungal activity of two *Centaurea* species was not observed against *C. albicans* and *C. tropicalis*.

The MIC method was applied on extracts that proved their high efficacy against microorganisms by the disc diffusion method. The MIC of ethyl acetate extract was shown in Table 2. The MICs of samples observed against sensitive strains ranged from 7.8 to 250 µg/ml. The final concentration of DMSO in the assays did not interfere with the microbial growth. Thus, we may conclude that the antibacterial activity in this assay is exclusively due to plant extracts. As seen in the table, flower extract showed a similar activity profile in *Staphylococcus* species and *L. monocytogenes* Li6 (MIC values = 62.5 µg/ml). The results observed confirmed that *E. coli* (MIC = 250 µg/ml) and *M. morganii* (MIC = 125 µg/ml) were the most resistant organisms to plant extracts. The flower extract of *C. cankiriense* was found to possess the strongest effect on *B. cereus* with 7.8 µg/ml concentration. The inhibition zones of the extracts on microorganisms showed a significant correlation with MIC values ($P < 0.01$).

When comparing the antimicrobial activity of the tested samples to that of reference antibiotics, the inhibitory potency of tested extracts could mostly be considered as important. This is due to the fact that medicinal plants are of natural origin, which means more safety for consumers, and are considered to have low risk for resistance development by pathogenic microorganisms. To the best of our knowledge, this study is the first report on the antimicrobial activity of *C. cankiriense* which is endemic in Turkey. It was found to inhibit the growth of microorganisms that cause infectious diseases and *C. cankiriense* can be used as a natural preservative in food against food-born disease. Future investigations will focus the research on the antioxidant activity and on chemical identification of the antimicrobial ingredients in the screened efficacious extracts. In summary, it might be said that *C. cankiriense*, especially the ethyl acetate extract of flower, could be used for protection against bacteria in ethno-medicine.

REFERENCES

Aslim B, Yucel N (2008). *In vitro* antimicrobial activity of essential oil from endemic *Origanum minutiflorum* on ciprofloxacin-resistant *Campylobacter* spp. Food Chem. 107: 602-606.

Baytop T (1995). *Türkiye'de Bitkilerle Tedavi*. Nobel Tip Kitabevi, İstanbul.

Barreroa AF, Oltraa JE, A´lvareza M, Raslanb DS, Sa´udec DA, Akssirad M (2000). New sources and antifungal activity of sesquiterpene lactones. Fitoterapia 71: 60-64.

Burt S (2004). Essential oils: Their antibacterial properties and potential applications in foods-a review. Int. J. Food. Microbiol. 94: 223-253.

Davis PH (1975). (ed.), Flora of Turkey and The East Aegean Islands Edinburgh University Press. 5: 465-585.

Duran A, Duman H (2002). Two new species of *Centaurea* (Asteraceae) from Turkey, Ann. Bot. Fennici, 39: 43-48.

Environment Waqf of Turkey (2005). The Biological Richnesses of Turkey, The Publication of Turkey Environment Waqf, Ankara p. 170,

Jones ME, Karlowsky JA, Draghi DC (2003). Epidemiology and antibiotic susceptibility of bacteria causing skin and soft tissue infections in the USA and Europe: a guide to appropriate antimicrobial therapy. Int. J. Antimicrob. Ag. 22: 406-419.

Kivrak I, Duru ME, Öztürk M, Mercan N, Harmandar M, Topçu G (2009). Antioxidant, anticholinesterase and antimicrobial constituents from the essential oil and ethanol extract of *Salvia potentillifolia*. Food Chemistry, 116: 470-479.

Masika PJ, Afolayan AJ (2002). Antimicrobial activity of some plants used for the treatment of livestock disease in Eastern Cape, South Africa. J. Ethnopharmacol. 83: 129-134.

Mercan N, Kivrak İ, Duru ME, Katircioglu H, Gulcan S, Malci S, Acar G, Salih B (2006). Chemical composition effects onto antimicrobial and Antioxidant activities of propolis collected from different regions of Turkey. Ann. Microbiol. 56: 373-378.

Panagouleas C, Skaltsa H, Lazari D, Skaltsounis A, Sokovic M (2003). Antifungal activity of secondary metabolites of *Centaurea raphanina* ssp. *mixta*, Growing Wild in Greece. Pharm. Biol. 41(4): 266-270.

Rennie RP, Jones RN, Mutnick AH (2003). Occurrence and antimicrobial susceptibility patterns of pathogens isolated from skin and soft tissue infections: report from the SENTRY antimicrobial surveillance program. Diagnostic Microbiol. Infect. Dis. 45: 287-293.

Skaltsa H, Lazari D, Panagouleas C, Georgiadou E, Garcia B, Sokovic M (2000). Sesquiterpene lactones from *Centaurea thessala* and *Centaurea attica*. Antifungal activity. Phytochemistry 55: 903-908.

Skliar MI, Toribio MS, Oriani DS (2005). Antimicrobial activity of *Centaurea diffusa*. Fitoterapia 76: 737-739.

Uysal T, Demirelma H, Ertuğrul K, Garcia-Jacas N, Susanna A (2007). *Centaurea glabro-auriculata* (Asteraceae), a new species from Turkey. Ann. Bot. Fennici, 44: 219-222.

Vaajs V, Todorovic N, Ristic M Tesevic V, Todorovic B, Janackovic P, Marin P, Milosavljevic S (1999). Guaianolides from *Centaurea nicolai*: antifungal activity. Phytochemistry 52: 383-386.

Wagenitz G (1986). *Centaurea* in South-West Asia: patterns of distribution and diversity. Proc. Roy. Soc. Edinburgh 89: 11-21.

Yayli N, Yasar A, Gulec C¸ Usta A, Kolayli S, Coskuncelebi K, Karaoglu S (2005). Composition and antimicrobial activity of essential oils from *Centaurea sessilis* and *Centaurea armena*. Phytochemistry 66: 1741-1745.

Yeşilada E, Gürbüz İ, Shibata H (1999). Screening of Turkish anti-ulcerogenic folk remedies for anti-*Helicobacter pylori* activity. J. Ethnopharmacol, 66: 289-293.