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Full Length Article

Comparison of Antimicrobial Activity of *Echinops viscosus* Subsp. *Bithynicus* and *E. microcephalus* Leaves and Flowers Extracts from Turkey

SEVİL TOROĞLU, DİLEK KESKİN[1]†, CEM VURAL‡, METİN KERTMEN¶ AND MENDERES ÇENET¶¶
Faculty of Science and Art, Department of Biology, Kahramanmaras Sutcu Lmam University, Kahramanmaras - 46100, Turkey
†*Cine High Vocational School, Adnan Menderes University, Cine Aydin - 09500, Turkey*
‡*Faculty of Science, Department of Biology, Erciyes University, Kayseri - Turkey*
¶*Faculty of Health, Department of Nursing, Siirt University, 56100, Siirt, Turkey*
¶¶*Faculty of Science and Art, Department of Biology, Osmaniye Korkut Ata University, Osmaniye, 80000, Turkey*
[1]Corresponding author's e-mail: dkeskin@adu.edu.tr

ABSTRACT

The antimicrobial activities of the ethyl acetate, acetone, methanol and ethanol extracts of *Echinops viscosus* DC subsp. *bithynicus* (Boiss) Rech. and *E. microcephalus* Sm. were studied by disc diffusion method. These extracts were tested against eight bacteria and four fungi, which revealed various levels of antimicrobial activity. The methanol, ethyl acetate, acetone extracts of EMF showed more antibacterial activity against *S. aureus* (18-19-18 mm 50 μL^{-1} inhibition zone) than standart antibiotics (f= 95.765; df= 18; p‹0.0001). The methanol extracts of EVF showed antibacterial activity against *E. coli* equal to standart antibiotics (V30 & E15). The ethyl acetate extracts of EVL showed antibacterial activity against *B. megaterium* equal to standart antibiotic (V30). The acetone extracts of EVF and EVL showed antimicrobial activity against *M. pusilus* close rate to standart antibiotic. *E. viscosus* and *E. microcephalus* contain antimicrobial components against different microorganisms, which could be in various pharmaceutical preparations. © 2012 Friends Science Publishers

Key Words: Antimicrobial activity; Plant extracts; *Echinops viscosus*; *E. microcephalus*

INTRODUCTION

Many people in developing or under developing countries use herbal medicine for their major primary health care needs (Farnsworth,1993). This has been the case of Turkey as well. During the last five years, extensive studies have been done on hundreds of medicinal plants in Turkey (Tepe *et al*., 2004; Yesil-Celiktas *et al*., 2007; Sengul *et al*., 2009). Turkey is an important floristic center internationally because of its geographic location, climate and the presence of nearly ten thousand plant species. The main part of Turkey, Anatolia, which has the appropriate climate, topography and soil properties, is the origin of many medicinal plants. Secondary metabolites are a major source of bioactive substances in plants. Nowadays, these metabolites have scientific interest because of antimicrobial resistance of microorganisms (Mbosso *et al*., 2010). In this age, microorganisms having ability to transmit and acquire resistance to antibiotics is important healthcare problem (Alanis, 2005). Antimicrobial compounds derived from plants can inhibit microorganims through different actions and are clinically important in the treatment of infections based on resistant microorganisms (Stein *et al*., 2005).

The genus Echinops (Asteraceae) consist of 18 species, 2 subspecies and 3 varieties in Turkey (Hedge, 1975; Gemici & Leblebici, 1992; Ozhatay *et al.*, 2009) and has medicinal importance. Therefore, in the present research, antimicrobial activity of two plants, i.e., *Echinops viscosus* and *E. microcephalus* were investigated.

MATERIALS AND METHODS

Plant collection and preparation of extracts: *Echinops microcephalus* was collected from Bursa, Bursa-Gemlik road at 1732 m altitude on 24. viii. 2005 (CV 3759) and *E. viscosus* subsp. *bithynicus* was collected from Bursa, Bursa-Gemlik, Armutlu road at 70 m altitude on 04. vii. 2006 (CV 4268). Voucher specimens of the plants are kept at the herbarium of Erciyes University, Faculty of Science. The taxonomic identities of these plants were confirmed by a taxonomist at the Botany Department, Faculty of Science, Erciyes University, Turkey.

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The plant parts used were dried and broken into small pieces under sterile conditions, and 20 g of each plant was extracted with 150 mL of ethyl acetate, acetone, methanol and ethanol extracts (Merck, Darmstadt) for 24 h by Soxhlet appartaus (Khan *et al*., 1988). Prepared extracts were dried at 30°C using a rotary evaporator until amount of each extracts was 1 mL.

Microorganisms and media: Eight bacteria (*Micrococcus luteus* LA 2971, *Escherichia coli* ATCC 8739, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus megaterium* DSM 32, *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* Cowan 1, *E. cloacae* ATCC 13047, *Mycobacterium smegmatis* CCM 2067) were obtained from the Biology Department of KSU, Science and Art Faculty. Cultures of these bacteria were grown in Nutrient Broth (NB) (Difco) at 37±0.1°C for 24 h. Four fungi (*Saccharomyces cerevisiae* WET 136, *Rhodotorula rubra*, *Mucor pusilus, Kluyveromyces fragilis* A 230). Cultures of these fungi were grown in Sabouraud Dextrose Broth (SDB) (Difco) at 25±0.1°C for 24 h.

Antibacterial activity: The disc assay described by Bauer *et al.* (1966) was used for antimicrobial activity. All of the extracts individually were injected into empty sterlized antibiotic discs having a diameter of 6 mm (Schleicher & Schül No:2668, Germany) in the amount of 50 µL. Discs injected with pure ethyl acetate, acetone, methanol and ethanol served as negative controls. The bacteria were incubated in Nutrient Broth (NB) (Difco) at 37±0.1°C for 24 h, and then inoculated [10^6 mL^{-1} (NCCLS, 2000)] into petri dishes containing homogenously distributed 15 mL of streilized Muller-Hinton agar (MHA, Oxoid) (Collins *et al.,* 1989). Disc injected with extracts were applied on the solid agar medium by pressing slightly. The treated petri dishes were placed at 4°C for 1-2 h and then the injected plates with bacteria were incubated at 37±0.1°C for 18-24 h, (Collins *et al*., 1989; Bradshaw, 1992; Toroglu, 2007; 2011). Vancomycin (30 µg/disc), Erythromycin (30 µg/disc) discs were used as standard antibiotics (as positive control). After incubation, all plates were observed for zones of growth inhibition, and the diameters of these zones were measured in millimeters. The experiments were conducted three times.

Antifungal activity: Antifungal assay was performed using disc diffusion method (Bauer *et al.,* 1966). The respective fungal cultures were inoculated [10^5 mL^{-1} (NCCLS, 2000)] into petri dishes containing homogenously distributed sterilized Saboraud Dextrose Agar (SDA) (Collins *et al*., 1989). Discs injected with extracts were applied on the solid agar medium by pressing slightly. The treated petri dishes were placed at 4°C for 1-2 h and then the injected plates with fungi were incubated at 25±0.1°C for 48 h. Nystatin 100 Units (10 µg/disc) discs were used as positive control. Different plant extracts were used to saturate the disc and placed on the seeded plates. Respective solvents act as a negative controls. After incubation period, the antifungal activity was evaluated by measuring the zone of inhibition against test organisms. The experiments were conducted three times.

Statistical analysis: Data from treatments for each plant were subjected to analysis of variance (one-way ANOVA) using the SPSS 13.0 (SPSS Inc., Chicago, IL) for Windows to find out the most effective plant extract and the most sensitive test microorganisms. Means were separated at the 5% significance level by the least significant difference test (LSD).

RESULTS AND DİSCUSSION

Antimicrobial activities of *E. viscosus* and *E. microcephalus* flowers and leaf extracts are presented in Table I. The ethanol, ethyl acetate, acetone and methanol used as negative controls did not show antimicrobial activity against the all tested microorganisms. In the present study, the methanol, ethyl acetate, acetone extracts of EMF showed more antibacterial activity against *S. aureus* (18-19–18 mm 50 μL^{-1} inhibition zone) than standart antibiotics (f= 95,765; df= 18; p‹ 0,0001). The methanol extracts of EVF showed antibacterial activity against *E. coli* equal to standart antibiotics (V30 & E15). The ethyl acetate extracts of EVL showed antibacterial activity against *B. megaterium* equal to standart antibiotic (V30). The acetone extracts of EVF and EVL showed antimicrobial activity against *M. pusilus* close rate to standart antibiotic.

The ethanol extracts of *E. viscosus* flowers (EVF) showed the best antibacterial activity against *M. luteus* (13 mm 50 μL^{-1} inhibition zone). The ethanol extracts of EVF presented the best antifungal activity against *K. fragilis* (12 mm 50 μL^{-1} inhibition zone). The methanol extracts of EVF displayed the best antibacterial activity against *E. coli, M. luteus B. megaterium, M. smegmatis* (10 mm 50 μL^{-1} inhibition zone. The methanol extracts of EVF displayed antifungal activity only against *M. pusilus* with 10 mm 50 μL^{-1} inhibition zone. The ethyl acetate extracts of EVF showed the best antibacterial activity against *S. aureus* and *M. smegmatis* (11 mm 50 μL^{-1} inhibition zone). The ethyl acetate extracts of EVF showed antifungal activity only *M. pusilus* with 12 mm 50 μL^{-1} inhibition zone. The acetone extracts of EVF showed the best antibacterial activity against *M. luteus* and *S. aureus* (10 mm 50 μL^{-1} inhibition zone). The acetone extracts of EVF showed antifungal activity against only *M. pusilus* with 13 mm 50 μL^{-1} inhibition zone.

When we compared to antimicrobial activity of *E. viscosus* leaves (EVL), the ethanol extracts of EVL showed the best antibacterial activity agianst *M. luteus* (13 mm 50 μL^{-1} inhibition zone). The ethanol extracts of EVL presented antifungal activity fungi only against *M. pusilus* with 12 mm 50 μL^{-1} inhibition zone. The methanol extracts of EVL displayed the best antibacterial activity against *M. luteus* (9 mm 50 μL^{-1} inhibition zone). The methanol extracts of EVL displayed antifungal activity only against *M. pusilus* with 7 mm 50 μL^{-1} inhibition zone.

Table I: Antimicrobial activity of different solvent extracts of *Echinops viscosus DC subsp. bithynicus* (Boiss) Rech and *E. microcephalus* Sm leaves and flowers

Microorganisms	Inhibition zone (mm)*																			
	Echinops viscosus (50 µL/disc)								*Echinops microcephalus* (50 µL/disc)								Standard antibiotics (µg/disc)			Control Discs
	Flowers				Leaves				Flowers				Leaves							
	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D	V30	E15	N10	ABCD
E. coli	7^{b}	10^{c}	0^{a}	0^{a}	0^{a}	0^{a}	7^{b}	7^{b}	7^{b}	0^{a}	0^{a}	7^{b}	7^{b}	8^{b}	0^{a}	8^{b}	11^{c}	10^{c}	NT	0^{a}
M. luteus	13^{e}	10^{cd}	10^{cd}	10^{cd}	13^{e}	9^{c}	10^{cd}	0^{a}	0^{a}	7^{b}	0^{a}	0^{a}	10^{cd}	10^{cd}	11^{d}	10^{cd}	21^{f}	34^{g}	NT	0^{a}
S. aureus	7^{b}	7^{b}	11^{cd}	10^{c}	7^{b}	8^{b}	14^{e}	11^{cd}	0^{a}	18^{g}	19	18^{g}	12^{d}	10^{c}	10^{c}	10^{c}	15^{ef}	16^{f}	NT	0^{a}
M. smegmatis	8^{bc}	10^{de}	11^{e}	7^{b}	0^{a}	7^{b}	11^{e}	0^{a}	0^{a}	14^{f}	0^{a}	0^{a}	7^{b}	7^{b}	9^{cd}	7^{b}	22^{g}	27^{h}	NT	0^{a}
P. aeruginosa	7^{b}	8^{bc}	8^{bc}	9^{cd}	11^{e}	0^{a}	9^{cd}	7^{b}	11^{e}	0^{a}	0^{a}	0^{a}	10^{de}	10^{de}	10^{de}	9^{cd}	17^{f}	35^{g}	NT	0^{a}
E.cloacae	8^{bc}	9_{cd}	9^{cd}	9^{cd}	0^{a}	0^{a}	8^{bc}	9^{cd}	7^{b}	8^{bc}	0^{a}	0^{a}	9^{cd}	8^{bc}	9^{cd}	10^{d}	27^{e}	28^{e}	NT	0^{a}
B. megaterium	8^{bc}	10_{d}	0^{a}	0^{a}	0^{a}	0^{a}	15^{f}	7^{b}	10^{d}	12^{e}	0^{a}	10^{a}	0^{a}	9^{cd}	8^{bc}	7^{b}	16^{f}	25^{g}	NT	0^{a}
E.faecalis	8^{bc}	7_{b}	9^{cd}	7^{b}	0^{a}	7^{b}	12^{e}	7^{b}	7^{b}	0^{a}	0^{a}	0^{a}	7^{b}	8^{bc}	10^{d}	9^{cd}	9^{cd}	20^{f}	NT	0^{a}
S. cerevisiae	10^{b}	0_{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	NT	NT	24^{c}	0^{a}
K. fragilis	12^{d}	0_{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	8^{c}	7^{b}	0^{a}	0^{a}	NT	NT	18^{e}	0^{a}
R.rubra	8^{b}	0_{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	0^{a}	8^{b}	7^{b}	11^{c}	12^{d}	NT	NT	18^{e}	0a
M. pusilus	11^{cd}	10_{c}	12^{de}	13^{ef}	12^{de}	7^{b}	11^{cd}	14^{f}	7^{b}	8^{b}	0^{a}	7^{b}	8^{b}	8^{b}	10^{c}	11^{cd}	NT	NT	16^{g}	0^{a}

A: Ethanol, B:Methanol, C:Ethyl acecate and D:Acetone extracts;
V30: Vancomycin (30 µg/disc), E15: Erytromycin (15 µg/disc), N10: Nystatin 100 Units (10 µg/disc), NT: Not tested
*Values, including diameter of the filter paper disc (6.0 mm), are means of three replicates
$^{a\text{-}h}$ Values specified in the same letters in the same row are not statisticaly significant

The ethyl acetate extracts of EVL showed the best antibacterial activity against *B. megaterium* (15 mm 50 µL^{-1} inhibition zone). The ethyl acetate extracts of EVL showed antifungal activity only *M. pusilus* with 11 mm 50 µL^{-1} inhibition zone. The acetone extracts of EVL showed the best antibacterial activity against *S. aureus* (11 mm 50 µL^{-1} inhibition zone). The acetone extracts of EVL showed antifungal activity against only *M. pusilus* with 14 mm 50 µL^{-1} inhibition zone.

When it comes to the antimicrobial activity of *E. microcephalus* , the ethanol extracts of EMF showed the best antibacterial activity *P. aeruginosa* (11 mm 50 µL^{-1} inhibition zone). The ethanol extracts of EMF presented antifungal activity only against *M. pusilus* with 7 mm 50 µL^{-1} inhibition zone. The methanol extracts of EMF displayed the best antibacterial activity against *S. aureus* (18 mm 50 µL^{-1} inhibition zone). The methanol extracts of EMF displayed antifungal activity only against *M. pusilus* with 8 mm 50 µL^{-1} inhibition zone. The ethyl acetate extracts of EMF showed antibacterial activity only against *S. aureus* with 19 mm 50 µL^{-1} inhibition zone. The ethyl acetate extracts of EMF showed no inhibition against the tested fungi. The acetone extracts of EMF showed the best antibacterial activity against *S. aureus* with 18 mm 50 µL^{-1} inhibition zone. The acetone extracts of EMF showed antifungal activity against only *M. pusilus* with 7 mm 50 µL^{-1} inhibition zone.

The ethanol extracts of EML exhibited the best antibacterial activity against *S. aureus* compared to *E. microcephalus* leaves (EML). The ethanol extracts of EML presented no antifungal activity only against *S. cerevisiae*. The methanol extracts of EML displayed the best antibacterial activity against *M. luteus, S. aureus P. aeruginosa* (10 mm 50 µL^{-1} inhibition zone). The methanol extracts of EMF displayed no antifungal activity only against *S. cerevisiae*. The ethyl acetate extracts of EML showed the best antibacterial activity against *M. luteus* with 11 mm 50 µL^{-1} inhibition zone. The ethyl acetate extracts of EML showed antifungal activity only against *M. pusilus* with 10 mm and *R. rubra* with 11 mm 50 µL^{-1} inhibition zone. The acetone extracts of EML showed the best antibacterial activity against *M. luteus, S. aureus, E. cloaceae* (10 mm 50 µL^{-1} inhibition zone). The acetone extracts of EML showed the best antifungal activity against only *R. rubra* with 12 mm 50 µL^{-1} inhibition zone.

In herbal medicine some species of the genus *Echinops* have been used to especially migraine, heart pain, mental, hemorrhoid, leprosy, kidney disease, diarrhea, malaria and many diseases (Abebe & Ahadu, 1993; Dawit & Ahadu, 1993). Some researchers reported that the genus *Echinops* are consist of flavonoids, alkaloids, saponins, phytosterols, polyphenols, carotenoids, sesquiterpene lactones/alcohols, lignans, acetylenic and thiophene compounds and essential oils (Tadesse & Abegaz, 1990; Singh & Pandey, 1994; Hymete *et al*., 2005). Flavonoids have two main role in plants, in flowers providing colours appealing to plant pollinators and in leaves, promoting physiological survival of the plant, protecting it from fungal pathogens and UV-B radiation (Middleton Jr. & Chithan, 1993; Harborne & Baxter, 1999; Harborne & Williams, 2000). Phytochemical preparations with high flavonoid content have also been reported to exhibit antibacterial activity (Aladesanmi *et al.*, 1986; Mahmoud *et al*., 1989; Torrenegra *et al*., 1989; Tarle & Dvorzak, 1990; Al-Saleh *et al*., 1997; Singh & Nath, 1999; Quarenghi *et al*., 2000; Rauha *et al*., 2000). Karou *et al.* (2006) reported that the alkaloids from *Sida acuta* displayed good antimicrobial activity against several test microorganisms. It can be suggested that saponins can display antimicrobial activity. This indication is in accordance with previous published reports that specific saponins could have antimicrobial activities (Fenwick *et al.,* 1992; Campbell, 1993). Haslam reported that polyphenols have antibacterial activities with important characteristics in their reactivity with proteins related polyamides polymers (Haslam,1996).

CONCLUSION

In vitro antimicrobial activities of *E. viscosus* and *E. microcephalus* the ethanol, methanol, ethyl acecate and acetone extracts have not been reported earlier. While the ethanol extracts of *E. viscosus* flowers (EVF) showed antibacterial activity against all the listed bacteria, the ethanol extracts of *E. microcephalus* flowers (EMF) showed antibacterial activity 5 out of 8 the listed bacteria. ethanol seemed to be better solvent for extracting the antibacterial substances from two medicinal plants used in this work. The results of this research clearly reported that the antibacterial and antifungal activity vary with the species of the plants, plant part and used extracts. And also the results from the present study have reported the scientific basis for traditional uses of the genus *Echinops* in the treatment of some illness. Different medicinal plant may be used for antibiotic resistance problem. Identifiying of active phytochemical compounds have done by reserachers. And also *in vitro* and *in vivo* studies should be done for their safety. After that stage, it can be produced commercially.

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