

Short Communication

Antibacterial and antifungal activity of *Heracleum sphondylium* subsp. *artvinense*

ERGENE, A.^{1*}, GULER, P.¹, TAN, S.¹, MİRİCİ, S.², HAMZAOĞLU, E.³ and DURAN, A.⁴

¹Kırıkkale University, Faculty of Science and Literature, Department of Biology, Kırıkkale, TURKEY.

²Akdeniz University, Faculty of Education, Department of Biology Education, Antalya, TURKEY.

³Erciyes University, Faculty of Science and Literature, Department of Biology, Yozgat, TURKEY.

⁴Selçuk University, Faculty of Education, Department of Biology Education, Konya, TURKEY.

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Ethanol and aqueous extract of *Heracleum sphondylium* subsp. *artvinense* was investigated for their antimicrobial activities against eight bacterial species (*Enterococcus faecalis*, *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Listeria monocytogenes*, *Shigella*, *Streptococcus pyogenes*, and *Corynebacterium diphtheria*) and two yeast (*Candida albicans* and *C. krusei*). Both ethanol and aqueous extract of *H. sphondylium* subsp. *artvinense* showed antimicrobial activity against the gram-positive bacterium (*S. aureus*).

Key words: *Heracleum sphondylium* subsp. *artvinense*, antibacterial and antifungal activities.

INTRODUCTION

Turkey is covered yearly with a huge number of plant species. About 9222 species are condensed on the region that between Asia and Europe. Many plant species have been used in folkloric medicine to treat various ailments (Baytop, 1994). Even today, plant materials continue to play a major role in primary health care as therapeutic remedies in many developing countries as well as in Turkey. Higher plants have been shown to be a potential source for new anti-microbial agents (Sokmen et al., 1999). The screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases (Dimayuga and Garcia, 1991).

Heracleum L (Apiaceae) includes more than 70 species in the world. In Turkey there are 17 species and 7 are endemic (Davis, 1972; Davis et al., 1988; Duman, 2000). *Heracleum sphondylium* subsp. *artvinense* is widespread in Turkey. It is in the VU (Vulnerable) category (IUCN; 2001). This species is of Euro-Siberian phylogeographic

region. *H. sphondylium* subsp. *artvinense* is grown in mixed forest, side of a mountain and side of the river at 1500 m, and is used for food or food additive. The roots and the stem are used as a folk medicinal herb. They are reported to be effective in menstrual problems, high blood pressure, dyspepsia and diarrhea (Baytop, 1994). This study determines if this plant has antimicrobial activities.

MATERIALS AND METHODS

Plant collection

Heracleum sphondylium subsp. *artvinense* used in this research was collected from Artvin region of Turkey. It was identified by Dr. Ergin Hamzaoglu and Dr. Ahmet Duran. These voucher specimens have been deposited at the Anadolu Herbarium (ADO) of the Department of Biology, Kırıkkale University, Turkey.

Preparation of extracts

Aqueous extract: Dried and powdered plant (100 g) was infused in distilled water until complete exhaustion. The extract was filtered using Whatman filter paper No. 1 and filtrate was evaporated in vacuum and dried using a rotary evaporator at 60°C. The final dried material was kept in the freezer at -20°C (Ali-shtayet et al., 1998).

*Corresponding author E-mail: ayergene@yahoo.com. Phone: 90 318 357 27 47. Fax: 90 - 318 - 357 24 76.

Table 1. Antibacterial and antifungal activity of *Heracleum sphondylium* subsp. *Artvinense*.

Microorganism	Inhibition zone diameter (mm) ^a	
	WE	EE
<i>Enterococcus faecalis</i> (Gram-negative)	8	11
<i>Shigella</i> (Gram-negative)	18	16
<i>Corynebacterium diphtheria</i> (Gram-positive)	10	14
<i>Staphylococcus aureus</i> (Gram-positive)	18	18
<i>Pseudomonas aeruginosa</i> (Gram-negative)	9	8
<i>Listeria monocytogenes</i> (Gram-positive)	10	14
<i>Escherichia coli</i> (Gram-negative)	10	12
<i>Streptococcus pyogenes</i> (Gram-positive)	10	12
<i>Candida krusei</i>	14	15
<i>Candida albicans</i>	11	13

Ethanol extract: Dried and powdered plant (100 g) was soaked in 95% ethanol until complete exhaustion. The extract was filtered using Whatman filter paper No. 1 and filtrate was evaporated under reduced pressure and dried using a rotate evaporator at 60°C. The final dried material was kept in the freezer at -20°C (Ali-shtayet et al., 1998).

Test microorganisms

The microorganisms used include *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 27853), *Listeria monocytogenes* (ATCC 7644), *Shigella*, *Streptococcus pyogenes*, *Corynebacterium diphtheria*, *Candida krusei* (ATCC 6258) and *Candida albicans* (ATCC 8459581). The bacterial cultures were maintained in nutrient broth (Oxoid), *Candida* species were maintained on sabouraud agar (Difco).

Simple susceptibility screening

The method used for screening for antimicrobial activities has been described elsewhere (Ali-shtayet et al., 1998). The dried plant extracts were dissolved in 10% aqueous dimethylsulfoxide (DMSO) to a final concentration of 200 mg/ml. Antimicrobial tests were carried out by the disc diffusion method (Ali-shtayet et al., 1998). The inoculums containing 10⁶ bacterial cells or 10⁸ yeast cells/ml were spread on Muller-Hinton agar plates (1 ml inoculums/plate). The discs (diameter, 6 mm) were each impregnated with 50 µl of extract (10 mg/disc) at a concentration of 200 mg/ml and placed on the inoculated agar and incubated at 37°C for 24 h for bacteria and 48 h for *Candida* species.

Statistical analysis

Data were analyzed and treatments compared using the analysis of variance (P>0.05).

RESULTS AND DISCUSSION

Antimicrobial activity of *H. sphondylium* subsp. *artvinense* was evaluated *in vitro* against eight bacterial species and two yeasts which are known to infections in humans (Table 1). Ethanol and aqueous extract of *H.*

sphondylium subsp. *artvinense* showed antimicrobial activity against the test microorganisms. These results showed that this plant is potentially a rich source of antimicrobial agents. *H. sphondylium* subsp. *artvinense* was least active against *P. aeruginosa*.

Ethanol and aqueous extract of *H. sphondylium* subsp. *artvinense* differed significantly in their activity against test microorganisms (df: 1; F: 3.750; p<0.05). Aqueous extract of *H. sphondylium* subsp. *artvinense* showed antimicrobial activity against the gram positive bacterium (*S. aureus*) and gram negative bacterium (*Shigella*) (Table 1). Ethanol extract showed similar antimicrobial activity against *S. aureus* only. The test microorganisms differed significantly in relation to their susceptibility to the different plant extracts used (df: 1; F: 6.060; p<0.05). Gram positive bacterium *S. aureus* was the most susceptible bacterium studied, while gram-negative bacterium *E. coli* was the least susceptible. The ethanol and aqueous extracts were observed to be more active against gram-positive bacteria tested. This may be attributed to the fact that cell wall in Gram positive bacteria consist of a single layer, whereas gram negative bacterial cell wall is a multilayered structure bounded by an outer cell membrane (Ali-shtayet et al., 1998; Yoa and Moellering, 1995).

The present work has shown that *H. sphondylium* subsp. *artvinense* is potentially a good source of antimicrobial agents which can be used in assisting primary health care in Turkey.

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