ANTIBACTERIAL POTENTIAL OF THE CAPITULA OF EIGHT ANATOLIAN *HELICHRYSUM* SPECIES

Mustafa ASLAN[1,*], Hikmet KATIRCIOĞLU[2], İlkay ORHAN[1], Tahir ATICI[2], Ekrem SEZİK[1]

[1] Gazi University, Faculty of Pharmacy, Department of Pharmacognosy, ,
06330 Etiler- Ankara, TURKEY
[2] Gazi University, Faculty of Education, Department of Biology,
06550 Beşevler- Ankara, TURKEY

Abstract

Sixteen extracts prepared with petroleum ether and ethanol from the capitula obtained from eight Helichrysum species including H. armenium subsp. *araxinum, H. armenium* subsp. *armenium, H. arenarium, H. pallasii, H. stoechas* subsp. barrelieri, *H. sanguineum, H. plicatum* subsp. *plicatum and H. graveolens (Asteraceae) growing in Turkey were screened for their antibacterial activity against ten different bacteria, six of which are the Gram-positive bacteria; namely Bacillus cereus, B. megaterium, Streptococcus mutans, Micrococcus luteus, Staphylococcus aureus and Listeria monocytogenes as well as four Gram-negative bacteria; Salmonella enteritis, Shigella sonnei, Pseudomonas aeruginosa, and Escherichia coli using disk diffusion method. Ampicilline, chloramphenicol, and erythromycin were employed as the reference drugs. Among the extracts screened, the ethanol extracts of H. pallasii, H. armenium subsp. armenium, H. plicatum subsp. plicatum and H. graveolens were found to be the most active ones with the wider antibacterial spectrum. The Helichrysum species screened herein also displayed remarkable inhibitory activity against the enteric-type of bacteria, S. enteritis and S. sonnei.*

Key words: *Helichrysum,Asteraceae, Antibacterial Activity, Disk diffusion*

Anadolu'da Yetişen Sekiz *Helichrysum* Taksonunun Kapitulumlarının Antibakteriyel Potansiyeli

H. armenium subsp. *araxinum, H. armenium* subsp. *armenium, H. arenarium, H. pallasii, H. stoechas* subsp. barrelieri*, H. sanguineum, H. plicatum* subsp. *plicatum and H. graveolens (Asteraceae) olmak üzere Türkiye'de yetişen 8 Helichrysum türünden petrol eteri ve etanol ile hazırlanan onaltı ekstre, 6'sı Gram-pozitif (Bacillus cereus, B. megaterium, Streptococcus mutans, Micrococcus luteus, Staphylococcus aureus ve Listeria monocytogenes), 4'ü Gram-negatif (Salmonella enteritis, Shigella sonnei, Pseudomonas aeruginosa ve Escherichia coli) olan 10 farklı bakteriye karşı antibakteriyel aktiviteleri açısından disk diffüzyon yöntemi kullanılarak taranmıştır. Ampisilin, kloramfenikol ve eritromisin referans ilaçlar olarak kullanılmıştır. Taranan ekstreler içinde, H. pallasii, H. armenium subsp. armenium, H. plicatum subsp. plicatum ve H. graveolens'e ait etanollü ekstreler, daha geniş antibakteriyel spektrumlarıyla en aktif ekstreler olarak bulunmuştur. Burada taranan Helichrysum ekstreleri aynı zamanda enterik tipte bakteriler olan S. enteritis ve S. sonnei'ye karşı da önemli bir inhibitör aktivite göstermişlerdir.*

Anahtar kelimeler: *Helichrysum, Asteraceae, Antibakteriyal Aktivite, Disk diffüzyon*

***Corresponding author:** Tel: +903122154467; E-mail: marslan@gazi.edu.tr

INTRODUCTION

Since not many people have access to benefit professional health services, particularly in rural areas of undeveloped and developing countries, plants used in traditional folk medicine for antimicrobial properties are still widely used to treat infections and, hence, constitute a great potential in the search for new antibiotics.

The genus *Helichrysum* (Asteraceae) is represented by 20 species in Turkey, 12 of which are endemic, namely *H. compactum*, *H. chasmolycicum*, *H. chionophilum*, *H. heywoodianum*, *H. noeanum*, *H. pamphylicum*, *H. artvinense*, *H. peshmanianum*, *H. arenarium* subsp. *aucheri* and subsp. *erzincanicum*, *H. kitianum* and *H. sivasicum* (1). Various *Helichrysum* species have been widely used as folk remedy in Turkish folk medicine for diuretic and anti-asthmatic properties as well as against kidney stones and stomache as decoction. Besides, powder of the capitula has been reported to be also used like pomade, prepared by mixing with barley flour, for wound healing (2-4). In fact, the genus of *Helichrysum* has been so far investigated well from the view point of antimicrobial activity.

In the present study, we report on antibacterial properties of the petroleum ether and ethanol extracts of eight *Helichrysum* species including *H. armenium* subsp. *araxinum* (Kirp.) Takht. and subsp. *armenium* D.C., *H. arenarium* L. (Moench), *H. pallasii* (Sprengel) Ledeb**.** , *H. stoechas* (L.) Moench subsp. *barrelieri* (Ten.) Nyman., *H. sanguineum* (L.) Kostel, *H. plicatum* D.C. subsp. *plicatum* and *H. graveolens* (Bieb.) Sweet growing commonly in Anatolia by disk diffusion method against the Gram-positive bacteria *Bacillus cereus*, *B. megaterium*, *Streptococcus mutans*, *Micrococcus luteus*, *Staphylococcus aureus* and *Listeria monocytogenes* as well as Gram-negative bacteria *Pseudomonas aeruginosa*, *Escherichia coli*, *Salmonella enteritis* and *Shigella sonnei*. In order to compare contributions of the plant components in different polarities, both petroleum ether and ethanol extractions were performed in the present study.

EXPERIMENTAL

Plant materials

Collection sites, dates and herbarium codes of the plant materials are given in Table 1. The voucher specimens were authenticated by Dr. M. Aslan and are preserved at the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey.

Preparation of the extracts

Prior to extraction, the plant materials were dried at room temperature in shade and consequently powdered in a mechanic grinder. After weighing the capitula of each species (approximately between 1-2 g for each extract), each material was independently extracted with petroleum ether (PE) and ethanol (EtOH) and the resultant extracts were obtained by evaporating to dryness *in vacuo*.

Microorganisms

The extracts were tested against a total of ten randomly selected bacteria, six of which are the Gram-positive bacteria, *Bacillus cereus* RSKK 863, *B. megaterium* RSKK 5117, *Streptococcus mutans* CNCTC 8177, *Micrococcus luteus* NRLL B-4375 and *Staphylococcus aureus* ATCC 25923 and *Listeria monocytogenes* LM1 as well as four Gram-negative bacteria *Salmonella enteritis* 171, *Shigella sonnei* RSSK 8177, *Pseudomonas aeruginosa* ATCC 29212

Table 1. Collection sites and dates of the *Helichrysum* species growing in Turkey

Species	Herbarium codes	Collection sites	Collection dates
H. armenium subsp. *araxinum* (Kirp.) Takht .	GUE 2349	Palandöken Mountain, Erzurum	July, 2000
H. armenium subsp. D.C.*armenium*	GUE 2350	Palandöken Mountain, Erzurum	August, 2000
H. arenarium L. (Moench)	GUE 2351	Palandöken Mountain, Erzurum	August, 2000
H. pallasii (Sprengel) Ledeb.	GUE 2352	Palandöken Mountain, Erzurum	August, 2000
H. stoechas (L.) Moench subsp. *barrelieri* (Ten.) Nyman.	GUE 2353	Vicinity of St. Pierre Church, Antakya	July, 2001
H. sanguineum(L.) Kostel	GUE 2354	Vicinity of St. Pierre Church, Antakya	July, 2001
H. plicatum D.C. subsp. *plicatum*	GUE 2355	Palandöken Mountain, Erzurum	July, 2003
H. graveolens(Bieb) Sweet	GUE 2356	Ilgaz Mountain, Kastamonu	August, 2002

and *Escherichia coli* ATCC 35218. All the strains used were obtained from the Laboratory of Biotechnology, Department of Biology, Faculty of Science and Art, Gazi University, Ankara, Turkey except for *S. enteritis*, *S. sonnei* and *L. monocytogenes* which were provided by Faculty of Agriculture, Ankara University, Ankara, Turkey.

Determination of antibacterial activity

The plant extracts were dissolved in 10% dimethylsulfoxide (DMSO) to a final concentration of 200 mg/ml and sterilized by filtration through a membrane filter with 0.45 µm diameter (Whatman No:1) according to Murray *et al.*'s method (5). Antimicrobial activity tests were then carried out by the disk diffusion method using an inoculum containing 10^6 bacterial cells to spread on Mueller-Hinton (Oxoid) agar plates (1 ml inoculum/plate). Each disk (5 mm in diameter) was impregnated with 50 µl of the extracts and placed on the inoculated agar. The plates were incubated at 37 ºC for 24 h. On each plate, an appropriate reference antibiotic disk was applied depending on the test bacteria. The tests were carried out in triplicate. Ampicilline (10µg/ml), chloramphenicol(30µg/ml), and erythromycin(15µg/ml) were employed as the reference antibiotics (Oxoid antibiotic disks, Germany). Antibacterial activity results were determined by measuring the diameter of the inhibition zone formed around the disks. 10% DMSO was used as negative control agent and did not cause any inhibition zone.

RESULTS AND DISCUSSION

The extracts were obtained as described in experimental part. Their yields (w/w) were given as follows: PE: 4.50%, EtOH: 13.32% for *H. armenium* subsp. *araxinum*, PE: 2.37%, EtOH: 12.01% for *H. armenium* subsp. *armenium*, PE: 5.03%, EtOH: 14.51% for *H. arenarium*, PE: 5.67%, EtOH: 17.76% for *H. pallasii*, PE: 4.06%, EtOH: 9.01% for *H. stoechas* subsp. *barrelieri*, PE: 16.54%, EtOH: 17.88% for *H. sanguineum*, PE: 3.25%, EtOH: 10.08% for *H. plicatum* subsp. *plicatum*, PE: 3.82%, EtOH: 15.10% for *H. graveolens*.

The results of antibacterial activity tests performed on the abovementioned *Helichrysium* species are displayed in Table 2. Most of the *Helichrysum* species screened herein displayed remarkable activity against the enteric-type of bacteria, *S. enteritis* and *S. sonnei* better than ampicilline and chloramphenicol, the antibiotics used widely for enteric infections. The EtOH extracts of all *Helichrysum* species exerted a significant inhibition towards *S. enteritis*, causing

the inhibition zones in range of 11.0-21.0 mm in diameter. In addition, the PE extracts of *H. armenium* subsp. *araxinum* and subsp. *armenium*, *H. arenarium* and *H. sanguineum* exhibited also some degree of inhibitory activity against *S. enteritis*, whereas all of the EtOH extracts also moderately inhibited the growth of *L. monocytogenes*. Differences were found among the inhibitory effects of the extracts from these *Helichrysum* species against *S. sonnei*.

Relevantly, up to date, a number of studies on antimicrobial activities of an assortment of *Helichrysum* species have been reported. For instance; *H. italicum* showed a strong inhibition against *S. aureus* (6), while *H. aespititium* of South African origin had a significant antibacterial activity against *Bacillus cereus*, *B. pumilis* and *B. subtilis* as well as *S. aureus*, which led to isolation of a new phloroglucinol derivative by bioactivity-guided fractionation (7). The dichloromethane extract of *H. pedunculatum* leaves also showed remarkable antibacterial activity against *S. aureus* and *Micrococcus cristinae* (8). Antibacterial activity-guided fractionation of the extract afforded linoleic and oleic acids as the active component.

H. aureonitens, used against infections topically in South African folk medicine, displayed high inhibitory activity against a series of Gram-positive bacteria and antibacterial activity-guided fractionation of acetone extract led to isolation of a 3,5,7-trihydroxyflavone (9).

On the other hand, there have been two studies on the antimicrobial activity of the Turkish *Helichrysum* species. The first one was carried out on *H. arenarium* (subsp. *aucheri* and subsp. *rubicundum*), *H. armenium*, *H. graveolens*, *H. noeanum*, *H. orientale*, *H. pallasii*, *H. plicatum* (subsp. *plicatum* and subsp. *polyphyllum*) and *H. sanguineum* as well as fifteen flavonoid derivatives isolated from different *Helichrysum* species (10). The later work on the antibacterial activity of *H. compactum* cleared this plant to be highly active against *E. coli* (11). The same result with *H.compactum* was also reported by Karahan et al.(12). In the present study, it was shown that the EtOH extracts of *H.graveolens* and *H.sanguineum* have possessed a potent inhibitory effect against *E.coli*, which is consistent with previously reported data.

The phytochemical investigations on divergent *Helichrysum* species indicated that this genus is quite rich in flavonoid content. In a report on the total flavonoid content of the Turkish *Helichrysum* species, capitula of the species that we studied were also found to have a rich flavonoid content such as 3.56% for *H. armenium* subsp. *araxinum*, 3.80% for *H. armenium* subsp. *armenium*, 1.00-1.95% for various subspecies of *H. arenarium*, 2.92% for *H. pallasii*, 0.83% for *H. stoechas* subsp. *barrelieri*, 6.83% for *H. sanguineum*, 4.83% for *H. plicatum* subsp. *plicatum*, and 4.80% for *H. graveolens* (13). Since flavonoids are well-known to possess antimicrobial properties, it may be important to know the flavonoid contents of these species in order to correlate their antibacterial properties.

In conclusion, the EtOH extracts of the *Helichrysum* species, which are the subjects of this research, could be evaluated as one of the alternative natural sources of antibacterial agents which support ethnobotanical utilization of the *Helichrysum* species and therefore, it deserve further phytochemical analysis for discovery of their active constituent(s). This procedure is progressively managed in our laboratory at present.

Table 2. Inhibition zone diameters (mm) of the petroleum ether and ethanol extracts from *Helichrysum* species

The Species	*H. armenium* ssp. *araxinum*		*H. arenarium*		*H. pallasii*		*H. armenium* ssp. *armenium*		*H. stoechas*		*H. sanguineum*		*H. plicatum* ssp. *plicatum*		*H. graveolens*		A	C	E
The Extracts / Test bacteria	Et	PE	Et	PE	Et	PE	Et	PE	Et	PE	Et	PE	Et	PE	Et	PE			
B. cereus	10.5	8.5	7.5	9.6	6.0	-	9.5	9.5	9.5	11.0	13.1	13.6	10.6	-	10.9	8.1	NT	28.6	23
B. megaterium	8.9	11.1	8.4	12.1	10.0	6.0	8.8	7.1	17.1	-	19.6	8.2	29.9	-	25.0	-	NT	15.4	18.4
S .aureus	19.5	7.6	22.1	11.0	20.4	-	22.1	8.7	10.2	-	9.2	-	9.4	-	-	-	NT	-	17.4
S. mutans	-	-	-	11.6	19.1	-	-	8.0	7.8	-	10.2	8.1	12.7	-	15.5	-	NT	-	13
M. luteus	8.2	9.0	16.7	10.8	1.43	-	8.0	10.6	-	-	17.4	-	-	-	-	-	NT	31.8	13
L. monocytogenes	17.7	9.3	13.5	10.8	7.2	-	12.9	10.0	8.4	6.7	11.7	8.5	10.1	-	13.4	-	NT	NT	20
E. coli	6.6	-	-	-	8.6	-	8.8	9.6	9.8	-	14.7	-	-	-	14.3	-	NT	-	-
P. aeruginosa	-	7.1	-	-	-	-	-	-	7.2	-	-	-	8.5	9.0	10.2	-	NT	-	-
S. enterititis	14.4	10.0	18.2	15.8	21.0	-	15.3	10.2	9.7	-	11.0	9.1	20.6	-	15.3	-	17	18	-
S. sonnei	-	9.0	16.0	11.7	-	-	-	10.5	10.4	11.9	11.2	10.7	16.4	-	13.4	6.6	17	16	-

Et: Ethanolic extract, **PE**: Petroleum ether extract, **A:** Ampicillin, **C:** Chloramphenicol, **E:** Erythromycin, **NT:** Not tested,

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