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Antimicrobial Activity of Endemic *Hypericum havvae* from Turkey

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n-Hexane, ethyl acetate, ethanol and aqueous extracts of *Hypericum havvae* A.Guner (Hyperiaceae) were tested for their antimicrobial activity against *Escherichia coli* ATCC 11230, *Enterobacter aerogenes* ATCC 13048, *Alcaligenes faecalis* CCM 3763, *Salmonella typhimurium* CCM 5445, *Citrobacter freundii* ATCC 8090, *Staphylococcus aureus* 6538P, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Bacillus brevis* ATCC 9999, *Pseudomonas aeruginosa* ATCC 27583, *Proteus vulgaris* ATCC 8427, *Micrococcus luteus* CCM 169, *Micrococcus flavus* ATCC 14452, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 70403 and *Kluyveromyces fragilis* ATCC 8608. While extracts of the plant have shown strong antimicrobial activity against the tested bacteria, they have weak activity against the tested yeast cultures.

Key Words: Antimicrobial activity, *Hypericum havvae*, Turkey.

INTRODUCTION

Hypericum species belonging to Cluciaceae (Hyperiaceae) family are widely found Europe, Asia, Northern Africa and America[1]. This genus encompasses various species used in traditional medicine around the world. Several antifungal, antibiotic, antiviral and anticancer compounds have been isolated from *Hypericum* species[2-4]. The majority of the active compounds isolated are phenolic in nature. These plants have a strong tendency to accumulate phenolic compounds with the pholoroglucinol substitution pattern.

The genus *Hypericum* comprises more than 400 species in the world but 77 species were found in Turkey[5,6]. Some these species are well known folk medicine used in Turkey and several countries, where they are employed in various curative treatments[7].

Hypericum havvae A.Guner is endemic to Turkey[5]. Information gathered from native healers: powdered or water macerate forms are used as an antidepressive drug. The plant is also used for therapeutic purposes by Turkish

native people. The aim of the present study is to assess the *in vitro* antimicrobial activities of various extracts of *H. havvae*. Although there are many investigations on *Hypericum* species[8-11], this plant has not been previously investigated. Therefore, this paper introduces *H. havvae* whose antimicrobial activities have not been reported before.

EXPERIMENTAL

Aerial parts of plant were collected from Camlikaya (Namrun), Cehennemdere, Icel, Turkey in September, 2006. Voucher specimens of the plants were deposited in the Biology Department at Canakkale Onsekiz Mart University, Canakkale, Turkey and identified by Dr. Emin Ugurlu from Celal Bayar University, Manisa, Turkey.

Preparation of extracts: The aerial parts of the plant were dried in an oven at 40 °C (12 h) and powdered. The powders (100 g) were separately extracted twice with 150 mL of *n*-hexane, ethyl acetate, ethanol and water[12]. The extracts were filtered using Whatmann filter paper no. 1 and the filtrates were then evaporated under reduced pressure and dried using a rotary evaporator at 55 ºC. Dried extracts were stored in labeled sterile screw-capped bottles at -20 ºC.

Microorganisms: *Escherichia coli* ATCC 11230, *Enterobacter aerogenes* ATCC 13048, *Alcaligenes faecalis* CCM 3763, *Salmonella typhimurium* CCM 5445, *Citrobacter freundii* ATCC 8090, *Staphylococcus aureus* 6538P, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Bacillus brevis* ATCC 9999, *Pseudomonas aeruginosa* ATCC 27583, *Proteus vulgaris* ATCC 8427, *Micrococcus luteus* CCM 169, *Micrococcus flavus* ATCC 14452, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 70403 and *Kluyveromyces fragilis* ATCC 8608 were used as test microorganisms.

Screening for antimicrobial activities: The dried plant extracts were dissolved in 10 % aqueous dimethyl sulfoxide to a final concentration of 200 mg/mL and sterilized by filtration through an 0.45 µm membrane filter. Empty sterilized antibiotic disks having a diameter of 6 mm (Schleicher & Schull No: 2668, Dassel, Germany) were each impregnated with 50 µL of extract (10 mg/disk) at a concentration of 200 mg/mL. All the bacteria mentioned above were incubated at 35 ± 0.1 ºC for 24 h by inoculation into nutrient broth (Difco Laboratories, MI, USA) and the yeast cultures studied were incubated in malt extract broth (Difco Laboratories, MI, USA) at 25 ± 0.1 ºC for 48 h an inoculum containing 10^6 bacterial cells or 10^8 yeast cells/mL was spread on Muller-Hinton Agar (Oxoid Ltd., Hampshire, UK) plates (1 mL inoculum/plate). The disks injected with extracts were placed at 4 ºC for 2 h, plaques injected with the yeast cultures were incubated at 25 ± 0.1 ºC and bacteria were incubated at 35 ± 0.1 ºC for 24 h[13]. At the end

of the period, inhibition zones formed on the medium were evaluated in millimeters. Studies were performed in triplicate. On each plate, an appropriate reference antibiotic disk was applied, depending on the test microorganism for comparison.

RESULTS AND DISCUSSION

The pholoroglucinol derivates found frequently in the lipophilic fractions of several *Hypericum* species have demonstrated antifungal and antibacterial activities against microorganisms such as *Staphyloccoccus aureus, Bacillus cereus, B. subtilis* and *Nocardia gardenen*[3,14-16]. Other substances present in some *Hypericum* have also shown antimicrobial activity against various bacteria and fungi including the benzopyrans[2,4], the xanthones[17], the flavonoids[18] and the tannins recognized antimicrobial compounds[19].

Table-1 shows antimicrobial activity of the plant extracts and the inhibition zones formed by standard antibiotic disks are indicated. As can clearly be seen from Table-1, the extracts of the *H. havvae* are shown strong antimicrobial

TABLE-1
SUMMARY OF ANTIMICROBIAL ACTIVITY OF *Hypericum havvae*

Microorganisms		Zone of inhibition (mm)*						
		A	B	C	D	Standards**		
						AK	CHL	NYS
Bacteria								
Staphylococcus aureus ATCC 6538P	(Gr+)	16.9	17.6	19.7	12.4	24.2	18.2	NT
Bacillus cereus ATCC 7064	(Gr+)	17.2	18.4	19.6	14.4	16.2	16.4	NT
Bacillus subtilis ATCC 6633	(Gr+)	18.4	17.6	20.2	15.0	16.4	16.2	NT
Bacillus brevis ATCC 9999	(Gr+)	16.8	18.6	20.0	14.0	16.0	16.6	NT
Micrococcus luteus La 2971	(Gr+)	14.4	13.6	16.8	11.0	24.4	16.4	NT
Micrococcus flavus ATCC 14452	(Gr+)	10.0	12.2	13.4	9.0	20.0	17.2	NT
Pseudomonas aeruginosa ATCC 27853	(Gr-)	11.8	12.4	13.8	11.2	20.2	24.0	NT
Proteus vulgaris ATCC 8427	(Gr-)	11.4	12.2	12.6	10.2	18.0	18.0	NT
Escherichia coli ATCC 11230	(Gr-)	10.2	11.2	10.8	9.8	17.2	18.4	NT
Enterobacter aerogenes ATCC 13048	(Gr-)	9.8	10.6	10.2	9.0	18.6	18.2	NT
Alcaligenes faecalis CCM 3763	(Gr-)	–	–	10.0	–	19.8	18.6	NT
Salmonella typhimurium CCM 5445	(Gr-)	9.4	9.4	10.8	9.0	19.2	16.0	NT
Citrobacter freundii ATCC 8090	(Gr-)	9.2	-	9.6	–	20.0	16.6	NT
Fungi								
Candida albicans ATCC 10231		–	–	10.6	–	NT	NT	18.4
Kluyveromyces fragilis ATCC 8608		–	10.4	11.2	–	NT	NT	17.8
Rhodotorula rubra DSM 70403		–	10.0	11.4	–	NT	NT	18.2

A = *n*-Hexane extract; B = Ethyl aceate extract; C = Ethanolic extract; D = Aqueous extract; – = no inhibition; NT = not tested; *Values, including diameter of the filter paper disc (6.0 mm), are means of three replicates; **AK = Amikacin (30 µg/disc); CHL = Chloramphenicol (10 µg/disc); NYS = Nystatin (25 µg/disc).

activity against the tested microorganisms. It is determined that all extracts except for aqueous extracts have more effective than those of chloramphenicol and amikacin against *Bacillus* species. In addition, *Staphylococcus aureus* and *Micrococcus luteus* are susceptible to the ethanolic extract, as compared to those of chloramphenicol. Notably, all extracts have weak antimicrobial activity against the tested yeast cultures.

The screening of plant extracts for antimicrobial activity has shown that higher plants represent a potential source of new antiinfective agents[20]. Herbal medicines are a valuable and rapidly available resource for primary health care and complementary health care systems. Undoubtedly, the plant kingdom still holds many species of plants containing substances of medicinal value which have yet to be discovered. A large numbers of plants are constantly being screened for their antimicrobial effects. Finally, the results of the present study provide evidence that *Hypericum havvae* continue to represent an important asset to the health care in communities in Turkey. More pharmacological investigations are necessary.

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