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Antibacterial Activity of Two Endemic *Hypericum* (*H. kazdaghensis* and *H. havvae*) Against Methicillin-Resistant *Staphylococcus aureus*

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Aqueous and ethanolic extracts obtained from endemic *Hypericum kazdaghensis* Gemici et Leblebici and *H. havvae* A. Guner have been investigated for its ability to inhibit 35 hospital isolated of methicillin-resistant *Staphylococcus aureus* (MRSA). Both aqueous and ethanolic extracts of the plant were effective on MRSA. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of the ethanolic extract with the greatest antibacterial activity were those of *H. kazdagensis* MIC 0.2-0.4 mg/mL and MBC 0.4-1.6 mg/mL, respectively.

Key Words: Antibacterial activity, Plant extract, *Hypericum* L.

INTRODUCTION

Hypericum has been used for centuries in the treatment of burns, bruises, swelling, inflammation and anxiety, as well as bacterial and viral infections[1,2]. One of the important species of this genus is *Hypericum perforatum* L. which has been in herbal medicine externally for the treatment of skin wounds eczema and burns and internally for diseases of the central nervous system, the alimentary tract and others[3].

Hypericum kazdaghensis and *H. havvae* are endemic to Turkey[4]. During our field exurcions, it was determined that these plants have been used for opthalmia, eczema, swelling, bruises and wounds. So, the aim of the present study is to screen these endemic plant extracts have been shown earlier to have antimicrobial activity against hospital isolated of methicillin-resistant *Staphylococcus aureus* (MRSA).

EXPERIMENTAL

Hypericum kazdaghensis Gemici et Leblebici was collected from Canakkale, Turkey and *Hypericum havvae* A. Guner was collacted from Icel, Turkey, during the month of September 2009. Voucher specimens of the plants in the Biology Department at Canakkale Onsekiz Mart University, Canakkale, Turkey.

Microorganisms: Thirty-five clinical isolated of MRSA (BD 01-BD 35) were kindly provided by Research Hospital of Medical Faculty of Canakkale Onsekiz Mart University, Canakkale, Turkey and from Trakya University, Edirne, Turkey.

Preparation of extract: Aerial plant parts were air-dried. Each dry powdered plant material (20 g) was extracted with 150 mL of 80 % ethanol (Merck, Darmstadt, Germany) for 24 h by using Soxhlet equipment[5]. The extracts evaporated under pressure and dried using a rotary evaporator at 55 °C. Dried extracts were stored in labeled sterile screw-capped bottles at -20 °C.

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 0.45 μm membrane filter. The MIC was determined by microdilution methods[6]. The reconstituted extract was serially diluted two-fold in Mueller Hinton Broth (Oxoid, Hampshire, UK) medium. Duplicate tubes of each dilution (ranging from 50.0-0.1 mg/mL) were inoculated with 5×10^5 cfu/mL of the best bacterial strain and cultures incubated at 37 °C for 48 h. Minimum inhibitory concentration (MIC) was taken as the highest dilution (least concentration) of extract or drug showing no detectable growth. Minimum bactericidal concentration (MBC) was determined by subculturing the test dilution on the fresh drug-free solid medium and incubating further for 18-24 h. The highest dilution that yielded no single bacterial colony on a solid medium was taken as MBC.

RESULTS AND DISCUSSION

Significant antibacterial activities, expressed as MICs and MBCs, of crude extracts obtained from *Hypericum kazdaghensis* and *H. havvae* against the 35 isolates of MRSA are listed in Table-1.

TABLE-1
ANTIBACTERIAL ACTIVITY (MIC AND MBC (mg/mL) OF THE EXTRACTS OF *Hypericum* SPECIES ON MRSA

Plant tested	Aqueous extract		Ethanol extract	
	MIC	MBC	MIC	MBC
Hypericum kazdaghensis	0.4-0.8	0.8-1.6	0.2-0.4	0.4-1.6
Hypericum havvae	1.6-6.3	3.2-25	0.8-3.2	1.6-6.3

MIC: Mininum inhibitory concentration, MBC: Minimum bactericidal concentration.

Ethanolic extracts of *H. kazdaghensis* were the most active against the MRSA isolates, with MICs and MBCs of 0.2-0.4 and 0.4-1.6 mg/mL, respectively. Notably, antibacterial assay indicated that the ethanol extracts of *Hypericum* species used in this study were more efficient than those of the aqueous extracts. In previous study, ethanol was observed as the best solvent for extracting antimicrobial substances[7]. The results obtained from this study with ethanol are parallel to those reported in the mentioned study. It is important to bear in mind that the concentration of extract used in this test may be correlated with high activity of its chemical components.

Hypericum species used in this study have been shown earlier to have antimicrobial activity against some bacteria and the yeast cultures. In previous study,

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. Also, the ethanolic extract of *H. havvae* have a stong antibacterial effect against *Staphylococcus aureus* ATCC 6538P strain (inhibition zone is 19.7 mm) as compared to standard antibacterial antibiotic chloramphenicol (10 µg/disc)[8]. In another study, the extracts of *H. kazdaghensis* show strong antimicrobial activity against the tested bacteria. When the results obtained are compared to those of gentamycin, it is determined that *B. subtilis* and *P. aeruginosa* are more susceptible to all extracts. Acetone extract of *H. kazdaghensis* has a strong antibacterial effect against *S. aureus* ATCC 6538P (inhibition zone is 17.6 mm)[9]. The results in this study are similar to those reported in the above studies.

Hypericum species contain many phenolic compounds (hypericin, hyperforin and their derivatives, rutin, hyperoside, quercetin, chlorogenic acid, flavonols and flavones), suggesting that they could have important antioxidant properties[10]. Hypericin has shown antibacterial, antiviral and antiinflammatory activity and hyperforin is the main compound involved in antidepressant activity[11]. Hyperforin exhibits effects against the methicillin-resistant strains of *Staphylococcus aureus* with a minimum inhibitory concentration (MIC) value of 1 µg/mL[12]. The result indicated that *H. kazdaghensis* and *H. havvae* possessed significant activity against MRSA. This activity may be indicative of the presence of metabolic toxins or the mentioned plant compounds. So, these plant extracts should be analyzed further, as it might provide a new compound effective against pathogens. According to the latest report from the National Nosocomial Infection Surveillance System, approximately 60 % of all *S. aureus* nosocomial infections in intensive care units were methicillin resistant in 2003, representing an 11 % increase in resistance compared to the preceding 5-year period[13]. The result supports the folkloric usage of the studied plants and suggests that the plant extracts possess certain constituents with antibacterial properties that can be used as antibacterial agents in new drugs for the therapy of infectious diseases caused by pathogens especially methicillin-resistant *Staphylococcus aureus*. The most active extracts can be subjected to isolation of the therapeutic antibacterial and carry out further pharmacological evaluation.

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