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Antimicrobial Activity of *Bacopa caroliniana*

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Ethanolic extract obtained from *Bacopa caroliniana* (Walt.) B.L.Robins (*Scrophulariaceae*) was investigated for its antimicrobial activity against *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Bacillus cereus* ATCC 7064, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* CCM 169, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 70403, *Kluyveromyces fragilis* ATCC 8608, *Debaryomyces hansenii* DSM 70238 and *Hanseniaspora guilliermondii* DSM 3432 by disc-diffusion method. The extract has strong antimicrobial effects against the tested microorganisms especially the bacterium *Staphylococcus aureus* and the yeast culture *Candida albicans*. The findings support the use of this plant in traditional medicine.

Key Words: *Bacopa caroliniana*, Plant extracts, Antimicrobial activity.

INTRODUCTION

Bacopa caroliniana (Walt.) B.L.Robins (*Scrophulariaceae*) has been used as an aquarium plant for many years. It is suitable for use as an aquatic plant in aquarium and garden pond. The leaf of *Bacopa* species has been used in the Indian medical system of Ayurveda since the 6th century AD. It has been used in treatment of insanity, epilepsy, hysteria and skin diseases[1]. Although there are many investigations on other *Bacopa* species especially *B. monnieri*[2-9], *B. caroliniana* has not been previously investigated. Therefore, our aim is to determine the antimicrobial effects of the ethanolic extract obtained from this plant against microorganisms.

EXPERIMENTAL

The plant is collected from different garden ponds in Turkey during early summer, 2008.

Preparation of extracts: The plant parts were air-dried. Each dry powdered plant material (50 g) was extracted with 150 mL of 95 % ethanol for 24 h by using Soxhlet equipment[10]. The extract was 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 extract were stored in labeled sterile screw - capped bottles at - 20 ºC.

Microorganisms: *Escherichia coli* ATCC 11230, *Staphylococcus aureus* ATCC 6538P, *Klebsiella pneumoniae* UC57, *Pseudomonas aeruginosa* ATCC 27853, *Proteus vulgaris* ATCC 8427, *Bacillus cereus* ATCC 7064, *Mycobacterium smegmatis* CCM 2067, *Listeria monocytogenes* ATCC 15313, *Micrococcus luteus* CCM 169, *Candida albicans* ATCC 10231, *Rhodotorula rubra* DSM 70403, *Kluyveromyces fragilis* ATCC 8608, *Debaryomyces hansenii* DSM 70238 and *Hanseniaspora guilliermondii* DSM 3432 were used as test microorganisms.

Screening for antimicrobial activities: 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 disc 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 Mueller Hinton Agar (Oxoid Ltd., Hampshire, UK) plates (1 mL inoculum/plate). The discs injected with extracts were placed on the inoculated agar by pressing slightly. Petri dishes were placed at 4 ºC for 2 h plaques injected with the yeast cultures were incubated at 25 ± 0.1 ºC for 72 h and bacteria were incubated[11] 35 ± 0.1 ºC for 24 h. 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 disc was applied, depending on the test microorganisms for comparison.

RESULTS AND DISCUSSION

Table-1 shows antimicrobial activity of the plant extracts and the inhibition zones formed by standard antibiotic discs. The ethanol extract obtained from *B. caroliniana* has antimicrobial activity against all tested microorganisms used in this study in different levels.

It is found that among different bacterial strains *Staphylococcus aureus* is most susceptible to the extract, as compared to all standard antibacterial antibiotics. Reports indicated that clinical isolates from different infectious sources from hospitals showed resistance against the drug methicillin[12]. Similarly, the ethanolic extract has strong antibacterial effect against the gram negative bacterium *Pseudomonas aeruginosa* than those of the standard antibacterial antibiotics. In comparison to P10 and SAM20 standard, it was seen that *Bacillus cereus* and *Listeria monocytogenes* are more susceptible. The growth inhibition was moderate against the other bacteria. The extract has greater antiyeast effect than the standard antifungal antibiotics Nystatin against *Candida albicans*. Against the other yeast cultures, the extract has a moderate antiyeast activity.

TABLE-1
ANTIMICROBIAL ACTIVITY OF *B. caroliniana*

Microorganisms	Inhibition zone (mm)*					
	Ethanolic extract	Standard antibiotics				
		P10	SAM20	VA30	CLT10	NY100
Bacteria						
Escherichia coli	16.2	18.2	12.2	22.0	-	-
Staphylococcus aureus	18.6	13.4	16.8	13.4	-	-
Klebsiella pneumonia	14.6	18.2	14.4	22.4	-	-
Pseudomonas aeruginosa	12.4	8.6	10.8	10.8	-	-
Proteus vulgaris	11.8	10.2	16.2	20.0	-	-
Mycobacterium smegmatis	14.7	15.8	21.0	20.0	-	-
Listeria monocytogenes	13.8	10.6	12.4	26.4	-	-
Micrococcus luteus	14.2	36.2	32.0	34.2	-	-
Bacillus cereus	16.2	14.4	12.4	18.6	-	-
Fungi						
Candia albicans	18.2	-	-	-	15.2	20.0
Kluveromyces fragilis	12.3	-	-	-	18.4	18.2
Rhodotorula rubra	13.2	-	-	-	16.0	18.0
Hanseniaspora guilliermondii	14.7	-	-	-	22.2	21.4
Debaryomyces hansenii	15.0	-	-	-	18.4	16.0

*Includes diameter of disc (6 mm)
P10 = Penicillin G (10 Units), SAM20 = Ampicillin 10 µg, V30 = Vancomycin 30 µg, CLT10 = Clotrimazole 10 µg, NY100 = Nystatin 100 µg.

Members of the family Scrophulariaceae have been reported to contain a group of unusual macrocyclic spermine alkaloids[13]. Antibacterial activity of Bacoside-A an active constituent isolated of *Bacopa monnieri* is previously been reported[2]. Bacoside-A and the methanolic extract exhibited a significant zone of inhibition against the clinical strain of *Staphylococcus aureus*. A moderate zone of inhibition was observed on the clinical strains of *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. In another study, the ethyl acetate and *n*-butanol fractions of ethanolic extract of *B. monnieri* aerial parts were screened for antimicrobial activities[3]. The fractions showed a strong antimicrobial effect. In addition, *B. monnieri* is also reported to contain tetracyclic triterpenoid saponins, bacosides A and B[14,15], hersaponin[16] and some flavanoids[17,18]. The results obtained in this study are similar to those reported in the mentioned studies. However, there are no literature data available on the phytochemistry of *B. caroliniana*. The mentioned substances may be responsible for the antimicrobial activity in *B. caroliniana*.

In conclusion, this preliminary evaluation indicated that *B. caroliniana* possessed significant activity against tested microorganisms especially *Stapylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans*. Microorganisms used in this study were chosen primarily on the basis of their importance as pathogens of humans. *Stapylococcus aureus* causes superfacial skin lesions such as boils and also more

serious infections such as pneumonia, mastitis, phlebitis and meningitis. *Pseudomonas aeruginosa* is an opportunistic pathogen of immunocompromised individuals. It typically infects the pulmonary tract, urinary tract, burns, wounds and also causes other blood infections. It's the most common cause of burn and external ear infections[19]. According to findings from National Nosocomial Surveillance System (NNIS), 61 % of reported nosocomial fungal infections were due to *Candida albicans*[20]. The findings obtained in this study support the use of this plant in traditional medicine for treating the mentioned diseases. Further phytochemical studies are required for the antimicrobial activities of this medicinal plant.

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