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ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF THE EXTRACT AND FRACTIONS OF AERIAL PARTS OF *HELIOTROPIUM BACCIFERUM*

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Abstract

Background: *H. bacciferum* belonging to the family Boraginaceae is an important medicinal plant. The current research was carried out to investigate the medicinal properties of this plant.
Methodology: The crude (methanol fraction) and *n*-hexane, ethyl acetate, butanol and aqueous fractions were subjected to antibacterial and antifungal activities by using standard methodology available in literature. Bacterial strains of *Salmonella typhi, Escherichia coli, Pseudomonas Aeroginosa, Staphylococcus aureus, Erwinia carotovora, Klebsiella Pneumoniae, Bacillus subtilis and Bacillus atrophaeus* were used for antibacterial activity.
Results: All the fractions were active against different bacterial strains but *n*-hexane and ethyl acetate showed (Zone of inhibition ranged from 18-30 mm) highest activity. The fungal strains, *Trichoderma longibrachiantum, Aspergillus flavus, Aspergillus niger, Fusarium solani* and *Candida albican* were used for antifungal activity. Excellent inhibitory effect was observed against all fungal strains. The minimum inhibitory concentration (MIC) against various fungal strains was determined. The minimum inhibitory concentrations (MICs) of the investigated plant fractions ranged from 0.5-2.00 mg/ml.
Conclusion: The plant showed significant antibacterial and antifungal activities. All tested plant extracts exhibit activities against different fungal strains. The result against various microorganisms shows the therapeutic potential of the plant *H. bacciferum*

Keywords: *H. bacciferum*, Medicinal Plant, crude fractions, Antimicrobial activities.

Introduction

Major sources of traditional medicines are plants with large variety of bioactive constituents, which are effective against different diseases. The significant biological activities of the plants are due to these bioactive constituents. Rich sources of antibacterial and antifungal agents are medicinal plants used in many countries as sources for potent and beneficial drugs (Mahesh, 2008). The genus *Heliotropium* belongs to the family Boraginaceae, which consists of about Hundred (100) genera and two thousand (2000) species (Ali and Nasir 1983). The distribution of Polyphenols and Flavonoids in family Boraginaceae has various pharmaceutical activities such as antibacterial, antiviral, antioxidant, hepato-protecting and anti-inflammatory (Iqbal et al., 2005). Species of Boraginaceae are distributed in temperate region, especially in tropical and Mediterranean area (Ali and Nasir, 1983).

H. indicum of the family Boraginaceae is used locally in Nigeria to treat ailments such as ulcer and fever. It is a common weed in waste places and settled areas. It's also found within tropical and non-tropical countries. The genus *Heliotropium* was derived from Helios (Sun) and trope (turn), that is, its flowers turn towards the sun. Other species of *Heliotropium* are *H. bacciferum, H. ovalifolium* and *H. pterocarpum*. *H. indicum* is an annual plant which grows in all parts of Nigeria and is given different names by the local communities. It bears different names, such as Cock's comb (Gambia), Indian Heliotrope, herb a verrues (France), Karkashen – koorama (Hausas Nigeria), Ogbe Akuko or Agogo Igun (Yorubas-Nigeria). Its chemical components include pyrrolizidine, alkaloids, tannins and saponins. Its alkaloid component confers on it anti-inflammatory, wound healing, antiseptic/ antimicrobial, febrifuge, secretagogue stimulation (of gall bladder functions) and menstruation activator properties. It's most important local application is for skin lesions, wounds, abscesses, gastric and varicose ulcerations, rashes and warts (Shoge et al 2011). The species of *Heliotropium* shows antimicrobial activities (Jain SC, 1998). *H. bacciferum* belongs to the family Boraginaceae. Rich sources of Pyrrolizidine alkaloids are present in *H. bacciferum*, some of which have antimicrobial, anti-hyperlipedemic, antitumor and anti-diabetic properties (Murugesh, 2006). From *H. bacciferum* four (4) pyrrolizidine alkaloids were isolated and identified as heliotrine, europine, heleurine, supinine. For their isolation, HPLC new preparative method was used (Farrage, 1996). The aim of the current study is to investigate the medicinal potential of the selected plant.

Materials and Method

Plant Collection and Identification

The plant *H. bacciferum* (HB4) was collected at Karak, KPK, Pakistan, in March, 2012. It was identified by plant taxonomist of the Department of Plant sciences of Kohat University of Science and Technology, KPK, Pakistan.

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Extraction and Fractionation:

The shade dried aerial parts of *H. bacciferum* (1.5 Kg) was taken and soaked in methanol for 15 days, extracted three times in the same solvent at room temperature and filtered. The resulting methanol extract (165g) was then suspended in water and partitioned successively to get n-hexane (26g), ethyl acetate (16g), *n*-butanol (23g), and aqueous (63g) fractions.

Antibacterial activity

Antibacterial activity of plant crude extract and their fractions were evaluated by Disc Diffusion Susceptibility method using the methodology of (Aida et al., 2001). Eight Bacterial strains, *Salmonella typhi, Escherichia coli, Pseudomonas Aeroginosa, Staphylococcus aureus, Erwinia carotovora, Klebsiella Pneumoniae, Bacillus subtilis* and *Bacillus atrophaeus* were used for antibacterial activity. The stock solution was prepared by the addition of 1 mg of crude and its fractions in 6µl (1mg/6µl) DMSO. The nutrient agar media (2.8 g/100 ml) and nutrient broth (1.3 g/100 ml) were prepared in distilled water in flasks and was sterilized with petri plates, Whattman filter paper discs, yellow tips, blue tips etc used in the activity were sterilized at 1.5 pounds pressure and 121 ^{0}C for 15 minutes. In a laminar flow hood, the media was poured into the petri plates, allowed to solidify and placed in an incubator at 37 ^{0}C. Into the sterilized nutrient broth, the microbial cultures were inoculated in flasks containing approximately 20-25 ml broth media and then in shaking water bath (GLSC-SBR-04-28) were incubated for Eighteen (18) hours at 200 rpm at 37 ^{0}C. For standardization, the sterilized nutrient broth in test tubes were then diluted with the microbial cultures from flasks and compared with 0.5 McFarland (turbidity) Standard. From standardized microbial cultures 50 µl were spread on each nutrient agar plates with help of a glass spreader. With the help of sterilized forcep, Whattman filter paper-1 discs (6mm in diameter) were then placed on agar media. The Crude, *n*-hexane, ethyl acetate, *n*-butanol and aqueous fractions were applied 6 and 12 µl/disc and then the plates were incubated at 37 ^{0}C for 24 hours. On separate plates, antibiotics (Ciprofloxacin, Azithromycin, Clotrimazole) were applied 6µl/disc as positive control for gram positive and gram negative bacteria. For each extract the zone of inhibition was then measured around each paper disc in millimeter (mm).

Minimum Inhibitory Concentration (MIC)

For Minimum inhibitory concentration, five dilutions (4, 2, 1, 0.5 and 0.25 mg/ml) of the crude and its fractions were prepared in Dimethyl sulfoxide (DMSO) for antifungal activity.

Antifungal activity: The Agar tube dilution method was carried out for antifungal activity. Five (5) fungal strains, *Trichoderma longibrachiantum, Aspergillus flavus, Aspergillus niger, Fusarium solani* and *Candida albican* were used for this activity. The fungal strains were cultured on Nutrient Agar. Five dilutions (4, 2, 1, 0.5and 0.25 mg/ml) of the crude and its fractions were prepared in Dimethyl sulfoxide (DMSO). Nutrient agar media (28 g/1000ml) was then prepared in distilled water and were sterilized with test tubes, yellow and blue tips etc at 1.5 pounds pressure and 121 ^{0}C for 15 minutes in an autoclave. After sterilization, 8 ml sterilized media and 1 ml from each five dilutions of all fractions were incorporated into sterilized test tubes. The test tubes were then kept in inclined position to make a slant for some time. Then in each test tube, a small piece of previously grown fungus was inoculated with the help of sterilized loop. All the test tubes were then incubated at 25 ^{0}C for seven days. After seven days, in each test tube the absence and presence of fungal growth was recorded (Aida et al., 2001)

Results and Discussion

In vitro antibacterial activities of crude and fractions of *H. bacciferum* and standard antibiotics are given in table 1. All extracts shows different inhibitory potentials. Ethyl acetate and *n*-hexane fractions show excellent activities (14-30 mm) against all bacterial strains. All the fractions were active against *S. typhi, E. coli, P. Aeroginosa, E. carotovora, K. pneumoniae, B. atrophaeus.* Butanol fraction shows good activity (15-22 mm) against all microorganisms except *S. aureus.* Crude extract was active (14-25 mm) against all bacterial strains. Aqueous fraction show activity against different bacterial strains (12-17 mm) but was inactive against *B. subtilis.*

In vitro antifungal activities of crude fractions of *H. bacciferum* are given in Table 2. The Minimum inhibitory concentrations (MICs) of all plant extracts are given in table 3. Excellent antifungal activity was shown by the plant *H. bacciferum.* All the fractions were active against all fungal strains. *Trichoderma longibrachiantum* were inhibited at 2 mg/ml concentration of crude, *n*-butanol and aqueous fractions, while on 1 mg/ml concentration of *n*-hexane and ethyl acetate. *A. flavus* were inhibited at 1 mg/ml concentration of crude, ethyl acetate and *n*-butanol, while on 0.5 and 2 mg/ml concentration of *n*-hexane and aqueous fractions respectively. At 1 mg/ml concentration of crude and aqueous fractions, 2 mg/ml concentration of *n*-butanol and 0.5 mg/ml concentration of *n*-hexane and ethyl acetate, *A. niger* was inhibited. *F. solani* was inhibited at 2 mg/ml concentration of crude, while at 1 mg/ml concentration of *n*-hexane, ethyl acetate, *n*-butanol and aqueous fractions. *C. albican* was inhibited at 1 mg/ml concentration of crude, *n*-hexane, *n*-butanol and 2 mg/ml concentration of ethyl acetate and aqueous fractions.

The plants and their extracts used in the treatment of diseases dates back to 460 to 370 BC when for healing the plant based drugs was used by Hippocrates (Soforowa, 1982). In this research, the obtained results indicated that the plant extracts inhibited the growth of different microorganisms. Therefore it showed that the plant extracts contained substances which can inhibit various microorganisms growth. At various concentrations, different researchers have also shown that the plant extracts inhibit the growth of different microorganisms (Nweze et al., 2004). The antibacterial activity of plant extracts is believed to be due to the presence of flavonoids, tannins and alkaloids (Draughon, 2004). Some researchers also observed that the antimicrobial effects of the extracts of plants are due to the presence of these different secondary metabolites (Nweze et al., 2004). Plant extracts are traditionally used in wound healing, sore and for boils treatment in the ear as ear drop. For dysentery and diarrhea control, they are also used. (Igoli et al., 2005). The large zones of inhibition exhibited by the Plant extracts showed significant activity against *P. aeruginosa* and *S. aureus,* which shows their use in the treatment of open wounds, sores and bores (Braude, 1982). The activity against *E. coli* by the plant extracts justifies their use in the treatment of dysentery and diarrhea. The mains cause of diarrhea and in humans other different diarrhoeagenic infections is *E. coli*. (Adams, 1999).

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Table 1: Zones of Inhibitions in millimeter (mm) of *H. bacciferum* against different bacterial strains
Noinhibition zone: Ciprofloxacin (*): 30µg/6µl. Azithromycin (**): 50µg/6µl. Clotrimazole (***):50µg/6µl, Ampicillin

Fractions mg/6µl	***E.coli***	***S. typhi***	***P. Aeroginosa***	***S. aureus***	***E. carotovora***	***K. pneumoniae***	***B. atrophaeus***	***B. subtilis***
Standard 50 or 30mg/6µl	28*	30*	30*	25**	24*	34*	26**	27**
Crude	14	14	16	16	18	21	15	25
n-hexane	22	22	20	21	18	24	20	14
Ethyl acetate	23	24	24	24	23	30	23	22
n-butanol	18	16	16	-	18	22	17	15
Aqueous	12	15	16	13	16	17	15	-

Table 2: Antifungal activities of crude fractions of *H. bacciferum* at dilutions (4, 2, 1, 0.5, 0.25 mg/ml)

Fractions	Concentration of the Extracts in mg/ml	*T. longibrachiantum*	*A. flavus*	*A. niger*	*F. solani*	*C. albican*
Crude	4	-	-	-	-	-
	2	-	-	-	-	-
	1	+	-	-	+	-
	0.5	+	+	+	+	+
	0.25	+	+	+	+	+
n-hexane	4	-	-	-	-	-
	2	-	-	-	-	-
	1	-	-	-	-	-
	0.5	+	-	-	+	+
	0.25	+	+	+	+	+
Ethyl acetate	4	-	-	-	-	-
	2	-	-	-	-	-
	1	-	-	-	-	+
	0.5	+	+	-	+	+
	0.25	+	+	+	+	+
n-butanol	4	-	-	-	-	-
	2	-	-	-	-	-
	1	+	-	+	-	-
	0.5	+	+	+	+	+
	0.25	+	+	+	+	+
Aqueous	4	-	-	-	-	-
	2	-	-	-	-	-
	1	+	+	-	-	+
	0.5	+	+	+	+	+
	0.25	+	+	+	+	+

Positive + indicates growth, Negative – indicates no growth

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Table 3: Minimum inhibitory concentrations (MICs) of crude fractions of *H. bacciferum* against fungal Strains.

Test Microorganisms	Minimum Inhibitory Concentrations (MICS) in mg/ml				
	Crude	*n*-hexane	Ethyl acetate	*n*-butanol	Aqueous
T. longibrachiantum	2	1	1	2	2
A. flavus	1	0.5	1	1	2
A. niger	1	0.5	0.5	2	1
F. solani	2	1	1	1	1
C. albican	1	1	2	1	2

Conclusion

The plant *H. bacciferum* was investigated for antimicrobial activities. The plant exhibits significant antibacterial and antifungal activities. All the plant extracts were active against different bacterial and fungal strains. Also, various gram positive and gram negative bacteria, plant showed significant activity. All the tested plant extracts exhibit activities against different fungal strains. The result of the study showed the importance of *H. Bacciferum* in the treatment of various diseases. The result against various microorganisms shows the therapeutic potential of the plant *H. bacciferum.*

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