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ANTIMICROBIAL AND PHYTOTOXIC EFFECTS OF THE PLANT *HELIOTROPIUM DASYCARPUM* L.

M. ABUZAR GHAFFARI*[1], S. BANO[2] AND K.HAYAT[3]

[1]Faculty of Pharmacy, Bahauddin Zakriya University, Multan, Pakistan,
2 Lecturer, University of Faisalabad, Faisalabad, Pakistan
[3]School of Pharmacy, Hajvery University, Lahore, Pakistan.

ABSTRACT

Heliotropium dasycarpum is an important desert plant of Boraginaceae family. The fresh extract of plant is used for the treatment of eye diseases. In the present study, dichloromethane and methanol crude extracts of plant was screened for antifungal, antibacterial and phytotoxic bioassay. Both extracts of *Heliotropium dasycarpum* inhibited the growth of *Lemna minor* L. and showed significant phytotoxic activities. The plantexhibited non-significant activity when tested against pathogenic gram positive and gram negative bacteria. The methanol extract of the plant showed 25% inhibition against a fungus, *Microsporum canis*. The phytochemical tests indicated the presence of alkaloids and cardiac glycosides in the plant.

KEYWORDS:*Heliotropium dasycarpum,* Phytotoxicity, LD50*, Lemna minor* L.

M. ABUZAR GHAFFARI
Faculty of Pharmacy, Bahauddin Zakriya University, Multan, Pakistan,

Corresponding author

INTRODUCTION

The beauty of this universe is nothing without plants. The key role of plants for the treatment of diseases is exemplified by their use right from the start of history. Our ancestors chewed the leaves of certain herbs to relieve pain and wound healing, so plants are being used to treat diseases and injuries from thousand years but also included much misrepresentation and irrational belief [1].Due to diverse medicinal potential, every civilization has accumulated experience and knowledge of their use of plants. Western medicine system was centered in Egypt and Mesopotamia, while the Unani (Islamic) and Ayurvedic (Hindu) systems were initiated in western Asia, the Indian subcontinent and those of the Orient from China, Tibet and Japan[2].The family, Boraginaceae comprises of hundred genera and two thousand species that are widely distributed in the temperate, mainly in Mediterranean or tropical regions. The plant, *Heliotropium dasycarpum* is very common in Iran, Afghanistan, Brazil and Turkmenistan. In Pakistan this species is found in Southern Punjab, Gilgit, Waziristan and Baluchistan[3,4]. Its local name is "Sagdaroo". The fresh extract of plant is locally used for eye diseases[5].

MATERIALS AND METHODS

The present work was done in natural product chemistry laboratory, Faculty of Pharmacy, Bahauddin Zakariya University, Multan campus (Pakistan), from January 2012 to January 2013.

(i) Collection and identification of plant

Collection of the plant was started in February-March, 2012 from desert area of Kot Mithan, District Rajanpur (Pakistan). The plant was identified by Institute of pure and applied Biology, Bahauddin Zakariya University, Multan and voucher was allotted.

(ii) Preparation of plant extracts

The whole plant of *Heliotropium dasycarpum* was dried under shade by placing on old newspapers for 15 days. When plant material dried, it was ground in grinding mill and weighed. The extraction of *Heliotropium dasycarpum* was carried out by simple maceration process. 200gm of ground plant material was taken in extraction bottle and measured volume of dichloromethane was added to it. To achieve maximum possible extraction, this mixture was shaken after some time then homogenized in ultrasonic bath. Filtration of this mixture was carried out after 24 hours. Then marc was macerated again by dichloromethane using same above procedure. After 3rd collection of this extract, the marc was extracted by methanol in the same manner. The extracts of dichloromethane and methanol were concentrated separately under reduced pressure by using a rotary evaporator. The extracts of dichloromethane and methanol were collected in separate sample bottles and weighed. Then they were designated codes as HDWPD and HDWPM respectively.

(iii) Determination of Phytochemical Constituents

The detection of alkaloids was carried out by using Dragendorff's reagent, Mayer's reagent and Wagner's reagent. For the identification of free and bound anthraquinones, Borntrager's test was performed. Similarly for cardiac glycosides, Keller Kiliani test; for tannins, Ferric chloride test and for saponins, Froth test was employed[6].

(iv) Antimicrobial Assay

Agar tube diffusion method

The methanol and dichloromethane extracts (HDWPM & HDWPD) of the plant were tested against six pathogenic bacteria *Eschericha coli* (NCTC 10418)*, Shigella flexinari, Bacillus subtilis* (NCTC 8236)*, Salmonella typhi, Staphylococcus aureus* (NCTC 6571) and *Pseudomonas aeruginosa* (ATCC 10145) by using Agar tube diffusion method. 10 ml of aliquots of the nutrient broth was inoculated with test organisms and incubated at 37ºC in oven

for 24 hours. A sterile pipette is used to add 0.6ml of broth culture of the test organism to 60 ml of molten agar which had been cooled to 45ºC that is mixed and poured into a sterile petri dish. For each organism, duplicate plates were prepared. The agar was allowed to set and harden. Then, Agar plugs were removed. 100 µl of the test samples of the plant were poured into labeled cups with a 0.1ml pipette. The same concentrations of the standard antimicrobial agents i.e. Imipenum 10 µg/ml and the solvent (as a control) were used. The plates were left at room temperature for 2 hours to allow diffusion of the samples of the plant and incubated face upwards at 37ºC for 24 hours. The diameter of the zones of inhibition was measured to the nearest mm[7,8].

(v) Antifungal Assay

Agar tube dilution method

Stock solutions of methanol and dichloromethane extracts of plant *(Heliotropium dasycarpum)* were dissolved separately into sterile Dimethyl sulfoxide. By mixing Sabouraud 4% glucose agar and agar in distilled water, Sabouraud dextrose agar was prepared and then it was stirred with a magnetic stirrer to dissolve it and a known amount of this agar was dispensed into a screw capped test tubes. Media containing test tubes were autoclaved at 121ºC for 15 min. Test tubes were cooled at 50ºC and then test sample of required concentrations was pipetted from the stock solution into the non-solidified sabouraud agar media. At room temperature, tubes were solidified in a slanting position. Each of the tube was inoculated with inoculum of 4mm diameter that was removed from seven days old culture of fungus. All tubes containing culture were inoculated for growth for 7-10 days at temperature of 28-30ºC. Humidity (40%- 50%) was maintained in the incubator. Cultures were examined two times in a week during the incubation period. After the incubation period of 7-10 days, test tube of no any visible growth of the microorganisms was taken to represent the MIC of the test sample that was expressed in µg/ml[9,10].

(vi) Phytotoxicity Assay

Lemna bioassay for Phytotoxicity

The E-medium was prepared and pH was maintained at 5.5-6.0 by the adding KOH pellets. 8 sets of 20 vials each for 500, 50, 5ppm and control were prepared for testing. 15mg of each of crude methanol and dichloromethane extracts of plant (*Heliotropium dasycarpum*) were dissolved in 15ml related solvents. 1000µl, 100µl and 10µl solutions of extract were added to 500ppm, 50ppm and 5ppm vials respectively. The solvent was placed for overnight to evaporate. 2ml of E-medium and a single plant of *Lemna minor* L. having a rosette of three fronds was added to each testing vial. These vials were placed in a glass dish that was filled with water up to 2cm and it was sealed with stopcock grease and glass plate. The glass dish was placed in a growth chamber for seven days at temperature of 26ºC under fluorescence and incandescent light. No. of fronds of each test vial were counted and recorded on 3rd and 7th day [11].

RESULTS AND DISCUSSION

1. Phytochemical tests

The Phytochemical tests were performed to evaluate the presence of secondary metabolites in drug sample. The results showed the presence of alkaloids and cardiac glycosides while the saponins, anthraquinone glycosides and tannins were absent in the plant extract. The results are given in the Table No 1.

Table 1
The Phytochemical constituents of Heliotropium dasycarpum.

Plant name	Alkaloids	Glycosides		Saponins	Tannins
		Anthraquinones	Cardiac glycosides		
Heliotropium dasycarpum	+	–	+	–	–

The presence of alkaloids in *Heliotropium dasycarpum* is continuous with the previous studies that were done in numerous plants of genus Heliotropium. Heliotropium species are extremely toxic in nature due to presence of pyrrolizidine alkaloids as major constituent that resulted in human deaths due to accidental consumption of these plants in different countries of the world. The pyrrole metabolites were formed from pyrrolizidine alkaloids by liver microsomal oxidation that causes liver damage to the effected people [12]. So the above positive result of alkaloid test is indicative in toxicity of the plant to the animals and human population.

2. Antibacterial activity

Antibiotic resistance is increasing worldwide. One possible source for new antimicrobials can be plants[13,14,15]. Various secondary metabolites especially pyrrolizidine alkaloids, saponins, tannins and triterpenoids were found to be responsible for the antimicrobial activitiesin the species of genus Heliotropium[16,17].Geranyl aromatic derivative named filifolinol obtained from *Heliotropium filifolium* and *Heliotropium Sclerocarpum* showed antimicrobial activity because these plants have ability to grow in extreme conditions of environment.So these constituents have key role in the defense mechanism of the plant[18,19,20].In this study, antibacterial activity of *Heliotropium dasycarpum* was determined. The zone of inhibition of methanol and dichloromethane extract of the plant was showed in the TableNo 2 and compared with standard drug named Imipenum. Both fractions were inactive against all bacterial cultures. So no antimicrobial activity was found against these tested organisms.

Table 2
Results of In vitro antibacterial bioassay of Heliotropium dasycarpum

Microorganism	Zone of inhibition (mm)		
	Methanol extract	Dichloromethane extract	Standard Drug *(Imipenum)*
Eschericha coli	0	0	25
Bacillus subtilis	0	0	50
Shigella flexinari	0	0	28
Staphylococcus aureus	0	0	48
Pseudomonas aeruginosa	0	0	23
Salmonella typhi	0	0	28

3. Antifungal activity

The Antifungal activity was carried out in plant *Heliotropium dasycarpum*. The results shown by methanol and dichloromethane fractions of plants are given in Table 3. The methanol fraction showed 25% inhibition against *Microsporum canis* while the dichloromethane fraction of plant was inactive against all fungal strains.The results of antifungal activity of the plant is in consistent with other plants of family Boraginaceae like *Echium rauwolfii*, *Echium horridum*[21], *Cordia alliodora*[22], *Cordia linnaei*[23], *Cordia morelosana*[24],*Arnebia euchroma*[25], *Arnebia hispidissima*[26], *Trichodesma amplexicaule*[27] and *Cynoglossum officinale*[28]. Moderate activity was reported by crude methanolic extract of *Onosma griffiithii* against *Aspergillus flavus* (55%) and *Fusarium solani*

(40%) while its n-butanol and ethyl acetate extracts shows no activity [29]. No antifungal activity was recorded when aqueous and ethanolic extract of the plant, *Colendia procumbents* was tested against *Candida albicans*[30]. Aqueous and methanol extracts of *Anchusa italic* and *Trichodesma zeylanicum* also displayed no activity[31].

Table 3
Results of In vitro antifungal bioassay of Heliotropium dasycarpum

Name of Fungus	Linear Growth (mm)		% Inhibition		Standard Drug	MIC (µg/ml)
	Sample	Control	Methanol extract	Dichloromethane extract		
Candida albicans	100	100	0	0	Miconazole	110.8
Aspergillus flavus	100	100	0	0	Amphotericin B	20.20
Microsporum canis	75	100	25	0	Miconazole	98.4
Fusarium solani	100	100	0	0	Miconazole	73.25
Candida glabrata	100	100	0	0	Miconazole	110.8

4. Phytotoxic activity

Weeds are one of the major causes of poor agricultural productivity in Pakistan. So there is a need to discover new natural weedicides (herbicides) because synthetic weedicides available in the market are expensive, toxic and non-specific. The *Lemna minor* assay is a quick measure of phytotoxicity of plants extracts[32].The results of phytotoxic bioassay of crude methanol and dichloromethane extracts of *Heliotropium dasycarpum*are given in the Table No 4.

Table 4
Results of In vitro phytotoxic bioassay of Heliotropium dasycarpum

Extract of Plant	Plant Name	Conc. of Compound (µg/ml)	No. of Fronds		% Growth Regulation	Standard Drug *(Paraquat)* Concentration (µg/ml)
			Sample	Control		
Methanol	*Lemna minor*	1000	0	20	100	0.015
		100	7		65	
		10	15		25	
Dichloromethane		1000	0		100	
		100	4		80	
		10	8		60	

The results showed that both extracts of plant *Heliotropium dasycarpum* showed 100% phytotoxic activity against *Lemna minor* at concentration of the 1000 µg/ml while dichloromethane extract of the plant showed 80% inhibition at concentration of 100 µg/ml. Moderate activity was reported by methanol (65%) and dichloromethane (60%) extract of plant at concentration of 100 µg/ml and 10 µg/ml respectively. The methanol plant extract exhibited lowest activity at 10 µg/ml concentration.The phytotoxic measurementswas previously done in Heliotropium species especially aqueous extract of *Heliotropium indicum* exhibited allelopathic growth regulation effectafter testing on wheat rootlet[33].The phytotoxic studies on ethyl acetate fraction of *Heliotropium strigosum* resulted in highest (LD_{50} 91µg/ml) activity whereas chloroform, crude plant extract and n-hexane fraction of the plant showed 50%, 30.76 ± 1.1% and 30.7 ± 1.1% inhibition at maximum concentration (1000µg/ml)[34].The species of Heliotropium genus have valuable tendency towards phytotoxicity but further work is required to be discussable.So above literature and experimental work provides scientific basis for the use of the plant *Heliotropium dasycarpum* as a potent herbicidal agent that provides new era of isolation of its phytochemical constituents. The plant can grow in extreme conditions of the environment so

less antifungal activity must be taken under consideration for the future study.

CONCLUSION

The present findings have clearly demonstrated that the plant has excellent potential towards the phytotoxic activity.The growth inhibition of the plant against *Microsporum canis* identified that the plant can be used as antifungal agent for the treatment of different fungal diseases. So the further studies are needed to purify the active constituents of the plants that are responsible for its phytotoxic and antifungal activity to get a valuable herbicidal and antifungal agent.

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