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Phytochemical Analysis, Antifungal and Phytotoxic Activity of Seeds of Medicinal Plant *Indigofera heterantha* Wall.

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Abstract: The seeds of Indigofera *heterantha* are used in folk medicine for the treatment of gastrointestinal disorder and abdominal pain. Biological evaluation on the seeds extracts were carried out. The antifungal activity was tested against six fungal strains viz., *Trichophyton longifusis, Candida albicans, Aspergilus flavus, Microsporum canis and Candida glaberata* shows non significant activity. The phytotoxic activity was tested against the specie *Lemna minor* shows significant activities (50-85%) Phytotoxicity.

Key words: Indigofera *heterantha* · Antifungal activity · Phytotoxic activity

INTRODUCTION

About 80% of the world population used medicinal plants for the basic needs of their health cares. The interaction between man, plants and drugs derived from plants describe the history of mankind. Plants are important factories of natural products used as drugs against various dieasess. The plants contain various chemical constituents, which have potential to be used in modern medicine to treat diseases which are not curable. Pakistan is selfsufficient in indigenous floras especially the Khyber pukhtoonkhwa province. The country has more than 6000 species of wild plants, out of which about 1500 are considered to be medicinally important. During the past decade, traditional system of medicine has become a raising issue of global importance. Current estimates shows that in many developing countries, a large number of population relies heavily on traditional practioners and medicinal plants to meet the primary health care needs, although modern medicine may be available in these countries. Herbal medicines are considered to be safe and have often maintained popularity for historic and cultural reasons. Currently, many people in the developed countries have started to turn to complementary and alternative medicine (CAM) therapies, including where conventional health care is provided. Indigofera *heterantha* (Fabaceae) wall commonly known an (Indigo Himalayan) is a deciduous shrub 30-60 cm tall widely distributed in the tropical region of the globe [1]. In Pakistan, it is represented by 24 species. The bark of this plant is used in folk medicine to treat gastrointestinal disorder and abdominal pain in the Swat Valley of Pakistan [2]. The plants as well as the whole genus are a rich source of bioactive compounds such as monoterpenes [3], tritepenes [3], steroids [3], lignins [3], tannins[4] and alkaloids [5]. Biological activites such as lipoxigenase [6], dehydrogenase [7], antiulcerogenic [8], antioxidant [9] and antibacterial [10] activities have also been reported. In the light of previous reported data and biological activities the present work was initiated to search for new bioactive constituents in the seeds of indigofera *heterantha* plant.

METERIALS AND METHODS

Plant Material: The Pods of Indigofera *heterantha* were collected in the month of May 2009 from lower Dir, northern part of Pakistan. Taxonomic identification of the plant was done by Mr. Samin Jan, Associate Professor, Department of Botany, Islamia University, Peshawar, KPK province, Pakistan. A voucher specimen (No.Sj-36) was deposited in the herbarium of Islamia University, Peshawar Pakistan.

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Extraction: The powdered pods (22 kg) were soaked (cold extraction) in water-methanol (1:19) for seven days. The crude water-methanol extract was filtered and concentrated at reduce pressure using rotary evaporator at 50 °C, afforded a crude semi solid mass of (2.92kg) F1. The F1 fraction was then dissolved in chloroform resulted in to (41g) soluble fraction F2 and remaining insoluble fraction F3.

The chloroform soluble fraction F2 was further partitioned with *n*-hexane and methanol afforded (3g) F2-X and (36g) F2-Y crude extracts respectively using Soxhlet extractor for one day. While the insoluble fraction F3 was further dissolved in ethyl acetate and concentrated which result in (1660g) crude fraction F4. The ethyl acetate soluble fraction F4 was further fractionated between diethyl ether and water which gave (400g) ethereal crude fraction F4-Z the water fraction (440g) F4-W and insoluble residue (360g) F4-I.

Micro-Organism Collection and Maintenance: The microorganism used in antifungal activity activity are: *Trichophyton longifusis, Candida albicans, Aspergilus flavus, Microsporum canis and Candida glaberata.* The phytotoxic activity was tested against the plant species *Lemna mino.*These organism and plant species were collected from stock culture in the PCMD, HEJ research laboratory, university of Karachi, Karachi Pakistan. The organisms were kept on agar in muller hantin agar in the refrigerator at 40°Cprior to subculture.

Antifungal Bioassay of the Seed of Indigofera Heteranta Against Selected Bacterial: The antifungal activity was determined by the Agar tube dilution Method. In this method the crude extract was dissolved in DMSO (24mg/1ml). After this the Sterile Sabouraud's dextrose agar medium (5ml) was placed in a test tube and inoculated with the sample solution (400 µg /ml) which was then kept in a slanting position at room temperature for one night. The fungal culture starts inoculating on the slant. The samples were incubated for 7 days at 29°C and growth inhibition was observed and percentage growth inhibition was determined by calculating with reference to the negative control by applying the formula:

$$\% \text{ inhibition of fungal growth} = \frac{100 - \text{linear growth and test (mm)}}{\text{in linear growth in control (mm)}} \times 100$$

In this study Miconazole and amphotericin B were used as standard drugs, while miconazole, amphotericin B and DMSO were used as positive and negative controls.

Phytotoxic Activity Activity of Seeds of Indigofera Heteranta: Phytotoxic activity was observed by adopting the modified protocol Of *Lemna minor* (Atta-ur-Rehman, 1991; Ali *et al.,* 2009; Rashid *et al.,* 2009). The Medium was made by mixing various constituents in 1000 ml distilled water and then its pH was adjusted (5.5-6.5) by mixing KOH solution. After this the medium obtained was autoclaved at temperature ranges from 121°C for a duration of 15 minutes. The extracts dissolved in ethanol (20 mg/ml) served as stock solution. After this nine sterilized flasks, three for each concentration, were inoculated with an amount of 1000 µl, 100 µl and 10 µl of the stock solution for 1000, 100 and 10µg/ml respectively. The solvent was allowed to evaporate for a night under sterile conditions. To each and every flask, medium (20 ml) and plants (10), each containing a rosette of three fronds of *Lemna minor* L., was mixes. All flasks were cotton plugged and placed in the growth cabinet for 7 days. The number of fronds per flask were counted and recorded on day seven and their growth regulation in percentage was determined by calculated with the formula given below:

$$\text{Growth regulation (\%)} = \text{Mortality (\%)} = \frac{\text{100 – number of the fronds in the test sample}}{\text{Number of the fronds in the control samples}} \times 100$$

The result was calculated with reference to the positive and negative control. In this study Paraquat was used as a standard drug, while paraquat and volatile solvent were used as positive and negative controls.

Table 2: Antifungal activities of indigofera *heterantha* seeds

		% inhibition						
Fungal species Resd	Standard	*n*-hexane	Chloroform	Et-acetate	Eether	methanol	Aqueous	
Trichophyton longifusis	Miconazole 70	-	-	-	-	-	-	-
Candida albicans	Miconazole 110.8	-	-	-	-	-	-	-
Aspergilus flavus	Amphotericin 20	-	-	-	-	-	-	-
Microsporum canis	Miconazole 98.4	-	-	-	10	15	-	10
Fusarium solani	Miconazole 73 10	-	-	30	-	-	-	-
Candida glaberata	Miconazole 110.8	-	-	-	-	-	-	-

Table 3: Phytotoxic activities indigofera *heterantha*

Conc. of Sample (µg/ml)	Conc. Of stand drug	% Growth regulation						
		n-hexane	Chloroform	Et-acetate	Ether methanol	Aqueous	Residue	
1000		85	50	35	85	75	05	35
100	0.015	05	15	-	10	05	-	05
10		-	05	-	-	-	-	-

DISCUSSION

The medicinal properties such as antifungal and phytototoxicity of ethyl acetate, ether and methanol extract may be attributed to the presence of steroid, terpenoids, tannins and reducing sugars [11]. The crude Di-Ethyl ether extracts obtained showed the presence of steroids, terpenoids, tannins and reducing sugars [11]. The presence of such components in this fraction may have antifungal activity but no work have been reported so far. Similarly the water extracts showed the presence of Steriods, tannins, saponins and reducing sugar only [11]. The antifungal activity of this fraction may be attributed to the chemical constituents present in this fraction. No work has been reported. The residue contains steroids, terpenoids and tannins [11]. These components in this fraction may be ascertained antifungal and phytotoxic activities but no work have been reported so far. The chloroform fraction contains Alkaloids, Steroids, Terpenoids, Flavonides, Tannins, Glycoside and Reducing sugars [11]. This fraction shows no antifungal activity but shows phytotoxictoxic activity. The specific activity that may be attributed to the presenc of alkaloids and flavonoids need futher investigation. The use of the species of this genus in gastrointestinal pain and to cure abdominal pain as well the presence of flavonids and tannins attribute its use in folk medicine.

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