Full Length Research Paper

**In-vitro pharmacological investigations of aerial parts of indigofera heterantha**

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**INTRODUCTION**

Genus indigofera, consist of 700 species. All the species are mostly herbs or shrubs distributed thoroughly the tropical region of the globe. In Pakistan it is represented by 24 species. The plant *indigofera heterantha* commonly known as Ghorega belongs to family leguminoseae (Fabaceae) (Hamayun et al., 2003). It is abundantly available in the northern region of Pakistan with wide varieties of medicinal uses. In northen parts of Pakistan, it is widely used as traditional medicine for treatment against abdominal pain, spastic pain and various skin infections and other infection disease (Nasir and Ali, 1997). Up to our knowledge considerable work is available on genus *indigofera*. *I. dalecoides* Benth is used for the treatment of diarrhea and various pathogenic bacterial infections (Mathabe et al., 2006). *Indigofera oblogifolia* possessed antimicrobial (Dahot, 1999), hepatoprotective (Shahjahan et al., 2005) and strong lipoxygenase inhibitory activity (Sharif et al., 2005).

*Indigofera pulchra* has showed snake-Venom neutralizing activity (Abubakar et al., 2006). *Indigofera tinctoria* showed antioxidant, free radical scavenging activity, and anti-dyslipidemic activity (Prakash et al., 2007). *Indigofera Mysore's* has showed anti diabetic activity. *Indigofera emarginella* showed *in-vitro* antimalarial activity against plasmodium falciparum (Chakrabarti et al., 2006).The aim of this study was to explore the antibacterial and antifungal activity of *indigofera heterantha* to determine the scientific basis for its use as folk medicine to treat microbial pathogen infected and other infectious diseases. Brine shrimps lethality bioassay was also done so as to assess its safety. Besides, we have make an attempt to investigate the importance of *I. heterantha* as an important medicinal plant for its insecticidal and phytotoxicity potential to be used as strong natural insecticide and herbicides.

**EXPERIMENTAL**

**Plant material**

The aerial parts of *I. heterantha* were collected during the month of May 2009 from Dir Lower, northern parts of Pakistan. The plant was identified and authenticated by Mr. Samin Jan, Associate Professor,
In this study, six fungal and six bacterial strain were used as standard antibiotics to compare extract and fraction with it. In this experiment 3 mL of all extract/fractions (1mg/mL) were applied to the filter papers with (90 mm diameter). After getting dried, each filter paper was allowed to place in a separate Petri dish along 10 adults of each of Tribolium castaneum, Sitophilus oryzae, Trogoderma granarium, callosobruchus analis and rhyzopartha dominica. Permethrin (235.71 µg/cm²) was used as a reference insecticide. These entire insect were allowed to stand without food for 24 h after which the mortality number was counted.

Phytotoxicity assay

In this bioassay the crude extract was tested against lemna minor (Ahn et al., 1995). In this study three flask were inoculated with a sufficient stock solution of (20 mg mg/mL) to obtained a final concentration of 500, 50, and 5 µg/mL, respectively. Each flask was then added a 20 mL medium 10 plants each one containing rosette of three fronds. Parquet was used as a standard growth inhibitor. The whole flasks were kept in growth cabinet for incubation up to seven days. After this growth regulation in percentage was determined with reference to the negative control. IC₅₀ was obtained by calculating through finny computer program.

Antibacterial activity

Crude extract and all fractions exhibited no antibacterial activity against all bacterial strains used for antibacterial activity.

Antifungal activity

N-hexane fraction (H), Ethyl acetate fraction (E), methanol fraction 1 (M1), methanol fraction 2 (M2), aqueous fraction (A) were studied (Table 1) for their antifungal activity against the Trichophyton longifusis, Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani, Candida glaberata. All fractions revealed no activity against selected fungal strains except methanol fraction 2 (M2), which showed low activity against M. canis as shown in Figure 1.

Insecticidal assay

In this bioassay a shallow rectangular plastic dish (22 x 32 cm), filled with artificial sea water was taken. The sea water was prepared with commercial salt mixture mixed with double distilled water. The Brine shrimp (Artemia salina leach) eggs were hatched the dish. The dish was made unequally partitioned by using an artificial perforated device. About 50 mg of the eggs were sprinkled into large compartment which becomes darken. The minor compartment was exposed to the ordinary light. After two days, nauplii were collected and removed by a pipette from lighted side. A sample of the compounds to be tested was prepared by dissolving 20 mg of each compound in Dimethylformamide (DMF) (2 ml). Three different stock solution that is, 550, 50, and 5 mg/mL were transferred to 9 vials (three for every dilution were used for each test sample and LD₅₀ is the average of the three values) with one vial containing DMF was reserved as a control. The solvent was allowed to evaporate keeping overnight. Two days, later when the shrimp larvae were ready, 1 mL of sea water and 10 shrimp were added to each vial (30 shrimps/dilution) with a volume adjusted with sea water to 5 mL per vial. After 24 h, the numbers of survivors were counted using standard procedure (McLaughlin et al., 1991; Meyer et al., 1982). The data was analyzed by the use of finny computer program to determine LD₅₀ values as reported earlier.
Table 1. Antifungal activities of indigofera heterantha aerial parts.

<table>
<thead>
<tr>
<th>Fungal strain</th>
<th>Std.</th>
<th>H</th>
<th>E</th>
<th>M1</th>
<th>M2</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichophyton longifusis</td>
<td>70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15</td>
</tr>
<tr>
<td>Candida albicans</td>
<td>110.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microsporum canis</td>
<td>98.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusarium solani</td>
<td>73.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida glaberata</em></td>
<td>110.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 Amphotericin, 2 Miconazole, H = n-hexane fraction, E = ethyl acetate fraction, M1 = Methanol fraction 1, M2: Methanol fraction 2, A = aqueous fraction.

![Graph](image)

Figure 1. Insecticidal activities of the extracts of *indigofera heterantha* aerial parts.

Table 2. Brine shrimp cytotoxic activity of the extracts of *Indigofera heterantha* aerial parts.

<table>
<thead>
<tr>
<th>Extract</th>
<th>Dose (µg/ml)</th>
<th>No. of shrimps</th>
<th>Mortality %</th>
<th>LD50 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>1000, 100, 10</td>
<td>30(t)</td>
<td>43.3, 23.3, 16.6</td>
<td>3314.79, 42152, 474.86</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>1000, 100, 10</td>
<td>30(t)</td>
<td>46.6, 26.6, 16.6</td>
<td>1775.28, 34458, 349.04</td>
</tr>
<tr>
<td>Methanol 1</td>
<td>1000, 100, 10</td>
<td>30(t)</td>
<td>46.6, 33.3, 13.3</td>
<td>1175.80, 32879, 290.74</td>
</tr>
<tr>
<td>Methanol 2</td>
<td>1000, 100, 10</td>
<td>30(t)</td>
<td>46.6, 33.3, 13.3</td>
<td>1175.80, 32879, 290.74</td>
</tr>
<tr>
<td>Aqueous</td>
<td>1000, 100, 10</td>
<td>30(t)</td>
<td>43.3, 23.3, 66.6</td>
<td>1653.13, 79477, 483.77</td>
</tr>
</tbody>
</table>

*Rhyzopertha dominica* and *Callosbruchuanalis*. The test sample was prepared by dissolving crude fractions in 3 ml acetone and placed in a Petri dish with the filter papers covered. After duration of 24 h, 10 test insects were added in each plate and incubated at 27°C for 24 h with 50% relative humidity in growth chamber. Results were obtained as percentage mortality, which was calculated with reference to the positive and negative controls. In this bioassay Permethrin was used as a standard drug, while Permethrin, acetone and test insects were used as positive and negative controls. All the fraction tested shows no activity the insects *castaneum, R. dominica* and *Callosbruchuanalis*, except n-hexane fraction (N1), Ethyl acetate fraction (E) which showed 20% activity against *R. dominica*. N-hexane fraction (H) and Methanol fraction 1 (M1) showed (Figure 1) 20% activity against *Callosbruchuanalis*. The residue fraction (A) showed 20% activity against *T. castaneum*.

**Cytotoxicity assay**

No significant (Table 2), cytotoxicity was found which indicates preliminary safety profile of the fractions of crude extract.
Cytotoxicity data supports ethnopharmacological use of this plant without causing significant cytotoxicity.

Phytotoxicity assay

Results of phytotoxicity assay of various fractions of the aerial parts of *I. heterantha* are shown in the Figure. n-hexane fraction (H), Ethyl acetate fraction (E) and methanol fraction 1 (M1) showed good phytotoxicity activity at 1000 μg/ml concentration. Similarly Methanol fraction 2 (M2) also showed moderate phytotoxicity. However aqueous fraction (A) showed low activity at 1000 μg/ml concentration. All fraction showed moderate activity at 100 μg/ml while low activity at 10 μg/ml.

DISCUSSION

The current investigation strongly supported the traditional use of *I. heterantha* for various pathological conditions. Interestingly no antibacterial activity was found while low antifungal activity was observed in case of fractions of *I. heterantha*. These activities may be attributed to the presence of alkaloids, phenols, polyphenols, saponins, tannins, anthraquinones, steroids and especially the diterpenes, found in the crude extract and the fractions thereof (Araruna and Carlos, 2010). These phytochemical groups/families of natural products are known to display various activities. Further purification and characterization of the active principles from fractions for insecticidal and phytotoxicity studies will provide a better understanding of the pharmacological mechanisms.

REFERENCES


