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# Phytochemical screening, anti-diabetic and antioxidant potential of methanolic extract of *Indigofera heterantha* roots

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## Abstract

The aim of the current study was to detect the Phytochemical present in the methanolic extract of the plant using the biochemical tests and to evaluate the anti-diabetic and antioxidant potential of the methanolic extract of *I. heterantha* roots using Glucose uptake yeast in cells assay and 1, 1-diphenyl -2-picrylhydrazyl (DPPH) free radical scavenging assay. The methanolic extract showed good anti-diabetic activity with minimum increase 5.31% at 10µg/ml and maximum 53.19% at 80µg/ml glucose concentration while moderate antioxidant activity with minimum radical scavenging activity 22.00% at 200µg/ml and maximum activity 56.35% at 1000µg/ml. The results obtained revealed that this plant is very important from medicinal point of view.

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## Introduction

*I. heterantha* locally known as ghoreja or kainthaye (in Pashtho), Jangli methi (in Urdu) and Himalayan Indigo (in English) extensively spread in Northern regions of Pakistan and possessing high medicinal importance in the indigenous system of medicine. It is a shrub of 30 to 60cm tall and the leaves are imparipinnately compound, while the fruits are long cylindrical 1.5cm with 10-12 seeds (Hamayun *et al.,* 2006).

The various constituents have been isolated from the *I. heterantha* exhibited inhibitory activity against the enzyme lipoxygenase (Rehman *et al.,* 2005). The phytotoxic and antifungal activity has been shown by the various crude fractions of extract of seeds of *I. heterantha* (Ghias Uddin *et al.,* 2011) as well as insecticidal and antimicrobial potentials (Ghias Uddin *et al.,* 2011).

To our knowledge, considerable work is available on genus *Indigofera*. *I. oblongifolia* has shown its antimicrobial (Dhot, 1999) hepato protective (Shahjahan *et al*., 2005) and lipoxygen as inhibitory activity (Sharif *et al.,* 2005). Abubakar *et al.* has reported the snake-venom neutralizing activity of *I. pulchra* (Abubakar *et al.,* 2006). Antioxidant and free radical scavenging and anti-dyslipidemic activities of *I. tinctoria* has been reported (Parkash *et al.,* 2007; Waako *et al.,* 2007).

Similarly, *I. emarginella* has shown *in-vitro* antimalarial activity against Plasmodium falciparum. Chakrabarti *et al.* have reported the antidiabetic activity of *I. mysorens* (Chakarbarati *et al.,* 2006). Whole plant is used in hepatitis, whooping cough (Shinwari *et al.,* 2006) antispasmodic (Khan *et al*., 2003), tonic (Gamble, 1972), the extract prevents the development of hypoglycemia in the mouse (Nyarko *et al.,* 1998); the leaves, flowers and tender shoots are cooling and demulcent, they are used in the form of liprosary and cancerous infection. The leaves are applied to abscesses. The roots are chewed in toothache and apathy (Gamble, 1972). The alcoholic extract of the dried shoots is reported anti-inflammatory activity (Amala Bhaskar *et al.,* 1982); the root bark is chewed in the mouth to relieve the abdominal pain (Esimon, 1999); leaves, bark and roots have anti-bacterial activity (Umar, 1999; Khan *et al.,* 2003).

*I. heterantha* has a widespread ethno-botanical uses, such as *I. heterantha* is used as herbal medicine as well as folk medicine to treat gastrointestinal disorder and abdominal pain (Hamayun *et al.,* 2006). To provide scientific evidence to the ethnobotanical uses of *I. heterantha*. The current study was attempt to detect the constituents and investigate the importance of *I. heterantha* roots as an important medicinal plant for its ant-diabetic and antioxidant potential.

## Materials and methods

### *Plant Material*

*I. heterantha* roots were collected during the month of September, 2015 from District Swat, K.P.K Pakistan. The identification of plant was done at the Department of Botany, Hazara University, Mansehra, Pakistan. The plant material was washed and dried in shade for fifteen days then chopped and powdered using a grinder.

### *Extraction*

The powdered plant materials (5Kg) was extracted by maceration in methanol for 10 days with (10L) solvent at room temperature, and the extract was concentrated in vacuum to yield 900g of residue.

### *Phytochemical Screening*

Phytochemical screening of methanolic extract of *I. heterantha* roots was performed using standard procedures as described by Sofowora, (1993), Trease and Evans, (1989) and Harborne (1973).

### *Alkaloids*

About 0.2g of the extract was warmed with 2% $H_2SO_4$ for two minutes. It was filtered and a few drops of Dragendroffs reagent were added. Orange red precipitate indicated the presence of alkaloids.

### *Anthraquinones*

About 0.5g of the extract was boiled with 10% HCl for few minutes in water bath. It was filtered and allowed to cool.

Equal volume of $CHCl_3$ was added to the filtrate. Few drops of 10% ammonia was added to the mixture and heated. Formation of rose-pink color indicates the presence of anthraquinones.

*Flavonoids*

Extract of about 0.2g was dissolved in diluted NaOH and HCl was added. A yellow solution that turns colorless indicates the presence of flavonoids.

*Reducing Sugars*

The extract was shaken with distilled water and filtered. Then boiled with few drops of Fehling's solution A and B for few minutes. An orange red precipitate indicates the presence of reducing sugars.

*Saponins*

About 0.2g of the extract was shaken with 5ml of distilled water and then heated to boil. Frothing shows the presence of saponins.

*Steroids*

2ml of acetic anhydride was added to 0.5g of the extract with 2ml of $H_2SO_4$. The color changed from violet to blue or green in some samples indicate presence of steroids.

*Tannins*

A small quantity of the extract was mixed with water and heated on water bath and filtered. A few drops of ferric chloride was added the filtrate. A dark green solution indicates the presence of tannins.

*Terpenoids (Salkowski Test)*

0.2g of the extract was mixed with 2ml of chloroform ($CHCl_3$) and concentrated $H_2SO_4$ (3ml) was carefully added form a layer. A reddish brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

*Anti-diabetic Activity*

The anti-diabetic activity of methanolic extract was measured on the basis of the Glucose uptake in yeast cells method. Commercial baker's yeast was dissolved in distilled water. It was kept overnight at room temperature. The yeast cell suspension was washed by centrifugation at 4200rpm (Microphage® 16 Centrifuge, FX241. 5P Rotor, 50/60Hz and 220-240V) in distilled water for 5 minutes. The process was repeated again and again until clear supernatant fluids were obtained. 1% suspension of yeast cells with distilled water was prepared. 5mm glucose solution was prepared in distilled water. 1mg of the methanolic extract was dissolved in DMSO for stock solution. Various concentrations (10µg, 20µg, 40µg, 60µg, 80µg) in DMSO, 1ml Glucose and 100µl of yeast was used to prepare reaction mixture for evaluation of the anti-diabetic activity. The reaction mixture was vortexes and incubated further for 60 minutes at 37 ˚C. After one hour of incubation of the reaction mixture, the tubes were centrifuged for 5 minutes at 3800rpm. Glucose left behind in the supernatant was estimated by measuring the absorbance via spectrophotometer (UV 5100B spectrophotometer) at 520nm. The percent increase in uptake was calculated by the formula:

$$\% \text{ increase in glucose uptake} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

*Antioxidant activity*

The antioxidant activity of the methanolic extract of the roots of *I. heterantha* was determined using the 1, 1-diphenyl -2-picrylhydrazyl (DPPH) free radical scavenging assay by the method of Blois, (1958). 1 mm 1, 1-diphenyl 2-picrylhyorazyl (DPPH) solution was prepared in methanol. 1mg Stock solution of methanolic extract solution was prepared in methanol. Different concentrations (200µg, 400µg, 600µg, 800µg and 1000µg) were taken with methanol along with 1ml of 1, 1- diphenyl 2-picrylhyorazyl (DPPH). Solution with final concentration of reaction mixtures was taken in falcon tubes and were thoroughly mixed. Ascorbic acid was used as a reference antioxidant. The DPPH solution without sample solution was used as control. For control 3 ml of methanol and 1 ml DPPH solution was taken in a falcon tube. The prepared solution mixtures were shaken vigorously and incubated at 37 ºC in dark for half an hour. After 30 minutes in the dark the solutions were taken out of the dark. By using UV 5100B spectro photometer the absorbance was measured at 517 nm. Increase in the DPPH radical scavenging percentages was correlated with the decrease in the absorbance by spectrophotometer. Using the following formula DPPH radical scavenging percentages was calculated.

$$(\%)\ \text{Scavenged DPPH} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100$$

## Results and discussion

The Phytochemical screening of methanolic extracts of roots of *I. heterantha* are listed in (Table1).

**Table 1.** Results of Phytochemical screening of *Indigofera heterantha* roots.

| Alkaloid | Anthraquinone | Flavonoid | Reducin | | Steroid | Tannin | Terpenoid |
|---|---|---|---|---|---|---|---|
| s | s | s | g Sugars | | s | s | s |
| + | - | + | + | + | + | + | |

(+): Present (-): Absent

*Anti-diabetic Activity*

The evaluation of anti-diabetic potential of plants can be exposed in vitro by a number of procedures such as study of glucose uptake, inhibition of alpha amylase, alpha glycosidase enzymes and effect on glycoslation of the hemoglobin. For assessing the hypoglycemic properties of different medicinal plants the method of glucose transport through the yeast cell membrane has been achieved an outstanding importance as an in vitro screening method (Maier *et al.,* 2002). The present study revealed that methanolic extract of the plant has good anti-diabetic activity. The glucose transport takes place in yeast through facilitated diffusion. After treating the yeast cells with the methanolic crude extract, the glucose uptake was found to increase in a dose dependent manner. In (Fig. 1) the results revealed that methanolic extract exhibited moderate activity at all glucose concentrations with minimum increase 5.31% at 10µg/ml and maximum increase 53.19% at 80µg/ml glucose concentration. The results clearly indicated that methanolic extract had good efficiency in increasing the glucose uptake by yeast cells as compared to standard drug Metronidazole 50% at 10µg/ml and 81.37 at 80µg/ml.

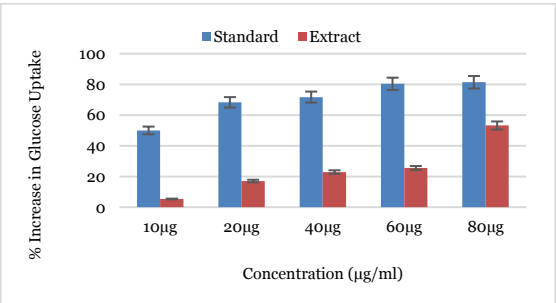


**Fig. 1.** % Increase in glucose uptake by yeast cells due to the effect of methanolic extract.

*Antioxidant Activity*

The methanolic extract of the roots of *I. heterantha* was subjected to DPPH free radical scavenging assay to evaluate its antioxidant properties with reference to ascorbic acid a natural antioxidant. Ascorbic acid showed the minimum radical scavenging activity 84.95% at 200µg/ml and maximum activity 91.07% at 1000µg/ml while the methanolic extract of the plant showed minimum radical scavenging activity 22.00% at 200µg/ml and maximum activity 56.35% at 1000µg/ml. The results obtained revealed that the scavenging activity of methanolic extract of the plant was a dose dependent as shown in (Fig. 2).

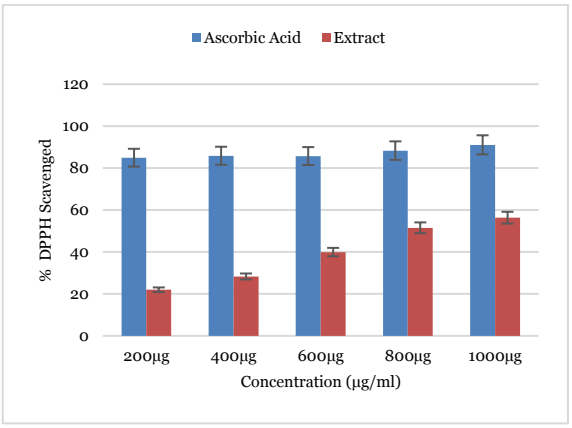


**Fig. 2.** Scavenging activity of methanolic crude extract compared with ascorbic acid.

## Conclusion

In the current study the roots of *I. heterantha* were investigated to explore its medicinal importance. The results obtained exhibit that this plant is very important from medicinal point of view, and it needs further phytochemical exploitation to isolate phytochemical constituents having antidiabetic and antioxidant activities.

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**Conflicts of Interest**

Authors have none to declare.

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