academicJournals

Vol. 12(8), pp. 77-80, 30 April, 2017
DOI: 10.5897/SRE2014.5814
Article Number: 3FADC8265368
ISSN 1992-2248

http://www.academicjournals.org/SRE

Scientific Research and Essays

Full Length Research Paper

Cytotoxicity of aerial parts of *Indigofera heterantha*

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Received 22 January, 2014; Accepted 5 May, 2016

In the ongoing phytochemical study, an effort was made to investigate cytotoxicity of various crude fractions of aerial parts of *Indigofera heterantha*. The results obtained revealed that all the fractions including *n*-hexane, ethyl acetate, methanol and residue showed brine shrimp (*Artemia salina* Leach) cytotoxicity activity. The data obtained revealed the medicinal importance of the plant and will help the researchers to exploit the phytochemicals for biological activities (cytotoxicity).

Key words: *Indigofera heterantha*, cytotoxicity, aerial parts.

INTRODUCTION

The genus *Indigofera* consists of about 700 species. All species are herbs or shrubs distributed throughout the tropical regions of the globe. In Pakistan, this genus is represented by 24 species. *Indigofera heterantha*, commonly known as ghorega belongs to family leguminosae (Fabeaceae) (Hamayun et al., 2003). This plant is abundantly available in the northern regions of Pakistan. The plant is widely used as traditional medicine for treatment against abdominal pain, spastic pain, skin problems and infectious dieses (Nasir et al., 1997). Some species of genus *Indigofera* such *Indigofera dalecoides* Benth is used for the treatment of diarrhea and pathogenic bacterial infections (Mathabe et al., 2006). *Indigofera oblogifolia* possess antimicrobial (Dahot, 1999), hepatoprotective (Shahjahan et al., 2005) and lipoxygenase inhibitory activity (Sharif et al., 2005). The species *Indigofera pulchra* has shown snake-venom neutralizing activity (Abubakar et al., 2006). *Indigofera tinctoria* showed antioxidant, free radical scavenging activity and anti-dyslipidemic activity (Prakash et al., 2007; Waako et al., 2007; Puri et al., 2007). *Indigofera mysorensis* has shown anti-diabetic activity. *Indigofera emarginella* have *in-vitro* antimalarial activity against *Plasmodium falciparum* (Chakrabarti et al., 2006). The leaves and flowers of *Indigofera aspalathoides* have cooling effect and used to treat leprosy and cancerous diseases. The leaves of this plant are also applied to abscesses and roots are used to treat dentifrice and mouth ulcers. The roots are chewed in for toothache and apathies. The oil extracted from whole plant, is used for

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Table 1. Brine shrimp cytotoxic activities of *n*-hexane fraction of aerial parts of *I. heterantha***.**

Dose (µg/ml)	No. of shrimps	No. of survivors	LD50 (µg/ml)	Std. drug	LD50 (µg/ml)
1000	30	16	1362.178	Etoposide	7.4625
100	30	24			
10	30	28			

dandruff (Nadkarni, 1996), syphilis and other skin infectious diseases. The plant is widely prescribed for psoriasis, eczema, burns, boils, ulcers, wounds and used also as antidote to snake venom (Kirtikar and Basu 1975) in Siddha system of medicine. Water soluble fraction of alcoholic extract of dried tender shoots of *I. aspalathoides* showed significant anti-inflammatory effect in experimental albino mice. Some new compounds which includes new ester, new indigoferamide-A and new isoflavone along with three new source compounds dotriacontanoic acid, quercetin and formononetin "4-hydroxy-4-methyl-2- pentanone" were isolated from the seeds of *I. heterantha* Wall exhibiting (Rahman et al., 2014, 2015). The aim of this study was to explore the cytotoxicity of the aerial parts of *I. heterantha* to determine the scientific basis for its use as folk medicine to treat various diseases. Brine shrimps (*A. salina* Leach) lethality bioassay was done so as to assess its safety.

MATERIALS AND METHODS

Plant

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, Department of Botany, Islamia College, Peshawar, KPK province, Pakistan. A voucher specimen (No.Sj-36) was deposited in the herbarium of Botany Department, Islamia College, Peshawar Pakistan.

Extraction and isolation

The powdered aerial parts (60 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 4.7 kg F1. It was then dissolved in chloroform resulting into 87 g soluble fraction F2 and remaining insoluble fraction F3 (370 g). The chloroform soluble fraction F2 was further fractionated with *n*-hexane and methanol afforded 9 g F2-X and 73 g F2-Y crude extracts, respectively using Soxhlet extractor for one day. While the insoluble fraction F3 was further dissolved in ethyl acetate and concentration afforded 270 g crude fraction F4. The ethyl acetate soluble fraction F4 was further partioned between diethyl ether and water gave 75 g ethereal crude fraction F4-Z, then water fraction (103 g) F4-W and insoluble residue (70 g) F4-I.

Cytotoxic assay

In this bioassay, a shallow rectangular plastic dish (22 × 32 cm) was filled with artificial sea water. The sea water was prepared by taking commercial salt mixture with double distilled water (3.8 g) in 1:1 ratio. The brine shrimp (*A. salina*) eggs were hatched in 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) an organic compound with the formula $(CH_3)_2NCH$ (2 ml) manufactured by china. 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 LD_{50} is the average of the three values) with one vial containing DMF was reserved as a control. The solvent was allowed to evaporate by keeping it 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 LD_{50} values (Finney, 1971; McLaughlin et al., 1991; Meyer et al., 1982; Uddin et al., 2011).

RESULTS AND DISCUSSION

Cytotoxicity activity

The result obtained indicated that *n*-hexane fraction showed high activities at 10 µg/ml, moderate activities at 100 µg/ml and low activities at 1000 µg/ml. Similarly, the ethyl acetate fraction showed activities in the same pattern as mentioned earlier that from high to low at 10, 100 and 1000 µg/ml, respectively. Methanol's fractions F1 and F2-X revealed high activities at 10 µg/ml, moderate activities at 100 µg/ml and low activities at 1000 µg/ml. The residue fractions showed low cytotoxic activities at 1000 µg/ml, moderate at 100 µg/ml and high at 10 µg/ml.

Tables 1 to 5 show that the active component display mostly high activity at low concentration and low activity at high concentration. The current investigation strongly supported the traditional use of *I. heterantha* for various pathological conditions. The current study proved that the extract fractions of *I. heterantha* have good cytotoxic activity. These activities may be accredited to the existence of alkaloids, polyphenols, phenols, tannins, saponins, anthraquinones, steroids and especially the diterpenes, found in the crude extract and the fractions thereof (Araruna and Carlos, 2010). The plant is commonly used for the treatment of various diseases.

Table 2. Brine shrimp cytotoxic activities of ethyl acetate fraction of aerial parts of *I. heterantha*.

Dose (µg/ml)	No. of shrimps	No. of survivors	LD50 (µg/ml)	Std. drug	LD50 (µg/ml)
1000	30	16	1500.66	Etoposide	7.4625
100	30	22			
10	30	26			

Table 3. Brine shrimp cytotoxic activities of water methanol fraction F1 of aerial parts of *I. heterantha*.

Dose (µg/ml)	No. of shrimps	No. of survivors	LD50 (µg/ml)	Std. drug	LD50 (µg/ml)
1000	30	16	2225.34	Etoposide	7.4625
100	30	22			
10	30	27			

Table 4. Brine shrimp cytotoxic activities of methanol fraction F2-X of aerial parts of *I. heterantha*.

Dose (µg/ml)	No. of shrimps	No. of survivors	LD50 (µg/ml)	Std. drug	LD50 (µg/ml)
1000	30	17	2225.63	Etoposide	7.4625
100	30	22			
10	30	26			

Table 5. Brine shrimp cytotoxic activities of residue fraction of aerial parts of *I. heterantha*.

Dose (µg/ml)	No. of shrimps	No. of survivors	LD50 (µg/ml)	Std. drug	LD50 (µg/ml)
1000	30	18	61	Etoposide	7.4625
100	30	23			
10	30	25			

This data strongly supports that widespread research should be steered to isolate and identify phytochemical constituents responsible for cytotoxic activities to intricate the hidden medicinal potential of the plant (Rahman et al., 2016).

Conclusion

In this study, an effort was made to scientifically authenticate cytotoxic bioassay of the crude extract fractions of aerial parts of *I. heterantha* to elaborate its medicinal importance. The results obtained discloses that it contains a variety of therapeutic agents, which need to be further explored phytochemically. The current study revealed that the aerial parts of the plant have strong cytotoxic activity. Further scientific investigation is needed to explore phytochemicals of this plant, which possess the activity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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