Pharmacognostic standardization and pharmacological study of *Sisymbrium irio* L.

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Abstract

Pharmacognostic study was carried out on *sisymbrium irio* (Family Brassicaceae) on the basis of quantitative microscopy, fluorescence characteristics, phytochemical screening tests and pharmacological study. Ethanolic extracts of *S. Irio* exhibited plant growth inhibition at 1000 µg/ml and 100 µg/ml concentrations. The insecticidal activity of crude ethanolic extract varied with the concentration and the test organisms. In cytotoxic bioassay the ethanolic extract exhibited high lethality, which was dose dependent.

Key words: *Sisymrium irio,* pharmacognosy, quantitative microscopy, insecticidal, cytotoxic, phytotoxic bioassays.

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Introduction

Sisymbrium irio is an annual herb of family Brassicaceae distributed in Pakistan. *S. irio* is used in treating coughs and chest congestion, rheumatism and to detoxify liver and spleen, reduce swelling and clean wounds (Lev, 2003). *S. irio* has many uses in folk medicine in treatment of inflammation and rheumatism (Bolus, 1983). *S. irio* can be used for dietary purposes (Guil *et al.,* 1998). Seeds are used as expectorant and as febrifuge (Ghazanfar, 1994). The seed of *S. irio* is used in treatment of voice disorders (Meyer *et al.*, 1982). *S. irio* has antipyretic, analgesic, anti-microbial and antioxidant potential (Vohora *et al*., 1980). The

phytochemical screening analysis revealed that the plant contained secondary metabolites like flavonoids, alkaloids, oils and glycosides (Arayno & Zafor 1983; Krets *et al.,* 1987). The following three bioassays: phytotoxic, cytotoxic and insecticidal activities were carried out which is inexpensive, rapid and easy to investigate their biological potential as well as to create pharmacological and pharmacognostic parameters for identification of this plant.

Materials and Methods

Plant Material

Healthy and fresh plants *of S. Irio* were collected at flowering stage from Peshawar university campus, Pakistan. The collected plant was washed with water and powdered by electric grinder.

Quantitative Microscopy

The following pharmacognostic parameters of the leaf of *S. Irio* worked out using light microscope, vein islet number, vein termination number, palisade cell ratio, stomatal number and Stomatal index, by following the standard procedure of (Trease & Evans 2002).

Fluorescence Characteristic

The fluorescence analysis of dried powder of *S. Irio* was carried out by treating 1 gm dried powder of each part with different chemicals (50% Hcl, 50% H_2SO, 50% HNO_3, picric acid and methanol) and each treated sample was observed under ordinary light and then under UV light (Evans, 2002).

Phytochemical Screening

The 70% etanolic extract was subjected to different qualitative chemical tests to find out the presence of different phytoconstituents i.e. alkaloids, glycosides, carbohydrate, phenolics and tannins, phytosterols, fixed oils, fats, proteins and amino acids, flavonoids, saponins, gums and mucilage by means of detection methods of (Trease & Evans 2002).

Preparation of Extracts

The whole plant was washed, cleaned under water to remove any adherent soil particles, shade dried and then grounded to 60 mesh diameter powder. Two hundred of powder was soaked in absolute ethanol for 72h and then filtered through Whatman filter paper No. 1823. The process was repeated three times. The filtrate evaporated through rotary evaporator to get the extract and preserved in refrigerator at 4^{o} C for pharmacological bioassays (Miliauskas *et al*., 2004).

Phytotoxicity

Phytotoxic activity of the extract was carried out against *Lemmna minor* following (Ahmad *et al.,* 2009). The medium was prepared in distilled water and autoclaved at 121 0C for about 20 min, and by adding KOH pellets the pH was adjusted to 5.4 – 5.5. Stock solution was prepared by dissolving 10 mg extract in 40ml ethanol. Than three different concentrations i.e. 10,100 and 1000 µ/ml were prepared from stock solution by taking 5, 50 and 500µl from stock solution respectively. The solvent was allowed to evaporate. In each petridish 20 ml of medium were separately added and 10 plants of *Lemmna minor* each with 2 or 3 fronds were added in each petridish. Methanol and paraquate were used as positive and negative controls respectively. For seven days the petri dishes were placed in growth chamber at 28 ^{0}C. On day seven the numbers of fronds in each petridish were counted.

Insecticidal Activity

Activity requirements

Test insects (*Tribolium castaneum, Sitophilus oryzea, Rhyzopertha dominica*, *Trogoderma granarium* and *Callosobruchus analis*), volatile organic solvent (ethanol), standard insecticide (Permethrin), Petri plates (9cm diameter), growth chamber, micropipette (1000µl), brush, glass vials, filter paper. The insecticidal activity of the crude extract was carried out by method of (Naqvi &Parveen, 1991).

Rearing Technique

The stored grain pests are reared in the laboratory under controlled conditions (temperature and humidity) in plastic bottles containing sterile breeding media. Insects of uniform age and size were used for the experiment.

Procedure

The filter paper was cut according to the size of Petri plate (9 cm or 90 mm) and was placed in each Petri. The sample was loaded over each filter paper through micropipette and to evaporate the solvent completely these plates were left for 24 hours. After 24h the solvent was evaporation completely then put 10 healthy and active insects of same size and age of each species in each plate (test and permethrin was used + *ve* and ethanol – *ve control,* respectively) by the help of a neat and clean brush. The plates were incubated for 24 hours at 27 ^{o}C. On the third day readings were noted and the percentage inhibition or percentage mortality with the help of the following formula was calculated.

$$\% \text{ Mortality} = 100 - \frac{\textit{Number of insects alive in test sample}}{\text{Number of insects alive in negative control}} \times 100$$

Cytotoxicity

The cytotoxic activity was tested following [5b] method.

Hatching Technique

Brine solution was taken in tray and 50 mg of shrimps eggs were sprinkled and incubated at 37 ^{0}C for 24 hours.

Sample Preparation

In 1ml of DMSO 10 mg of test sample was dissolved, that was used as stock solution and from this stock three concentrations 10, 100 and 1000 µg/ ml were prepared by taking 5, 50 and 500 µl of the stock solution respectively. After 2 days of hatching and maturation using a Pastuer pipette through which 10 larvae/vials were placed. The volume was made about 5ml with seawater. These were then incubated at 25 – 270C for 24 hours under illumination. In other vials ethanol and standard cytotoxic drug was taken which served as negative and positive controls

respectively. The data was analyzed to determine LD50 values with 95% confidence intervals by Finney computer program.

Results

Quantitative Microscopy

The epidermal study of *S. Irio* showed the presence of tetrahedral shape upper epidermis walls with anomocytic and mostly anisocytic stomatas. The results revealed 17- 26 vein islet number, 32-43 vein termination numbers, 163-216 stomatal numbers, 2.1-2.9 palisade ratio and 18.4-21.2 stomatal index/mm area.

Table 1. Quantitative Microscopy of *sisymbrium irio*

Parameter	**Range**
Vein islet number	17-26
Vein termination	32-43
Stomatal number,Lower epidermis	163-198-216
Palisade ratio	2.1-2.9
Stomatal index,Lower epidermis	18.4-19.1-21.2

Fluorescence Analysis

The powdered material of *S. Irio* produced orange and lim twist colour when treated with HNO_3 and Picric acid respectively, while black, spring green and leaf green colouration produced when treated with H_2SO_4, HCl and methanol respectively.

Table 2. Fluorescence characteristics of *sisymbrium irio*

Powder and Reagents	**Ordinary light**	**UV light**
Powder treated with 50% HNO3	Tile red	Orange
Powder treated with Picric acid	Yellowish green	Lim twist
Powder treated with 50%$H2SO_4$	Spice	Black
Powder treated with 50% Hcl	Lim twist	Spring green
Powder treated with Methanol	Spring leaf	Spring leaf

Note: Orignal colour of powder drugs was spring green.

Phytochemical Analysis

Ethanolic extract was subjected to qualitative chemical test and results be exposed in Table 3. The result shows that maximum constituents found ethonalic extract of *S. Irio* including protein, carbohydrate, flavonoids, tannins, fixed oil and alkaloids.

Table 3. Phytochemical study of *sisymbrium irio*

CONSTITUENTS	TEST NAME	ETHANOLIC EXTACT	WATER EXTRACT
Carbohydrates	Fehling test	+	+
	Molish test	+	+
	Benedict test	+	+
Protein	Ninhydrine	-	-
	Birurets test	-	-
	Wagner test	+	+
Alkaloids	Mayer test	+	+
	Hagens test	+	+
Phytosterol and Triterpenoids	Salkoskii,s	+	+
	Liebermann test	+	-
Phenol	Shinoda,s test	+	+
	Lead acetate test	+	+
Flavonoids	Alkali test	+	+
	Zn – Hcl acid	+	+
	Reduction test	+	+
Tannins	Alkali test	+	+
Saponine	Frothing test	-	-
Anthocyanins	Hcl test	+	+
Glycosides	Killaer killini	-	-
Fixed oil and fats	Spot test	+	+
Volatile oil	Spot test	-	-

Keys + = Present and - =Absent

Phytotoxic Bioassay

Phytotoxic activity of ethanolic extract of *S. Irio* was carried out at three different concentrations i.e.1000 µg/ml, 100µg/ml and 10µg/ml. At the concentrations 1000 µg/ml and 100µg/ml showed significant fronds inhibition 23% and 16 % respectively, while 10µg/ml concentration shows weakly active inhibition of 13% (table 04).

Table 4. *Lemna* Phytotoxicity of *S. irio*

Parts used	Concentration (µg/ml)	No. of Fronds		% inhibition of fronds
		Initial reading	After 7 days	
Whole plant	1000	30	23	23%
	100	30	25	16%
	10	30	26	13%
	control	30	30	0%

Insecticidal activity

The ethanolic extract of *S. Irio* exhibited high activity against *Callosobruchus analis* with 359.35 LD_{50} value moderate activities against *Trogoderma granarium* and *Rhyzopertha dominica* with LD_{50} values 561.70 and 20819.2 respectively. While *Rhyzopertha dominica* and *Tribolium castaneum* showed low sensitivity at all concentrations with LD_{50} values 1398.84 and 677.91 respectively (table 05).

Cytotoxic Bioassay

The ethanolic extract *S. Irio* was carried out at three different concentrations i.e.1000 µg/ml, 100µg/ml and 10 µg/ml against shrimps larvae. It was evident from the results that 1000 µg/ml, exhibited highly lethality with 83.3%, 100µg/ml, exhibited moderate lethality about 70% and 10 µg/ml showed low lethality which was 43.3%. All the tree concentrations showed112.39 LD_{50} value with 95% confidence interval (table 06).

Table 5. Insecticidal activity of *S. irio*

Conc. mg/ml	Insect pests	Total insects	Dead insect	% mortality	Mean % mortality	LC50	Intercept	χ^2 (p)
0	***Callosobruchus analis***	30	0	0	50	359.35	4.69	0
10		30	9	30.0				
100		30	15	50.0				
1000		30	21	70.0				
0	***Trogoderma granarium***	30	0	0	32.1	561.70	4.299	0
10		30	5	16.6				
100		30	11	36.6				
1000		30	13	43.3				
0	***Rhyzopertha dominica***	30	0	0	27.7	1398.84	4.183	0.2
10		30	4	13.3				
100		30	9	30.0				
1000		30	12	40.0				
0	***Sitophilus oryzea***	30	0	0	47.7	20819.2	4.74	0.12
10		30	11	36.6				
100		30	13	43.3				
1000		30	19	63.3				
0	***Tribolium castaneum***	30	0	0	29.9	677.91	4.22	0.122
10		30	3	10.0				
100		30	10	33.3				
1000		30	14	46.6				

Table 6. Cytotoxic activity of *S. irio*

Extract Conc. (µg/ml)	T. No. of Larvae	No. of dead	% mortality	LD_{50}	95% CL		χ^2 (p)
					LCL	UCL	
0	30	30	0	112.39	440.2	215.4	0.055
10	30	13	43.3				
100	30	21	70.0				
1000	30	25	83.3				

Discussion

Quantitative Microscopy

Anatomical features of leaf epidermis such as stomatal index, palisade ratio, stomatal number, vein islet number and vein termination number etc are useful tools for identification of plant material. In *S. Irio* anomocytic and anisocytic stomatas were present. Many other researchers like *Vitex negundo* (Ahirrao *et al.,* 2011), *Dillenia indica* (Kumar *et al*., 2011) and *Clitoria ternatea* (Taur & Patil, 2010) studied the leaf surface area of the respective plants. So our current parameters were strongly supported by these investigators.

Fluorescence Analysis

For resolution of doubtful specimen fluorescence analysis is a rapid method. When chemical and physical methods are inadequate, than on the basis of fluorescence characteristics the plant materials may be identified from their adulterants. The observation showed there was variation in coloration under ordinary light and UV light. The plant powdered material of *S. Irio* glow differently when treated with different chemicals, which create a pharmacognostic parameter for identification of this plant. Many other researchers like *Crocus sativus* and *Hygrophila auriculata* (Hussain *et al.,* 2011) carried out fluorescence analysis for different medicinal plants which were helpful in our findings.

Phytochemical Analysis

. Such preliminary phytochemical screening was helpful in prediction of nature of drugs and also useful for the detection of different constituents by solvent.

Many phytochemist carried out these tests like (Shivalingam *et al.,* 2009] analyse *Nothosaerva brachiata* and *Aerva lanata* and (Roe & Kuhr, 2011) carried out phytochemical screening of ethanolic extracts of *Teph.* So our results revealed that *S. Irio* contain all of the above secondary metabolites which make it suitable for pharmaceutical purposes.

Phytotoxic Bioassay

The ethanolic extract of *S. Irio* showed significant inhibition at 1000 µg/ml, moderate inhibition at100 µg/ml while at 10µg/ml weakly inhibition recorded. The results revealed that inhibition of fronds was dose dependent. Similar results were also obtained by (Hussain *et al*., 2010) reported phytotoxicity of different species of *Rumex*, (Khan *et al*., 2011) observed phytotoxicity of *Euphorbia prostrata* against wheat seeds, (Ayatollahi *et al.,* 2010) reported phytotoxicity of *Euphorbia Aellenii,* (Onocha *et al.,* 2011) tested the methanolic extract of *Acalypha torta* for phytotoxic potential. So our results are strongly supported by their research worked.

Cytotoxic Bioassay

The bioactive compounds present in plants extracts are toxic against shrimps larvae (Kivack et al., 2001; Hussain *et al.,* 2010) reported the cytotoxicity of *Rumex* species, (Koba et al., 2099) investigated the cytotoxic potential of *Cymbopogon citratus* L. *and Cymbopogon nardus* L leaves, (Ali *et al.,* 2009) studied the cytotoxic potential of *Euphorbia wallichii.* Our experimental data are similar to the worker of these workers.

Insecticidal Bioassay

Cuñat *et al.,* 1990 described juvenilising effects of *Juniperus thurifera* after topical extract application to pupae of *T. castaneum* at 10 *µ*g/insect and also 50% of mortality in *Oncopelthus fasciatus* when a fraction of *Genista tinctorea* was applied at 10 *µ*g/cm^2, (Hussain *et al.,* 2010) reported insecticidal activity of different species of *Rumex,* (Vinayaka *et al.,* 2009) investigated methanolic extracts of *Abrus pulchellus* leaves for insecticidal potential, (Islam *et*

al., 2011) studied *Suregada* against *Tribolium castaneum* insect. The results are closely similar to the findings of these researchers.

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