

# Cytotoxic and Phytotoxic Activities of *Reinwardtia trigyna* (ROXB.)

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**Abstract:** The present study was carried out to check the cytotoxic and phytotoxic activities of the ethanolic extract of leaf of *Reinwardtia trigyna*. The cytotoxic potential checked through *Artemia salina* mortality test which showed low cytotoxic effect (30% and 43.33%) at the doses of 10µg/ml, and 100µg/ml, while moderate cytotoxic effect (53.33%) was observed at 1000µg/ml with highest LD<sub>50</sub>, value 4496.29µg/ml as compared to the control having normal saline that shows no mortality. The same extracts showed significant dose dependent phytotoxicity 32.25%, 61.29% and 70.96% inhibition of *Lemna minor* at the doses of 10, 100 and 1000µg/ml as compared to the positive control "Atrazine sulphate" that shows 100% frond inhibition. The FI<sub>50</sub> value of plant extract was very low as 58.448 µg/ml. it is concluded from the present work that *Reinwardtia trigyna* could be used as a natural herbicide and weedicide as it shows a good phytotoxic activity.

**Keywords:** *Reinwardtia trigyna*, Ethanolic Extract, Leaf, Cytotoxic Activity, *Artemia salina*, Phytotoxic Activity, *Lemna minor*

## 1. Introduction

*Reinwardtia trigyna* (Roxb.) Planch, commonly known as yellow flax belonging to family Linaceae is a sub shrub upto 1m tall with lanceolate leaves, yellow flower and Globose capsule fruit [1]. It is also known as spring indicator because it blossoms early in the spring [2]. The plant is cosmopolitan in distribution while in Pakistan it is found in Khyber Pakhtunkhwa, Chitral, Swat, Hazara, Rawalpindi, Murree, AJK [2, 3]. The plant is well known for its ethno botanical uses and it is locally used for the treatment of different ailments i.e. paralysis backache, boils, headache, paralysis and pimples [3]. The root paste is applied to headache and its juice is given to treat scabies, fever, indigestion and wounds. Petals of the flowers are chewed to wash tongue [3, 5].

Bioassays are used to screen the plant extracts and to test their biological activities [5]. In plant extracts many therapeutically active compounds are still unidentified but their existence is detected by various bioassays [6]. Weeds are the main problem in agriculture because these reduces the

crop yield by competing with it for the available resources i.e. minerals, water and space. To control the reduction in crop yield, farmers use synthetic herbicides, which are dangerous for human beings and also causes soil and water pollutions and also increases the resistance of weeds [7]. The lethality bioassay of brine shrimps is considered to be the easiest and valuable process for initial evaluation of toxicity. This assay was first developed by Michael *et al.* [8]. Plants contain bioactive compounds, which are toxic to the larvae of brine shrimps (*Artemia salina*) and this test is used to test the cytotoxic effect of the natural products found in plants. This is a simple, inexpensive, rapid and general bioassay used to screen the physiologically active compounds [8]. Using brine shrimps, the cytotoxic potential of the *R. trigyna* will have been investigated.

## 2. Materials and Methods

### 2.1. Cytotoxic Activity

The ethanolic extract of the *R. trigyna* was tested for the

cytotoxic activity based on brine shrimps lethality by following the procedure of Meyer *et al* [9].

### 2.1.1. Hatching

The hatching tray was filled with sea salt solution and an unequal partition was made in it, with the help of a perforated partition wall. In the smaller part 50mg of brine shrimp eggs were sprinkled and was covered with black paper. The electric lamp was fitted above the uncovered part of the hatching tray to attract the brine shrimp larvae. The shrimp eggs hatched after 24 hours and the larvae moved to the larger illuminated part of the tray, through the perforated partition.

### 2.1.2. Procedure

For cytotoxic activity, 20mg of the extract was dissolved in 2ml of ethanol and from it 5, 50 and 500  $\mu$ l was added to the vials, which were equal to 10, 100 and 1000 $\mu$ g/ml respectively. Five vials were used for each concentration. The solution was allowed to evaporate overnight and then 5ml of sea salt solution was transferred to each test tube. For positive and negative controls, the salt solution and a reference drug were used respectively. Ten nauplii were transferred to each test tube with the help of Pasteur pipette and were kept at room temperature for 24 hours. After 24 hours the number of dead and live larvae was counted and the percent mortality of larvae was determined using the following formula

$$\% \text{ Mortality} = 100 \frac{(\text{number of shrimps alive test samples})}{(\text{number of shrimps in negative control})} \times 100$$

After Percent mortality result was subjected to statistical analysis and LD<sub>50</sub> value was calculated by through statistical software Biostat Version 05, AnalystSoft, USA), using Survival, Probit-analysis (Finney and MLS algorithms) with cumulation coefficient estimation. Experiment was performed in triplicates where n=5.

## 2.2. Phytotoxic Activity

The phytotoxic activity of the *Reinwardtia trigyna* was evaluated by using *Lemna minor* as a test sample by following the procedure of Anwar *et al* [10].

### 2.2.1. Media Preparation

The E-medium was prepared by mixing different minerals nutrients in different amounts, for *Lemna minor* phytotoxicity bioassay. E-medium was made by mixing the chemical reagents given in table. 1

**Table 1.** List of chemical reagents for E-Medium.

S. No	Name Of Chemicals	g/l
1.	Boric acid (H <sub>3</sub> BO <sub>3</sub> )	0.00286
2.	Copper sulfate (CuSO <sub>4</sub> .5 H <sub>2</sub> O)	0.00022
3.	Calcium nitrate (Ca(NO <sub>3</sub> ). 4H <sub>2</sub> O)	1.180
4.	Ethylene diamino tetra acetic acid (EDTA)	0.01120
5.	Ferric chloride (FeCl <sub>3</sub> .4H <sub>2</sub> O)	0.00540
6.	Manganese Chloride (MnCl <sub>2</sub> .4H <sub>2</sub> O)	0.00362
7.	Magnesium Sulfate (MgSO <sub>4</sub> .7H <sub>2</sub> O)	0.492
8.	Potassium Nitrate (KNO <sub>3</sub> )	1.515

S. No	Name Of Chemicals	g/l
9.	Potassium dihydrogen Phosphate (KH <sub>2</sub> PO <sub>4</sub> )	0.68
10.	Sodium molybdate (Na <sub>2</sub> MoO <sub>4</sub> .2H <sub>2</sub> O)	0.00012
11.	Zinc Sulfate (ZnSO <sub>4</sub> .5H <sub>2</sub> O)	0.00022

### 2.2.2. Procedure

Three different concentrations i.e. 10, 100 and 1000  $\mu$ g/ ml of the extract were prepared and then 20ml of E-medium was put into the petridishes. The petridishes were grouped into five. In the first group the atrazine was added as a standard phytotoxic drug to act as positive control. In the second group only E-medium was used for the growth of *Lemna minor* as a negative control, while the other three groups were used as tests for the three concentrations of extract. Ten *Lemna minor* plants with 2-3 fronds were kept in each petridish. Five replicas were used for each group. All the petridishes were kept under about 12 hour's day light. The growth of plants was examined daily and on the seventh day, final reading was taken by counting the number of fronds in each petridish. The percent growth inhibition was calculated with reference to the negative control by using the formula:

$$\% \text{inhibition} = 100 \frac{(\text{number of fronds in test samples})}{(\text{number of fronds in negative control})} \times 100$$

FI<sub>50</sub> value was calculated by through statistical software Biostat Version 05, AnalystSoft, USA). Using Survival, Probit-analysis (Finney and MLS algorithms) with cumulation coefficient estimation. Experiment was performed in triplicates where n=5.

## 3. Results and Discussion

### 3.1. Phytotoxic Activity

In present study the phytotoxic activity of the ethanolic extract of *R.trigyna* leaf was analyzed. The concentrations used were 10, 100 and 1000 $\mu$ g/ml. Standard phytotoxic drug "atrazine" was used as positive control, while blank E-media was used as negative control respectively. Five replicates were used for each concentration. The data was recorded as Mean  $\pm$  SEM and the percent frond inhibition and LD<sub>50</sub> values was also calculated. The extracts showed dose dependent phytotoxicity e.g. 10 $\mu$ g/ml showed 32.25% inhibition, 100 $\mu$ g/ml showed 61.29% inhibition and 1000 $\mu$ g/ml showed 70.96% frond growth inhibition. The percent frond inhibition value calculated for 10, 100 and 1000 were 32.25, 61.29 and 70.96 respectively, as compared to the positive control having only E-Medium that shows increase in frond number 31 and positive control "Atrazine sulphate" that shows 100% frond inhibition. The FI<sub>50</sub> value of plant extract was 58.448, given in the Table. 2.

Many researchers had carried out the phytotoxic activity of plants e.g. Ali *et al.*, [18] carried out phytotoxic activity of the chloroform, ethyl acetate and n-butanolic root extract of *Euphorbia wallichii* and reported good phytotoxic (60-100%) effect at dose of 1000  $\mu$ g/ml. Atta-Ur-Rehman *et al* [11], Malla *et al* [12] also reported similar phytotoxic potential of the various plants like *Chrozophora tinctoria*, *Fagonia*

*cretica Ricinus communis, Tribulus terrestris* and *Peganum harmala* showed phytotoxic activity at all the dilutions.

Various other workers also reported significant phytotoxic effects of various plants extracts [12, 13, 14, 15, 16, 17] on various medicinal plants like *Alpinia galangal*, *Curcuma longa*, *Zizyphus jujube*, *Grewia optiva*, *Euphorbia granulata* against *Lemna minor* and confirmed these plants as a phytotoxic agent. The weeds compete with crop plants that reduce the productivity, the plants obtained herbicide can be used as environmental friendly anti-herbicidal drugs [19, 20]. The secondary metabolites present in plants can act as allelochemicals for other plants and these metabolites are released by plants through exudations or by the decomposition of the plant material in soil and it may affect the growth of neighboring plants [21, 22]. Our results also suggest that this plant can be used as phytotoxic agent at doses of 100 and 1000µg/ml ratio. The plants originated compounds should be used as herbicide in order to reduce the hazards of synthetic herbicides.

### 3.2. Cytotoxic Activity

In the present study the brine shrimps lethality bioassay was carried out to evaluate the cytotoxic potential of the ethanolic extract of the *R. trigyna*. Three doses (10µg/ml, 100µg/ml and 1000µg/ml) of the extract were used. All the doses were applied in five replicates and each replicates have 30 *Artemia salina*. The data was recorded as Mean  $\pm$  SEM and the percent mortality and LD<sub>50</sub> values was also calculated. The results showed that the plant has cytotoxic activity, based on criteria of Ali *et al* [19].

30-40% lethality – Low activity

50% lethality – moderate activity

60-70% lethality –good activity

Above 70% lethality – significant activity

From the above criteria, it is can be concluded that the 10µg/ml and 100µg/ml doses showed low cytotoxic effect (30% and 43.33% respectively), while the 1000µg/ml dose showed moderate cytotoxic effect with 53.33% value as compared to the control having normal saline that shows no mortality, while the LD<sub>50</sub> value was 4496.29µg/ml (Table 3). Many other researchers also worked out the cytotoxic activity of various plants such as, Patil, and Magdum [2] Parveen and Qaisar, [22] evaluated the cytotoxic activity of the 14 angiosperm (wild plants) plants and the results showed that among these plants, only 2 plants showed

cytotoxic activity while remaining had no or very low cytotoxic effect. Rawat *et al* [23] and Richardson [24] also reported non significant activity for methanol extract of the leaves and roots of the *Cajanus cajan* (L.) Millsp. Sarkar *et al* [25], Uddin *et al* [26] screened 118 plants (aqueous extract) for their cytotoxic potential through brine shrimps lethality bioassay and they concluded that 11 out of 118 plants showed significant cytotoxic activity (<60 µg/ml) while remaining was completely non-significant were inactive in cytotoxicity test. Similarly, various other investigators [2, 7, 24, 27, 28, 29] reported no cytotoxic potential while studying *Azadirachta indica*, *Melia azedarach*, *Sandoricum indicum* and *Swietenia macrophylla*. These workers concluded that these plant does not have anticancerous substances, due to of anticancerous substances, these plants have not showed cytotoxic activity. In light of these reports, it can be concluded that *R. trigyna* is not a promising cytotoxic plant. Hence it can be suggested that the plant does not contain the antitumor and anticancer phytochemical which are responsible for cell-line toxicity.

## 4. Conclusions and Recommendations

Pakistan is an agricultural country producing good superior quality and amount of various vegetables, crops, fruits and Cereals But due to very low weeds and pests control policies, huge amount of these useful products may be spoiled and not available to people. Artificial weedicides and herbicides may be used for prevention and protection of these economic sources, which are haphazard both for the environment, causing air, water and soil pollution and also responsible for accumulation of carcinogenic heavy metal in vegetable crops and cereals. This may cause very serious diseases in human. Hence, there is a need to produce environment friendly and nontoxic herbicides and weedicides from natural sources like plants. In light of the present research work it is concluded that the ethanolic extract of leaf of *Reinwardtia trigyna* showed a very low non-significant cytotoxic potential against *Artemia salina* and showed a good significant effect of *Limna minor* fronds inhibition in the phytotoxic bioassay. Hence, it is recommended that further advance work should be done on the present research plant and the specific phytotoxic and herbicidal phytoconstituents should be identified, isolated and use as environment friendly herbicides instead of haphazard synthetic herbicides.

Table 2. Phytotoxic activity of *Reinwardtia trigyna* leaf.

S. No	Part used	Dose (µg/ml)	Number of fronds in test	Number of fronds in (-) control	% Fronds inhibition	FI <sub>50</sub>
1.	Leaf	10 (µg/ml)	21 $\pm$ 2.03	31	32.25	58.44
2.		100 (µg/ml)	12 $\pm$ 1.9		61.29	
3.		1000(µg/ml)	9 $\pm$ 3.06		70.96	

FI<sub>50</sub> = The concentration that inhibit 50% of frond proliferation. Data was represented as (Mean  $\pm$  SEM;) All the experiments were performed in triplicate where n= 5. And each replicate has 30 fronds.

Table 3. Cytotoxic activity of leaves of *Reinwardtia trigyna*.

S. No	Part used	Dose (µg/ml)	Number of larvae in –ve control	Number of larvae survive	% Mortality	LD <sub>50</sub>
1.	Leaf	10 (µg/ml)	30	21±1.69	30%	496.29
2.		100 (µg/ml)		17±2.32	43.33%	
3.		1000(µg/ml)		14±1.01	53.33%	

LD<sub>50</sub>= Lethal dose that causes 50% inhibition of Brine shrimp larvae. Data was represented as (Mean ± SEM) All the experiments were performed in triplicate where n= 5. And each replicate have 30 larvae.

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