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CYTOTOXICITY AND PHYTOTOXICITY OF SOME SELECTED MEDICINAL PLANTS OF FAMILY SOLANACEAE

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Abstract

The methanolic extracts of the *Datura innoxia* Miller, *Solanum nigrum* Lin, *Solanum surattense* Burm. f, *Withania somnifera* L and *Withania coagulans* (Stocks) Dunal are more cytotoxic as compared to the acetone extracts of these plants except *W. coagulans.* Acetone extracts of *S. surattense* had very low activity as compared to other tested plants. Phytotoxic activity of the both extract had greater activity at 10 µg/ml as compared to at 100µg/ml except *W. somnifera* (methanolic) and *W. coagulans* (acetone) as well as 1000µg/ml activity which was low at the both solvent of the studied plants.

Introduction

Bioactive compounds are often toxic to the eggs of the brine shrimp *Artemia salina* shrimp larvae (Kivack *et al.,* 2001; Carballo *et al.,* 2002). When placed in artificial seawater, the eggs hatch within 48 h, providing large numbers of larvae. This is a rapid, inexpensive, general bioassay, which has been developed for screening, fractionation and monitoring of physiologically active natural products (Carballo *et al*., 2002). Members of the family Lemnaceae are suitable organisms for assays. Phytotoxicity, due to the miniature size under normal conditions, reproduces exponentially with budding of daughter fronds. It is observed that natural antitumor compounds inhibit *Lemna* growth while some substances might stimulate frond proliferation; the assay is also useful in detecting new plant growth stimulants. The commercial need for such natural, biodegradable, herbicides and plant growth stimulants may someday be filled with natural products detected by the simple and convenient *Leman* bioassay (Atta-ur-Rehman, 1991). Khan *et al*., (2009) reported that leaves of *Datura innoxia* are applied to swollen limbs. Evans (2002) reported *Solanum nigrum* as narcotic, antispasmodic, diuretic, and laxative. Root of *Solanum surattense* is expectorant, and is used in curing cough, asthma and chest pain. The seeds are used for blood purification and for increasing blood level (Manan *et al*., 2007). The tubers of *Withania somnifera* are tonic, useful in inflammations, bronchitis, asthma, consumption and ulcers, aphrodisiac, leucoderma, lumbago arthritis, favors conception (Ahmad, 2007). Wazir *et al*., (2007) reported that *Withania coagulans* is used for curing stomach ulcer and as poultice. Vijayan *et al.,* (2004) reported the total alkaloid fractions of the methanolic extracts of the leaves, ripe fruits, roots, seeds and stem of *Solanum pseudocapsicum* were subjected to *In vitro* cytotoxicity. All the 5 fractions exhibited potent activity. The total alkaloid fraction of leaves was found to be the most potent. Hussain *et al*., (2010) worked on the cytotoxicity and phytotoxicity of the crude extracts of *Rumex hastatus*, *Rumes dentatus*, *Rumex nepalensis*, *Rheum australe*, *Polygonum persicaria* and *Polygonum plebejum* were determined against *Artemia salina* and *Lemna minor*, respectively. It was observed that these plants, except *R. australe,* possessed cytotoxic activity against it and also these plants showed phytotoxic activity against *Lemna minor*. Lim *et al.,* (2004) and Heo *et al.,* (2004) reported that a glycoprotein isolated from *Solanum nigrum* has a cytotoxic effect against MCF-7 and HT-29 cells, even at low concentrations. Knox *et al*., (2010) reported the phytotoxic activity of *Cassia occidentalis, R. dentatus, Calotropis procera* and *W. somnifera* against *Parthenium hysterophorus*. Ihsan-ul-Haq *et al.,* (2012) reported the brine shrimp cytotoxic activity of *Artemisia dubia* extract. Majeed *et al.*, (2012) studied the phytotoxic activity of *Chenopodium album* against wheat. Jabeen *et al*., (2013) reported the phytotoxic effect of *Asphodelus tenuifolius, Euphorbia hirta* and *Fumaria indica* against wheat. Assay of cytotoxicity and phytotoxicity is the process of screening plants for their bioactivity for knowing new substances. The proceeded study is a step forward in this direction.

Materials and Methods

Preparation of extract: Whole plants of *Datura innoxia* Miller, *Solanum nigrum* Lin, *Solanum surattense* Burm. f, *Withania somnifera* L and *Withania coagulans* (Stocks) Dunal were ground to 60 mesh powder using an electric grinder. 50 g of each sample was separately soaked in 250ml methanol and acetone for 72 h. Thereafter each plant extract was passed through Whatman filter paper No. 1823. This process was repeated for 3 times. Evaporating in a rotatory evaporator at 40^{o}C was carried out to concentrate the extracts. These extracts were stored at 4^{o}C priors to use. The methanolic and acetone extracts and the standard drug were dissolved in dimethylsulphoxide (DMSO) at the concentration of 10 and 1 mg/ml for cytotoxic and 30 and 1 mg/ml for phytotoxic activities, respectively.

Cytotoxic activity: The materials and reagents used for cytotoxicity includes test sample *Artemia salina* (shrimps eggs), sea salt (38 g/L of D/W, pH 7.4), hatching tray with perforated partition, lamp to attract brine-shrimp larvae, micro pipette (5, 50,500µl),vials tray, 30 vials, organic solvents methanol and acetone. The cytotoxic activity of the crude extracts of the plants was carried out by following the method of Meyer *et al.,* (1982).

Hatching techniques: The hatching tray (a rectangular dish 22x32 cm) was half filled with filtered brine solution and 50 mg (eggs of brine shrimp were sprinkled in it). It was incubated at 37°C and after 24 h brine shrimp hatched. The plant extracts were applied to see the cytotoxicity of these extracts.

Sample preparation: Test sample was dissolved (10 mg) in 1 ml of DMSO and from this solution 5, 50 and 500µl was transferred to vials (3vials/concentration). The concentration was 10, 100 and 1000 µg/ml respectively. After 2 days of hatching and maturation, 10 larvae/vials were placed, using a Pasteur pipette. The volume was made 5ml with seawater. It was incubated at 25-27°C for 24 h under illumination. Other vials were supplemented with DMSO and etoposoid was used as reference cytotoxic drug which served as negative and positive controls, respectively. The data were analyzed with Probit Analysis program to determine LD_{50} values (Finney, 1971).

Phytotoxicity: Phytotoxic activity of the extracts was carried out against the *Lemna minor* following McLaughlin *et al.,* (1991). The medium was prepared by mixing various constituents in distilled water (1000 ml) and the pH was adjusted (5.5-5.6) by adding KOH pellets. The medium was then autoclaved at 121°C for 15 min. The extracts (30.0 mg) dissolved in methanol (1.0 ml) served as stock solution. 18 petri plates, three for each concentration, were inoculated with 1000, 100 and 10 µl of the stock solution to give the final concentration of 1000, 100 and 10 ppm, respectively. The solvent was allowed to evaporate overnight under sterile conditions. To each plate, medium 20ml and 10 plants, each containing a rosette of 3 fronds, of *Lemna minor*, were added. Other plates supplement with solvent and reference growth inhibitor (Paraquate), served as a negative control. All plates were kept in the growth cabinet for seven days. The number of fronds per plates were counted and recorded on day 7.

Results and Discussion

Cytotoxic activity: Cytotoxic drugs are known to be highly toxic to cells, mainly through their action on cell reproduction. Many have proved to be carcinogenic, mutagenic or teratogenic. These drugs are increasingly being used in a variety of diseases e.g., for the treatment of cancer, rheumatoid arthritis, multiple sclerosis and auto-immune disorders. Generally, cytotoxic materials are identified by a purple symbol that depicts a cell in late telophase (Edgar-Hughes *et al*., 2012). The methanolic extract of the *Datura innoxia* showed 2.176 intercept value, 0.126 slope, 0.909 R square and LD50 was 94.86. Similarly, the methanolic extract of *S. nigrum* showed intercept value 2.151, slope 0.139, R square 0.823 and LD50 93.73. The methanolic extract of the *Solanum surattense* showed that intercept value 2.109, slope 0.159, R square 0.812 and LD50 95.18. While the methanolic extract of the *Withania somnifera* showed that intercept value 2.190, slope 0.119, R square 0.838 and LD50 93.66. And the methanolic extract of the *Withania coagulans* showed that intercept value 2.176, slope 0.126, R square 0.909 and LD50 94.86. The acetonic extract of the *Datura innoxia* showed that intercept value 2.142, slope 0.145, R square 0.852, and LD50 was 87.75. Similarly, the acetonic extract of the *Solanum nigrum* exhibited that intercept value of 2.155, slope 0.139, R square 0.786 and LD50 86.78. The acetonic extract of the *S. surattense* showed that intercept value 2.177, slope 0.132, R square 0.862 and LD50 74.31. While the acetonic extract of the *Withania somnifera* showed that intercept value 2.181, slope 0.126, R square 0.871 and LD50 86.56. And the acetonic extract of the *Withania coagulans* showed that intercept value 2.190, slope 0.119, R square 0.838 and LD50 93.66 (Table 1). Son *et al*., (2003) reported that the ethanolic extract of *Solanum nigrum* has cytotoxic effects on MCF-7 cells. Vijayan *et al*., (2004) reported the total alkaloid fractions of the methanolic extracts of the leaves, ripe fruits, roots, seeds and stem of *Solanum pseudocapsicum* were subjected to *In vitro* cytotoxicity. All the five fractions exhibited potent activity. The total alkaloid fraction of leaves was found to be the most potent. Lim *et al*., (2004) and Heo *et al.,* (2004) reported that a glycoprotein isolated from *Solanum nigrum* has a cytotoxic effect against MCF-7 and HT-29 cells, even at low concentrations. Hussain *et al*., (2010) reported the positive cytotoxicity of the crude extracts of *Rumex hastatus*, *R. dentatus*, *Rumex nepalensis*, *Rheum australe*, *Polygonum persicaria* and *Polygonum plebejum* against *Artemia salina.* The solanaceous plants contained cytotoxic compounds which had been reported by so many workers in different plants e.g. saponin from *Solanum surattense* (Lu *et al.,* 2011), glycoprotein isolated from *S. nigrum* might have shown anti-cancer abilities by blocking the anti-apoptotic pathway of NF-kappaB, activating caspase cascades reaction and increasing the production of nitric oxide (An *et al*., 2006), solanolide, solanolide, yamogenin and neochlorogenin from *Solanum torvum* had found *In vitro* cytotoxic against a panel of human cancer cell lines (Lu *et al*., 2009), Pan *et al.,* (2007) isolated ten withanolides from *D. metel* flowers and demonstrated that 4 were cytotoxic at 10 µM or lower against three human cancer cell lines. Withanolide D and 17α-hydroxywithanolide D were isolated from the stems, roots, and leaves of *Tubocapsicum anomalum* exhibited cytotoxicity against Hep G2, Hep 3B, A-549, MDA-MB-231, MCF-7, and MRC-5 cell lines (Hsieh *et al.,* 2007) which agree with the present study.

Phytotoxic activity: In all countries including Pakistan, there is great reduction in crop yield due to weeds. The extent of losses caused by weeds was found to be more as compared to the insects and other diseases but their effects are usually ignored. Weeds reduce productivity, because of competion for availiable natural resources such as sunlight, water and minerals etc. Also weeds might provide habitat for insects which demage the crops by eating them or spreading diseases. Weeds control through synthetic drugs has caused various human health problems and soil water pollution (Barkatullah *et al*., 2011). So weeds control through harmless means is indespensible, to increase yeild of various crops and to protect environments. Phytotoxicity of the methanolic extract of *Datura innoxia* (Fig. 1) and *Withania coagulans* (Fig. 5) showed significant activity at 10 µg/ml, good activity at 100µg/ml and moderate activity at 1000 µg/ml. *S. nigrum* (Fig. 2), *Solanum surattense* (Fig. 3) and *Withania somnifera* (Fig. 4)

extract showed significant activity at 10 and 100 µg/ml and moderate activity 1000 µg/ml. At the concentration of 10µg/ml % inhibition was high in *Datura innoxia* and was low in *Withania somnifera.* At the concentration of 100µg/ml % inhibition was high in *Withania somnifera* and was low in *Datura innoxia* and *Withania coagulans.* At the concentration of 1000 µg/ml % inhibition was high in *Solanum surattense* and was low in *Datura innoxia* and *Solanum nigrum.* The acetonic extracts of all the 5 plants showed significant activity at 10 and 100µg/ml while the extracts of all these plants showed moderate activity at 1000 µg/ml. However, at 10µg/ml the percent inhibition was high in *Solanum nigrum* (Fig. 2) and low in *Withania somnifera* (Fig. 4) and at 100 µg/ml concentration percent inhibition was high in *Withania coagulans* (Fig. 5) and was low in *Datura innoxia* (Fig. 1). At the concentration of 1000 µg/ml percent inhibition was high in *Solanum surattense* (Fig. 3) and was low in *Datura innoxia* and *Solanum nigrum* (Table 2). Knox *et al*., (2010) reported the phytotoxic activity of *Cassia occidentalis, Rumex dentatus, Calotropis procera and Withania somnifera* against *Parthenium hysterophorus.* Javaid *et al*., (2009) repoted that phytotoxic activity of aqueous extracts of two *Withania somnifera* and *Datura alba* was evaluated against *Rumex dentatus*. Aqueous extracts of root and shoot of both test species resulted in pronounced suppression in germination as well as seedling growth of target weed species. Application of aqueous extracts caused 68% reduction in germination, 62% in shoot length, 96% in root length and 68% in seedling biomass. Hussain *et al*., (2010) reported the phytotoxicity of the *R. hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Rheum australe*, *Polygonum persicaria* and *Polygonum plebejum* of the family Polygonaceae against *Lemna minor*. Moderate activity was shown by *Rumex nepalensis*, *Rheum austral* and *Polygonum persicaria* at the concentration of 100 µg/ml. All the plants showed low activity at the concentration of 10µg/ml. And all the plants except *R. hastatus* showed high activity at the concentration of 1000 µg/ml which agree with the present study.

Table 1. Cytotoxicity of some selected medicinal plants of family Solanaceae.

Plant name	Dose	Log 10 (Dose)	% to Probit	Intercept	Slope	R square	LD_{50}
Datura innoxia Miller.	10	1	2.279				
	100	2	2.473	2.176	0.126	0.909	94.86
	1000	3	2.530				
Solanum nigrum Linn.	10	1	2.252	2.151	0.139	0.823	93.73
	100	2	2.502				
	1000	3	2.530				
Solanum surattense Burm.f.	10	1	2.224	2.109	0.159	0.812	95.18
	100	2	2.516				
	1000	3	2.543				
Withania somnifera Linn.	10	1	2.279	2.190	0.119	0.838	93.66
	100	2	2.488				
	1000	3	2.516				
Withania coagulans (Stock) Dunal.	10	1	2.279	2.176	0.126	0.909	94.86
	100	2	2.473				
	1000	3	2.530				
Datura innoxia Miller.	10	1	2.252	2.142	0.145	0.852	87.75
	100	2	2.502				
	1000	3	2.543				
Solanum nigrum Linn.	10	1	2.252	2.155	0.139	0.786	86.78
	100	2	2.516				
	1000	3	2.530				
Solanum surattense Burm.f.	10	1	2.279	2.177	0.132	0.862	74.31
	100	2	2.502				
	1000	3	2.543				
Withania somnifera Linn.	10	1	2.279	2.181	0.126	0.871	86.56
	100	2	2.488				
	1000	3	2.530				
Withania coagulans (Stock) Dunal.	10	1	2.279	2.190	0.119	0.838	93.66
	100	2	2.488				
	1000	3	2.516				

Standard was etoposoid. The data was analyzed through Probit Analysis for the calculation of LD_{50}.

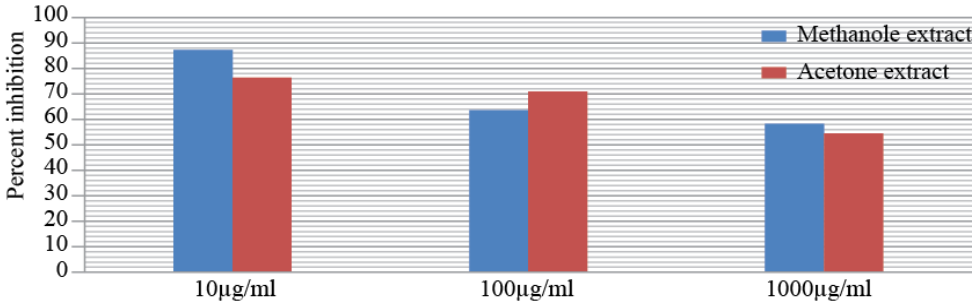

Fig. 1. Phytotoxic activity of *Datura innoxia* Miller.

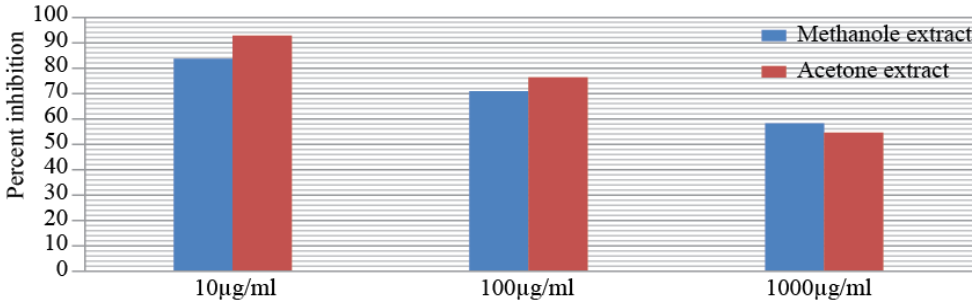

Fig. 2. Phytotoxic activity of *Solanum nigrum* Linn.

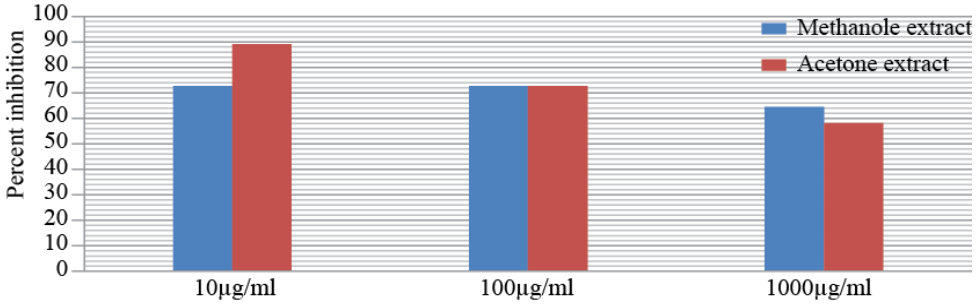

Fig. 3. Phytotoxic activity of *Solanum surattense* (Burm.) f.

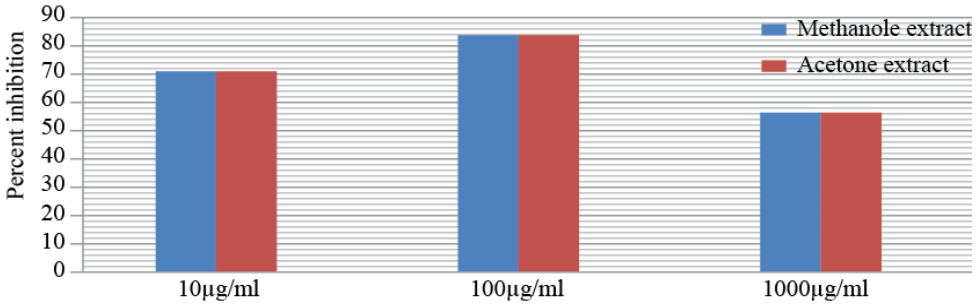

Fig. 4. Phytotoxic activity of *Withania somnifera* Linn.

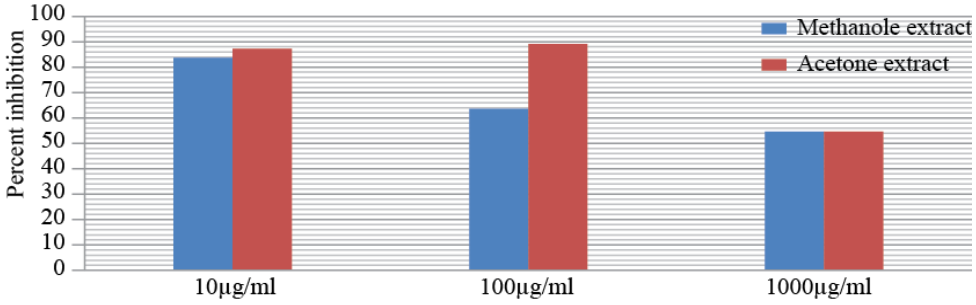

Fig. 5. Phytotoxic activity of *Withania coagulans* (Stock) Dunal.

Table 2. Phytotoxicity of 5 selected medicinal plants of family Solanaceae against *Lemna minor* on % fronds inhibition.

Plant name	% Inhibition					
-ve Control	**55**					
+ve Control	**30**					
	Methanolic extract			**Acetone extract**		
	10 µg/ml	**100 µg/ml**	**1000 µg/ml**	**10 µg/ml**	**100 µg/ml**	**1000 µg/ml**
Datura innoxia Miller.	87.27	63.63	58.18	76.36	70.90	54.54
Solanum nigrum Linn.	83.63	70.90	58.18	92.72	76.36	54.54
Solanum surattense Burm.f.	72.72	72.72	65.45	89.09	72.72	58.18
Withania somnifera Linn.	70.90	83.63	56.36	70.90	83.63	56.36
Withania coagulans (Stock) Dunal.	83.63	63.63	54.54	87.27	89.09	54.54

Criteria:	
0-39%inhibition	Low activity
40-59% inhibition	Moderate activity
60-69% inhibition	Good activity
Above 70%	Significant activity

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