Original Research

IN-VITRO BIOLOGICAL EVALUATION OF CRUDE EXTRACT OF AERIAL PARTS OF *APHELANDRA SQUARROSA*

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ABSTRACT

Purpose: In present studysamples of dichloromethane and methanol extracts of Aphelandra squarrosa of family Acanthaceae were screened forcytotoxic and phytotoxic activity.
Methods: Cytotoxicity was evaluated by using 'Brine-shrimp Lethality Assay' and Phytotoxicity was determined by using 'Lemna bioassay for phytotoxicity'.
Results: Dichloromethane crude extract exhibited highly cytotoxic activity with LD_{50} 0.6903µg/ml. The methanol extract showed significant activity in phytotoxic bioassay while dichloromethane extract showed good activity.
Conclusion: The present study assures the possible potential of cytotoxic activity as well as phytotoxic activity ofextracts of aerial parts of Aphelandra squarrosa of family Acanthaceae.
Keywords: Cytotoxic activity, Aphelandra squarrosa, Phytotoxic activity, crude extracts.

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INTRODUCTION

Some ornamental species are of huge medicinal importance [1].Pharmacologically active compounds from natural sources are used for those diseases which do not have effective treatment[2].A large number of drugs which are still being consistently used are derivatives of plants isolated compounds [3]. A trend towards increased screening of plantderived material for cytotoxic activity has been rapidly developing in recent years.Generally, driven by the ethno-traditional uses of different herbal remedies, the screening of plant extracts for cytotoxicpotential towards is usually accomplished by differentbioassays [4]. Medicinal plants often contain active phytochemical components with eminent therapeutically abilities, which encounter essential functions in preventing various prolonged diseases, thus having beneficial effects on the community [5-6].The source of phytochemicals have commonly found in leaves, barks, roots, flowers, fruits and seeds of the plants [6].Aphelandra squarrosa is found mostly in South Asia, Australia, India, USA and Pakistan. It is small, erect, evergreen shrub with elliptic leaves. Main veins in ivory. Flower yellow in four sided terminal inflorescence, enclosed in deep yellow bracts which turn green with age .The main objective of this study is to take into account the important aspect ofcytotoxic, phytotoxic potential of *Aphelandra squarrosa*.

EXPERIMENTAL

Chemicals and reagents

Methanol, Dichloromethane, Etoposide, Paraquat,Sabouraud, dextrose, agar, Nutrient broth, Potasium hydroxide pellets, Sae salt, All the chemicals used were either of Merck-Germany or of BDH-United Kingdom. Distilled water was prepared in our own laboratory.

Plant collection and extraction

From the Botanical Garden of BahauddinZakariya University Multan campus, the plant material was collected. The plant was identified as *Aphelandra squarrosa* (Catalog No. FCV #1, 1.). Aerial parts of plant were shade dried for a month.Then dried plant material was ground and weighed. The extraction of this finely ground plant material was affected by simple maceration. The weighed amount (300gm) of plant material was taken in bottle and measured volume (1200ml) of dichloromethane was added to it. In order to achieve maximum possible extraction, container having this mixture was placed in ultrasonic bath. Filtration was carried out after 24 hours of addition of solvent. The process was repeated two times with dichloromethane (600ml+300ml). The extraction of the marc was done by methanol (550ml+250ml+250ml) in the same manner. The Dichloromethane and methanol extracts were concentrated separately under reduced pressure by using rotary evaporator. These dried extracts were then stored in labelled sterile screw capped bottles. The extract stored in desiccator in order to prevent microbial growth.

Test organisms.

Lemnaminoe L for Phytotoxic assay and Brine Shrimp (Atemiasalina) for Cytotoxic assay.

Assay methodology

Phytotoxic activity was determined by *Lemna* bioassay.The E-medium was prepared and its pH was maintained at 5.5-6.0 by the addition of KOH pellets. 4 sets of 10 test vials each for 500, 50, 5ppm and control were prepared. 15mg of each of crude methanol and dichloromethane extracts were dissolved in 15ml corresponding solvents. 1000, 100 and 10µl extract solutions were added to 500, 50 and 5ppm vials respectively. The solvent was allowed to evaporate overnight. 2ml of E-medium was added to each vial and a single *Lemna minor* L. plant having a rosette of three fronds was added to each test vial. The test vials were placed in glass dish filled with water up to 2cm level. The glass dish was sealed with stopcock grease and glass plates. The glass dish was placed in growth chamber for seven days at 26ºC under fluorescent and incandescent light. No. of fronds in each test vial were counted and subsequently recorded on third and seventh day. The data obtained was analyzed data as percent of control with ED_{50} computer program to determine FI_{50} values and 65% confidence intervals [8].Cytotoxic activity was evaluated by Brine-Shrimp Lethality

assay.Artificial sea water was prepared by mixing of 3.8g sea salt per liter of water and filtered. It was added in small unequally divided tank, and to the larger compartment of the tank shrimp eggs were placed and it was darkened by covering it with aluminium foil. Through perforations in the dam the illuminated compartment attracts shrimp larvae. This was kept at room temperature so that shrimps should hatch and mature. For testing, vials were prepared for each fraction initially at 1000, 100, and 10 µg/ml and 3 replicates for each concentration making a total of 9 vials. 2 ml of organic solvent was added to 20 mg of sample and from this solution, 500, 50, or 5 µl, was transferred to vials corresponding to 1000, 100, or 10 µg/ml, respectively. Solvent was evaporated under nitrogen and then placed under high vacuum for about 30 minutes. Volatile organic solvents evaporate overnight. After 2 days, 5 ml of sea water was added to each vial and 10 shrimps per vial with the help of Pasteur pipette were added. The vials were kept under illumination. Numbers of surviving shrimps were counted after 24 hours with the help of a 3×magnifying glass and data was analyzed [7].

Statistical analysis

For Brine-Shrimp Lethality bioassay the data was analyzed with a Finney computer program (Probit analysis) to determine LD_{50} values and 95% confidence intervals [7].

RESULTS

The dried aerial parts of *Aphelandra squarrosa* were macerated successively with dichloromethane and methanol. The results are shown in the table 1.*In vitro* phytotoxic bioassay against *Lemna minor* was performed for evaluation of phytotoxic activity of DCM and MeOH extracts of aerial parts of *Aphelandra squarrosa.* The DCM and MeOH extracts exhibited significant activity. Results are described in table 2. Brine shrimp (*Artemiasalina*) lethality bioassay was performed for the evaluation of cytotoxic activity of the DCM and MeOH extracts of aerial parts of *Aphelandra squarrosa*. The MeOH extract exhibited no cytotoxic activity while DCM extract exhibited cytotoxic activity with LD50 0.6903µg/ml. Results are described in table 3.

Table 1. Results of extraction of aerial parts of *Aphelandra squarrosa* with dichloromethane and methanol.

Plant Name	Solvent Used	Extract obtained (g)	Sample codes
Aphelandra squarrosa	Dichloromethane	4.7	ASAPD
	Methanol	10.25	ASAPM

Table 2. Results of *In vitro* phytotoxic bioassay of dichloromethane and methanol extracts of aerial parts of *Aphelandra squarrosa*.

Extract	Plant Name	Conc. of Compound (µg/ml)	No. of Fronds		% Growth Regulation	Conc. of Standard Drug (Paraquat)(µg/ml)
			Sample	Control		
DCM	*Lemna minor*	1000	3	20	85	0.015
		100	8		60	
		10	11		50	
MeOH		1000	0	20	100	
		100	3		85	
		10	5		75	

Table 3. Results of Brine Shrimp *(Artemiasalina)* Lethality Bioassay of dichloromethane and methanol extracts of aerial parts of *Aphelandra squarrosa*.

EXTRACT	Dose (µg/ml.)	No. of Shrimps	No. of Survivors	LD_{50} (µg/ml.)	STD.Drug	LD_{50} (µg/ml.)
DCM	1000	30	00	0.6903	Etoposide	7.4625
	100	30	02			
	10	30	06			
MeOH	1000	30	22	10246.36	Etoposide	7.4625
	100	30	28			
	10	30	29			

DISCUSSION

In Brine-Shrimp Lethality Assay, the dichloromethane extract depicted high cytotoxic activity. The LD_{50} of the extract is 0.6903µg/ml as compared to standard drug i.e. Etoposide with LD_{50}, 7.4625 µg/ml. It is revealed that the extract is almost 10 times more potent than the standard. Further systematic investigations may be converged on separation of cytotoxic agents with improved, specific and safe therapeutic range.

The results showed that the dichloromethane extract exhibited good phytotoxic activity and methanol extract exhibited significant phytotoxic activity. The *Lemna* assay is a quick measure of phytotoxicity investigation. The phytotoxicty assay is a useful primary screen for weedicide research. As weeds is one of the major factors of poor agricultural productivity in the developing countries. Synthetic weedicides are expensive, toxic and non specific. Weedicides from natural sources having improved characteristics could have a promising future.

CONCLUSION

The evidence of cytotoxic and phytotoxic activities in *Aphelandra squarrosa* helps to discover new chemical classes of cytotoxic and phytotoxic agentswhich could be helpful for human.Furthermore, studies may be carried out to explore the cytotoxic and phytotoxic components of the plant by isolation, purification and structure determination leading to the development of an effective herbicide.

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