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RESEARCH ARTICLE

ANTIFUNGAL, CYTOTOXIC AND PHYTOTOXICITY OF AERIAL PART OF *RANUNCULUS MURICATUS*

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Abstract

Objectives: The present research is preliminary biological screening of aerial plant of *Ranunculus muricatus* (Ranunculaceae). Dichloromethane and methanol extracts of the aerial plant were investigated for their antifungal, phytotoxic and cytotoxic activities.

Methods: Anti-fungal, cytotoxicity and phytotoxicity activities were performed by agar tube dilution assay, brine shrimp lethality bioassay and lemna bioassay respectively. Dichloromethane and methanolic extracts exhibited significant phytotoxicity against Lemna minor having Paraquat as standard drug and incubation condition (28±1ºC).

Results: None of extracts presented any significant cytotoxic activity having Imipenum and Etoposide as standard drug respectively. Both extract had non-significant antifungal activity but it has been noted that methanol extract showed 30% inhibition with linear growth at 70 mm, when compared with control; only against *Microsporum canis*. *Ranunculus muricatus* showed significant phytotoxicity.

Conclusion: The phytotoxicty assay is a valuable major screen for weedicide investigation. Additionally, modern studies are currently carried out to identify the allelopathic constituents by isolation, purification and structure elucidation to find out as effective herbicidal.

Keywords: Biological screening; cytotoxicity, phytotoxicity, *Ranunculus muricatus*.

INTRODUCTION

Ranunculus muricatus also known as *Ranunculus pseudo-muricatus Baltter* and Hallb is indigenous to Atlantic, S. Europe, W. and S. W. Asia, Crimea, Caucasus, S. Siberia, Pakistan and India. Flowering period is between March and April[1]. It is recognised with different names in different part of world such as spiny buttercup (English); Chambul, jaghagha, Latokari, Korgandal (Folk). This plant is slightly poisonous. Whole plant is traditionally used as decoction for periodic fever and asthma[2]. The plant reported to contain stigmasterol-4-ene-3, 6-dione, stigmasterol, Anemonin, Aescin lactone dimethyl ether, beta-valley sterol, protocatechuic aldehyde, protocatechuic acid, and luteolin factors[3]. The findings of anemonin as chemical constituents in *R. muricatus* may justify the uses of these species against fever, rheumatism and rubefacient in Asian traditional medicines[4]. Present study is conducted to document the *in vitro* biological activities of *R. muricatus* conducted to document the *in vitro* biological activities of *R. muricatus*. Antifungal, phytotoxic and cytotoxic bioassay has studied.

METHODS

Plant material

R. muricatus was collected from Jallo pind, Lahore. Total weight of wet plant collected was 15 Kg while 5 kg of dried plant was obtained after drying. The plant was identified by Dr. Altaf Hussain Dasti, Professor, Institute of pure and applied Biology, Bahauddin Zakariya University, Multan.

Extraction

The air-dried aerial part of plant material was grounded and extracted successively with dichloromethane and methanol (thrice with each solvent) at room temperature occasional shaking for 24 hrs. Extracts were concentrated by Rotavapor-R20 at 35ºC.

Anti-fungal bioassay

Test fungi such as *Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani, Candida glabrata* were employed for preliminary screening. Extracts were dissolved in sterile DMSO to serve as stock solution. Sabouraud dextrose agar was prepared by mixing Sabouraud 4% glucose agar and agar in distilled water. Known amount of media was dispensed into screw capped test tubes. Test tubes containing media were autoclaved 121°C for 15 minutes. Tubes were allowed to cool to 50°C and the desired concentration of extract was added into non-solidified media. The tubes were allowed to solidify at room temperature. Each tube was inoculated with a 4mm diameter piece of in culture of fungi. All culture-containing tubes were inoculated at optimum temperature of 28-30°C for growth for 7 to 10 days. Culture was examined at least twice a weekly during the incubation. With no visible growth of microorganism is taken to represent the MIC of the test sample which is expressed in µg[5].

Phytotoxicity bioassay

Prepared inorganic medium of 5.5-6.0 pH attained with KOH pellets. Total 10 vials per dose 500, 50, 5 and control were prepared. Total 15 mg of the extract was dissolved in 15 ml of the solvent. 1000, 100 and 10 µl of solution to vials for testing allow the solvent to evaporate overnight. Total 2 ml of medium was added in each vial containing a single plant a rosette of three fronds. The vials were placed in a glass dish filled with 2 cm of water, sealed the container with stopcock grease and glass plate. Placed the dish along with vials in growth chamber for seven days at 25°C under fluorescent and incandescent light. Count the number of fronds per vials on day 3 and 7. Analyzed the data as percent of control with ED 50 computer program[5].

Brine shrinp lethality bioassay

Brine shrimp cytotoxicity assay was accomplished according to the standard procedure described by McLaughlin[6]. Three concentrations (1000, 100, and 10 ppm) of the plant extracts were used in this assay. Brine shrimp larvae were hatched in a small partitioned tank in artificial seawater. Illumination was provided on one side to attract newly hatched larvae. Brine shrimp larvae with second in star stage were used in this assay. Plant extracts of respective concentrations were added to dram vials. To each dram vial ten brine shrimp larvae were added. Negative control was prepared by evaporating 0.5 ml of methanol in dram vials and then by adding sea salt solution to it. Following 24 hrs of incubation, sur*vivo*rs were counted by using magnifying glass. The experiment was repeated three times. Mortality data was transformed by Probit analysis in finny computer program to estimate ED50 value. Percentage of mortality was also calculated at all concentrations[6].

RESULTS AND DISCUSSION

Dichloromethane and methanol extracts of the aerial plant of *R. muricatus* were studied for their antifungal, phytotoxic and Brine Shrimp lethality bioassay. *Candida albicans, Aspergillus flavus, Microsporum canis, Fusarium solani* and *Candida glabrata* were employed for fugitoxic effect of the extracts.

Table 1: *In-vitro* antifungal bioassay of *R. muricatus.*

Extract	Name of Fungus	Linear Growth (mm)		% Inhibition	Standard Drug	Mic (µg/ml)
		Sample	Control			
MeOH	*C. albicans*	100	100	0	Miconazole	110.8
	A. flavus	100	100	0	Amphotericin B	20.20
	M. canis	70	100	30	Miconazole	98.4
	Fusarium solani	100	100	0	Miconazole	73.25
	Candida glabrata	100	100	0	Miconazole	110.8
DCM	*C. albicans*	100	100	0	Miconazole	110.8
	A. flavus	100	100	0	Amphotericin B	20.20
	M. canis	100	100	0	Miconazole	98.4
	Fusarium solani	100	100	0	Miconazole	73.25
	Candida glabrata	100	100	0	Miconazole	110.8

Table 2: *In vitro* phytotoxic bioassay of *R. Muricatus.*

Extract	Plant Name	Conc. of Compound (µg/ml)	No. of Fronds		% Growth Regulation	Conc. of Standard Drug (µg/ml)
			Sample	Control		
MeOH	*Lemna minor*	1000	0	20	100	0.015
		100	9		55	
		10	17		15	
DCM		1000	0		100	
		100	9		55	
		10	17		15	

It has been noted that MeOH extract of *R. muricatus* showed 30% inhibition with linear growth at 70 mm, as compared with control; only against *Microsporum canis* at the concentration of 400 µg/ml for incubation period of seven days at 27ºC with reference to Miconazole as standard. While dichloromethane extract does not showed any activity as shown in Table 1. Dichloromethane and methanolic extracts of the

aerial part of *R. muricatus* showed significant phytotoxicity at concentrations of 1000 µg/ml, 100 µg/ml and 10 µg/ml against Lemna minor. Dichloromethane and methanolic extract of *R. muricatus* does not showed cytotoxicity even at highest level having Etoposide as standard drug containing 28±1ºC as incubation condition.

Table 3: *In-vitro* cytotoxic bioassay of *R. muricatus.*

Extract	Dose (µg/ml)	No. of Shrimp	No. of Survivors	LD 50 (µg/ml)	STD Drug	LD 50 (µg/ml)
MeOH	1000	30	14	857.73	Etoposide	7.4625
	100	30	25			
	10	30	29			
DCM	1000	30	24	45456.4		
	100	30	28			
	10	30	29			

CONCLUSIONS

The phytotoxicity assay is a valuable major screen for weedicide investigation. Weeds are one of the main issues of poor agronomic efficiency in the developing countries. Expensive, lethal and non-specific synthetic pesticides are used now. Using natural sources pesticides will not only reducers the adverse effect of pesticides but also help us to return to natural flora. Natural herbicides destroy specific targets, while leaving the wanted crop comparatively undamaged. Additionally, modern studies are currently carried out to discover the phytotoxic constituents of the plant by isolation, purification and structure elucidation to find out as effective herbicidal.

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DATA AVAILABILITY

The data supporting the findings of this study are not currently available in a public repository but can be made available upon request to the corresponding author.

AUTHOR'S CONTRIBUTION

Aslam MS: writing original draft, conceptualization, methodology, investigation. **Rehman R:** Writing, review, and editing, supervision, resources. **Choudhary BA:** writing, review, and editing. **Ijaz AS:** writing, review, and editing, project administration. **Uzair M:** methodology, investigation, formal analysis. **Ahmad MS:** conceptualization, methodology, investigation, data curation, writing, review, and editing. All authors revised the article and approved the final version.

CONFLICT OF INTEREST

None to declare.

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