

## Original Research Article

### Antifungal, Cytotoxic and Phytotoxicity of Aerial part of *Ranunculus muricatus*

#### Abstract:

**Objective:** 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.

**Method:** Anti-fungal, cytotoxicity and phytotoxicity activity was performed by Agar tube dilution assay, Brine Shrimp Lethality bioassay and Lemna bioassay respectively.

**Result:** Dichloromethane and methanolic extract exhibited significant phytotoxicity against Lemna minor having Paraquat as standard drug and incubation condition ( $28 \pm 1\text{C}^\circ$ ). None of extracts presented any significant cytotoxic activity having Imipenem 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 70mm, when compared with control; only against *Microsporum canis*. **Conclusion:** *Ranunculus muricatus* showed significant phytotoxicity. The phytotoxicity 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:** *Ranunculus muricatus*; Biological screening; Phytotoxicity; Cytotoxicity

#### INTRODUCTION

*Ranunculus muricatus* also known as *Ranunculus pseudo-muricatus* Baltter & Hallb is indigenous to Atlantic, S. Europe, W. & 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). It is slightly poisonous plant. 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 *Ranunculus 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 *Ranunculus muricatus*. Antifungal, phytotoxic and cytotoxic bioassay has studied.

#### EXPERIMENT

##### PLANT MATERIAL:

*Ranunculus muricatus* was collected from Jallo pind, Lahore. Total wet weight of plant collected was 15kg. It was then reduced to 5 kg of dried plant. 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 ground 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 35°C.

#### **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 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 4 mm diameter piece of inoculum removed from a seven-day-old culture of fungi. All culture-containing tubes were inoculated at optimum temperature of 28-30°C for growth for 7-10 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. 10 vials per dose 500, 50, 5 and control were prepared. 15 mg of the extract was dissolved in 15 ml of the solvent. 1000, 100 and 10µl of solution to vials for testing allow the solvent to evaporate overnight. 2ml of medium was added in each vial containing a single plant a rosette of three fronds. Placed the vials in a glass dish filled with 2cm 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 SHRIMP LETHALITY BIOASSAY:**

Brine shrimp cytotoxicity assay was accomplished according to the standard procedure described by McLaughlin, (1991). 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 instar 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 h of incubation, survivors 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].

#### **RESULT AND DISCUSSION:**

Dichloromethane and methanol extracts of the aerial plant of *Ranunculus 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. It has been noted that MeOH extract of *Ranunculus muricatus* showed 30% inhibition with linear growth

at 70mm, when compared with control; only against *Microsporium canis* at the concentration of 400 µg /ml for incubation period of seven days at 27°C with reference to Miconazole as standard. While dichloromethane extract does not showed any activity as shown in table 1

**Table 1: Results of In vitro antifungal bioassay of *Ranunculus muricatus***

| Extract | Name of Fungus            | Linear Growth (mm) |         | % Inhibition | Standard Drug  | Mic (µg/ml) |
|---------|---------------------------|--------------------|---------|--------------|----------------|-------------|
|         |                           | Sample             | Control |              |                |             |
| MeOH    | <i>Candida albicans</i>   | 100                | 100     | 0            | Miconazole     | 110.8       |
|         | <i>Aspergillus flavus</i> | 100                | 100     | 0            | Amphotericin B | 20.20       |
|         | <i>Microsporium canis</i> | 70                 | 100     | 30           | Miconazole     | 98.4        |
|         | <i>Fusarium solani</i>    | 100                | 100     | 0            | Miconazole     | 73.25       |
|         | <i>Candida glabrata</i>   | 100                | 100     | 0            | Miconazole     | 110.8       |
| DCM     | <i>Candida albicans</i>   | 100                | 100     | 0            | Miconazole     | 110.8       |
|         | <i>Aspergillus flavus</i> | 100                | 100     | 0            | Amphotericin B | 20.20       |
|         | <i>Microsporium canis</i> | 100                | 100     | 0            | Miconazole     | 98.4        |
|         | <i>Fusarium solani</i>    | 100                | 100     | 0            | Miconazole     | 73.25       |
|         | <i>Candida glabrata</i>   | 100                | 100     | 0            | Miconazole     | 110.8       |

Dichloromethane and methanolic extracts of the aerial part of *Ranunculus muricatus* showed significant phytotoxicity at concentrations of 1000 µg/ml, 100 µg/ml and 10 µg/ml against *Lemna minor*.

**Table 2: Results of In vitro phytotoxic bioassay of *Ranunculus muricatus***

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

Dichloromethane and methanolic extract of *Ranunculus muricatus* does not showed cytotoxicity even at highest level having Etoposide as standard drug containing 28°± 1°C as incubation condition.

**Table 3: Results of In vitro cytotoxic bioassay of *Ranunculus muricatus***

| Extract | Dose( $\mu\text{g/ml}$ ) | No. of Shrimp | No. of Survivors | LD 50 ( $\mu\text{g/ml}$ ) | STD Drug  | LD 50 ( $\mu\text{g/ml}$ ) |
|---------|--------------------------|---------------|------------------|----------------------------|-----------|----------------------------|
| 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               |                            |           |                            |

**CONCLUSION:**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 reduce 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 an effective herbicide.

#### **CONFLICT OF INTERESTS**

Declared none

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