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# Phytotoxic and cytotoxic characterization of crude methanolic extract of *Plumeria Obtusa*

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**Abstract**

*Plumeria Obtusa* belongs to the family Apocynacea; it has been utilized in tropical regions as medicine for the treatment of itches, swellings and fever. In this research investigation, phytotoxic characterization of crude methanolic extract of *P.obtusa* was tested on the germination of maize seeds. The extract showed inhibitory effect on the germination of the growth of root and shoot of the seedlings. The inhibitory result showed dose dependency. The maximum concentration of 1000 µg/ml showed maximum inhibitory effect on the growth of root and shoots. Similarly the presence of anti-tumorous compounds was also studied on Brine Shrimps by performing cytotoxic activity. The extract showed 100% shrimps deaths at 1000µg/ml. This confirmed the presence of anti-tumorous compounds in the plant which can further be isolated for the preparation of numerous drugs.

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## Introduction

There are several studies which have reported the potentiality of allelopathy in natural and agricultural ecosystem. In this regard, extensive researches have been done to explore the phenomenon. A potential way to exploit the allelopathic phenomenon is to screen the plants for their allelopathic and medicinal potential and to select the most bioactive ones for chemical analysis (Fujii *et al*., 1990, 1991, 2003).

The medicinal properties of plants remained a matter of concern for human since ancient times. Since modern medicine has progressively benefitted from discovering compounds existing in traditional medicine, researches should distillate on medicinal plants which are rich cradles of natural therapies (Abdallah and El-Ghazali, 2013). In linking to medicinal plants, the term "allelopathy" was 1st familiarized in 1937 by Molisch to suggest the detrimental and advantageous effects of one plant on the other by releasing certain chemical substances (Molisch, 1937). In recent years, medicinal plants are investigated for their allelopathic potential (Khan *et al*., 2009). Nature has gifted Pakistan with a vast variety of medicinal plants which are continuously in process of investigation for their allelopathic and or medicinal potential (Fujii *et al*., 2003, Khan *et al*., 2010c). It is need of the present time to make use of the therapeutic potential of higher plants to get new, less expensive, more effective and safer natural drugs. As an expedient scrutinizer for screening and fractionation in the discovery and evaluation of bioactive natural product, *invivo* lethality in a simple zoologic organism can be used (McLaughin & Rogers L, 1998). The brine shrimp assay is proved to be a rapid (24 hours), inexpensive, and simple procedure for the test described aforesaid. In this procedure, we can easily utilize a large number of organisms for the desired objectives accomplishment and requires no special equipment. In this procedure, relatively small amount of sample is required. Furthermore, it does not require animal serum as is needed for cytotoxicities. (McLaughin & Rogers L, 1998). *Plumeria obtuse* belongs to Apocynacea family which contains several poisonous compounds. The present study was aimed to investigate the phytotoxic as well as cytotoxic potential or lethality of the crude extract in order to isolate the potential compounds present in the extract for the purpose of synthesis of noval drugs later on.

## Materials and methods

*Plant Collection*

Fresh leaves of *Plumeria obtusa* were collected from the Cantonment board dispensary, supply lines Bannu in the month of March 2014.The plant was properly identified by a veteran taxonomist in the department of botanical sciences, faculty of biological sciences, University of science & technology Bannu, KPK-Pakistan. The plant material was washed by deionized water and was shade dried at room temperature for two weeks. The dried leaves were then milled mechanically into fine powder by using a local grinder machine. The powder was then subjected to further process.

*Preparation of plant extract*

200 g fine powder was socked in 1 liter of 80% methanol for seven days at room temperature. Initially after socking, it was continuously shaked for 5 hrs by using an automatic shaker machine. After 7 days, the powder mixture was filtered by using whatman filter paper no 1. The filtrate was collected and volume was noted. The filtrate was subjected for evaporation under reduced pressure in rotary evaporator at 50°c. The concentrated extract obtained was then applied to freeze drying using Lyophilizer (volume of the concentrated extract was noted prior to action). The extract was treated with liquid Nitrogen before applying the sample to lyophilizer. After 4 hrs operation, a very fine powder was resulted which was collected and stored at 4°C in the refrigerator for further investigation (weight was noted down).

*Phytotoxic assay*

*Requirements*

Methanolic crude extract of *Plumeria obtusa*, wheat seed, dis:H2O, $HgCl_2$, methanol, micro pipettes, tips, autoclaved Petri plates, filter paper, beakers,

electronic digital balance, disposable glasses, spray bottle.

*Assay Procedure*

Phytotoxic assay was preceded according to the modified protocol of McLaughlin and Rogers (1998). First of all stock solution was prepared by dissolving 5mg of powder into 5ml of methanol. From this stock solution, different sub solutions were prepared in a concentration of 100 and 1000 µg/ml. The experiment was performed in triplicates. Autoclaved petri plates were taken and filter paper was kept in the plates. The plates were properly marked/labeled for control and extract's different concentrations. 5ml from each concentration (100, and 1000 µg/ml) was sprayed/poured on the filter paper of each Petri plates very carefully by micro pipette. But for the control petri plates were not treated by the sample solution. All the treated Petri plates were placed in the oven at 40°C for complete evaporation of methanol from the filter papers. After the complete evaporation of methanol, then 5ml of distilled water were sprayed in all the treated petri plates as well as the three controlled (not treated) petri plates. Then, four maize seeds, washed with distilled water, were placed in each plate at equal distance. All the Petri plates were incubated in growth room for five days. Readings were taken after $5^{th}$ day, shoot and root inhibition was noted by scale in millimeters in comparison to control.

*Cytotoxic assay*

A cytotoxic activity of methanolic crude extract of P.Obtusa was carried out according to the standard procedure of (Meyer-Albert *et al.*, 1992).

Sample was prepared by dissolving 5mg of crude plant extract in respective solvent (methanol) to form stock solution of 5mg/5ml and further diluted into 100, 500, and 1000 µg/ml. 2.8 gm commercial sea salt (Sigma) was liquefied in 100ml of $dH_2O$ with constant stirring for 2 hrs. About 1g of Brine shrimp (Artemia salina) eggs was aerated in 1L capacity glass container (separating funnel) containing filtered sea salt solution. After 48 hours of incubation at room temperature (25-29°C), under continuous illumination of fluorescence lamp free-swimming pink colored larvae were hatched. The freshly hatched free-swimming larvae were used for the cytotoxic assay. 1ml form each sub-solution was taken in three test tubes. These test tubes were placed in the oven at 40°C for the complete evaporation of methanol for 24 hours. After the complete evaporation of methanol, 3ml saline solution (sea salt) was poured in each test tube. For the control, 3ml saline solution was taken in the separate test tube. 10 nauplii were transferred to each test tube and the setup was allowed to remain for 24h, under constant illumination of florescent lamp. Numbers of survived nauplii were counted with the help of magnifying glass after several time intervals. After 24 hours survival percentage was calculated.

**Results and discussion**

*Phytotoxic activity of Plumeria Obtusa*

Three concentrations (100, and 1000 µg/ml) of methanolic plant extract were used in this activity. The results shows that the extract inhibited the growth of shoot and root as compared to the control as shown in Figure A after five days.

**Table 1.** Cytotoxic activity of *Plumeria obtuse*.

| Concentrations (µg/ml) | Number of Shrimp | Number of survival | Number of death | %age activity |
|---|---|---|---|---|
| Control | 10 | 10 | 10 | 0% |
| 100 µg/ml | 10 | 7 | 3 | 70% |
| 500 µg/ml | 10 | 0 | 0 | 100% |
| 1000 µg/ml | 10 | 0 | 0 | 100% |

Cytotoxic activity of *Plumeria Obtusa*

From the assay it was found (Table 1) that in the control sets almost all the shrimp survived throughout the observed period (24 h). The table shows 100% cytotoxic activity at highest treated concentration 1000µg/ml, at the 500µg/ml extract

also shows 100% cytotoxic activity while at 100µg/ml, it shows 70% cytotoxic activity.

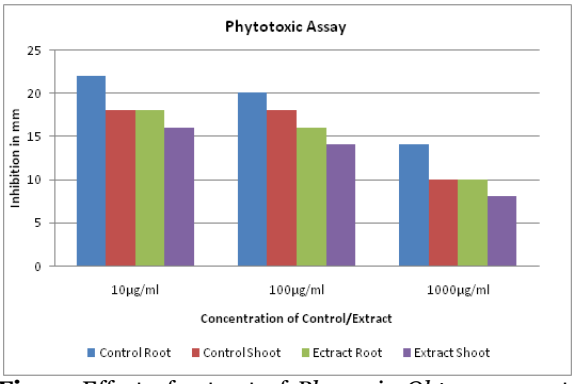


**Fig. 1.** Effect of extract of *Plumeria Obtuse* on root and shoot growth of maize seedlings.

Kanegusuku *et al.* (2001) reported organic fraction of Rubus imperialis (C) which showed more cytotoxicity. Zaidi *et al.* (2006) studied that methanolic fraction of Arceuthobium oxycedri possessed100% lethality for brine shrimps at high dose which are in accordance with our results. The results of present study suggest that methanolic fraction possess some bioactive constituents having anticancer activities that can be the point of interest for new drugs possessing anticancer and protective role against different pathogens.

## Conclusion

The results of the present study reveal that crude Methanolic extract of *Plumeria Obtusa* play a vital role in phytotoxicity so it can be used as phytotoxic agent. The plant also showed antitumor property and thus may be utilized for raising antitumor drug. A lot of therapeutic potential is hidden in the plant which is still to be explored using various types of bio-assays and procedures.

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