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BRINE SHRIMP BIOASSAY OF *PHOENIX SYLVESTRIS*

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ABSTRACT

Brine shrimp bioassay is a simple method for natural product research. The procedure determines LD_{50} values in μg/ml of active compound and extract in the brine medium. Three different extracts (methanol, chloroform and butanol) of the male flower inflorescence of *Phoenix sylvestris* were subjected to this bioassay. The results showed that the methanolic and butanolic extracts exhibit significant toxicity to the shrimps, while the chloroform extract was found to be non-toxic.

INTRODUCTION

Phoenix sylvestris Roxb. which belongs to the family Palmae is a moderate sized dioecious tree of 7.5-15 m height. It is indigenous in the Indus basin and also cultivated in Sind and Punjab (Malik, 1984), common in many parts of India, either wild or cultivated (Krishnamurthi, 1969). The fruit of the plant are edible and are considered as restorative and tonic. The kernels are made into a paste with the root of *Achyranthe saspera*, eaten with betel leaves as remedy for ague. The roots are reported to be useful in toothache (Chopra, 1956). The central tender part of the palm is useful in gonorrhoea and gleet (Watt, 1892). Economically this palm is of considerable importance, particularly as a source of sugar, that is obtained by refining the "*gur*" or treacle and toddy or "*tari*". This fluid is extracted from the cuts made in the trunk from which the juice exudes (Malik, 1984). The leaves are widely used for thatching and for making mats, fans, baskets, bags, brooms and fishing nets. They also yield a soft fibre which bleaches well. The petioles are beaten and made into ropes, used for drawing water from wells. The leaves are also lopped for fodder. The wood is used for temporary construction, bridges and piers and for tent pegs; trunks freed from pith make excellent water conduits. The tree also yields a gum (Krishnamurthi, 1969).

Brine shrimp bioassay is a rapid, reliable, inexpensive and convenient method in which natural product extracts, fractions or pure compounds are tested at an initial concentrations of 10, 100 and 1000 ppm (or μg/ml) in vials containing 5 ml of brine and ten shrimps in each of three replicates. Survivors are counted after 24 hours. These data are processed in a simple programme on a personal computer to estimate LC_{50} values with 95% confidence intervals.

A positive correlation has been found between brine shrimp toxicity and 9KB cytotoxicity, and have been observed that ED_{50} values for general cytotoxicities are about one-tenth LC_{50} values in the brine shrimp test (Meyer *et al*., 1982, McLaughlin *et al.*, 1990).

MATERIALS AND METHODS

Plant Material

The male flower inflorescence of *Phoenix sylvestris* were collected from Malir garden, Karachi.

Extraction and Fractionation

Fresh plant material was soaked in methanol, three times each for a period of ten days. The combined methanolic extract was evaporated under reduced pressure which afforded a dark brown gummy residue. A portion of the residue was partitioned first between chloroform and water. Both layers were separated and the chlorofrm layer was evaporated to obtain chloroform extract. The aqueous layer then extracted with presaturated n-butanol. As a result two layers were obtained. Aqueous layers was discarded and the butanol layer after evaporation of the solvent under reduced pressure afforded a dark brown extract.

Bioassay

The procedure described in the literature (Meyer *et al*., 1982; McLaghlin *et al.,* 1990) was adopted for this work. Samples of three different concentrations 10, 100, 1000 μg/ml were prepared according to the direction as given in the literature. Brine shrimp (*Artemia salina* Leach) nauplii were hatched in a specific tank. Ten shrimps were transferred to each sample vial and then sea water was added to make the volume 5 ml. Later on dry yeast suspension was added as food to each vial including control. The vials were kept for 24 hours, thereafter the active nauplii were counted with the aid of a 3 x magnifying glass and the percent deaths at each dose and control were determined. The data so obtained were processed in a simple programme on a personal computer to estimate LC_{50} values with 95% confidence intervals.

Observations:

The observations are given in Table 1.

Table 1
Brine Shrimp Bioassay of the Crude Extracts of *Phoenix sylvestris*

Extract	10 μg/ml		100 μg/ml		1000 μg/ml		LD_{50} μg/ml	95% confidence interval
	Alive	Dead	Alive	Dead	Alive	Dead		
MeOH	30	0	25	5	4	26	200.8038	119.54 – 342.43
$CHCl_3$	30	0	30	0	22	8	> 1000	–
BuOH	30	0	19	11	2	28	135.5239	83.78 – 222.27

RESULTS AND DISCUSSION

Brine shrimp nauplii have been used in a number of bioassay systems (McLaughlin *et al.*, 1990) and the crude extracts of the male flower inflorescence of *Phoenix sylvestris* were subjected to this evaluation. Results of brine shrimp bioassay are given in Table 1. The extracts were tested at concentrations of 10, 100 and 1000 μg/ml. Survivors were counted after 24 hours and the percentage of death caused by each dose was recorded as 0.0%, 16.66%, 86.66% for methanolic extract, 0.0%, 0.0%, 26.66% for chloroform extract and 0.0%, 36.66% and 93.33% for butanolic extract. The LD_{50} for methanolic extract was found to be 200.80 μg/ml with an upper limit of 342.43 μg/ml and lower limit of 119.54 μg/ml. Similarly the upper limit for butanolic extract was 222.27 μg/ml and lower limit 83.78 μg/ml with LD_{50} value of 135.52 μg/ml were observed. Thus on the basis of these results it may be concluded that the methanolic and butanolic extracts

exhibited significant toxicity, while the chloroform extract was found to be non-toxic. Altogether butanolic extract exhibited the highest toxicity as the results of brine shrimp bioassay.

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