Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Der Pharmacia Lettre, 2013, 5 (1):162-167
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Brine Shrimp bioassay of *Pentapetes phoenicea* Linn. and *Ipomoea carnea* jacq. leaves

Nisha Sharma[1,2*], Prakash Chandra Gupta[1], Anju Singh[1] and ChandanaVenkateshwara Rao[3]

[1]*University Institute of Pharmacy, Chhatrapati Shahu Ji Maharaj University, Kanpur, Uttar Pradesh, India*
[2]*Institute of Pharmaceutical Sciences and Research Centre, Bhagwant University, Ajmer, Rajasthan, India*
[3]*Pharmacognosy and Ethnopharmacology Division, National Botanical Research Institute, Lucknow, Uttar Pradesh, India*

ABSTRACT

The aim of the study was to investigate the biosafety of various fractions of the leaves of P. phoenicea and I. carnea by using brine shrimp lethality bioassay. Evaluation of brine shrimp toxicity assay was carried out for various fractions of leaves of P. phoenicea and I. carnea. None of extracts of P. Phoenicea were found to be toxic upto a dose level of 600 µg/ml. However, the chloroform extract was found to be weakly toxic with LC_{50} 659.8 µg/ml. The various fractions of Ipomoea carnea hexane (LC_{50} 141.4 µg/ml), chloroform (LC_{50} 211.28 µg/ml) and ethyl acetate (LC_{50} 307.28 µg/ml) showed significant toxicity. In the assay the positive control showed LC_{50} less than 100 µg/ml. The study establishes an addition to the scientific literature, although detailed investigations for the pharmacological activities and active ingredients could provide lead molecules to natural products discovery.

Keywords: Biosafety, Brine shrimps, In-vitro assay toxicity, LC_{50}, Lethality assay.

INTRODUCTION

Pentapetes phoenicea Linn. (Sterculiaceae), commonly known in hindi as Dopa-hariya, an annual erect herb. The capsules are mucilaginous and used for treatment of diseases of bowels. The water of boiled leaves of plant have been reported to be used traditionally for treatment of inflammatory glands, cough and cold, Juice of the leaves is applied on inflammatory glands. The roots have been reported to be astringent, mildly thermogenic, constipating and febrifuge, and are useful in fever, diarrhea, burning sensation, psychopathy and vitiated conditions of vata and pitta. The fruits are mucilaginous and used in gastropathy, fever and vitiated conditions of vata and pitta. Root decoction is administered for treatment of burning micturition. The various fractions of leaves of the plant have been reported to contain Alkaloids, carbohydrates, saponins, tannins, steroids and triterpenoids.[1]

Ipomoea carnea, belongs to family convolvulaceae, commonly known as behsaram in Hindi, is widely distributed in India. The milky juice is used by the local healers for the treatment of several skin diseases, Leucoderma. It is used as aphrodisiac, purgative and cathartic, Anti-inflammatory, decreases teratogenic effect resulting from

cyclophosphamide. Aqueous extract shows neuromuscular blocking activity.[2] It has been reported to show depressant activity on Central nervous system, HIV -1 RT inhibitory potential, immunomodulatory activity etc.[3] Considering the traditionally claimed uses of the plant, the aim of the study was to investigate the biosafety of the leaf extract fractions of *Pentapetes phoenicea* and *Ipomoea carnea* against *Artemia salina.* The assay is considered a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, pesticides and cytotoxicity testing of dental materials.[4-7] Brine shrimp lethality assay consists of exposing larvae to test sample in saline solution and lethality is evaluated after 24 hrs. The commercial availability of inexpensive brine shrimp eggs, low cost and ease of performing the assay make the brine shrimp, lethality assay, a very useful bench top method.[8]

MATERIALS AND METHODS

Plant material: The leaves were collected from the local areas of Kanpur, in the month of September, 2011. The plants were identified & the specimen vouchers have been preserved in the Dept. of Pharmacognosy, C.S.J.M. University, Kanpur for future reference.

Extraction and Fractionation:
The fresh leaves of *P. phoenicea* and *I. carnea* were collected, washed with water to remove dirt and shade dried at room temperature. The dried plant material was pulverized using electric blender and sieved. Weighed portion of the sample was subjected to cold hydro-alcoholic extraction.

Portions (100 g) of the powdered sample were weighed into a conical flask. Mixture of methanol & water in the ratio 80:20 was added and left for 72 hours. The mixture was filtered and the filtrate was concentrated using rotary evaporator and the concentrate was subjected to partitioning with hexane, chloroform and ethyl acetate. All the fractions were subjected to activity studies.

Hatching Brine shrimps:
The Brine shrimp eggs were gifted by Aquatic Enterprise Co. Malaysia. The *Artemia salina* eggs were hatched in artificial seawater prepared by dissolving 40 g sodium chloride in 1lt. of distilled water. Two unequal compartments plastic chamber with several holes on the divider was used for hatching[9]. After 36 h incubation at room temperature (22-29°C) under light source, the active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay.

Brine shrimp assay:
Biosafety of the *P. phoenicea* and *I. carnea* extracts was measured by brine shrimp lethality test.

Test plant extracts (20mg of each) were dissolved in 10ml of pure dimethyl sulfoxide (DMSO) to get stock solutions of 2mg/ml. Each of the stock solution (250, 500, 750, 1000, 1250, 2500, 3750 µL) were transferred into plates and 5ml of prepared sea water was added to each plates. The corresponding concentrations were 100, 200, 300, 400, 500, 1000, 1500 µg/ml respectively.[10]

DMSO was used as negative control and Thymol [11] used as positive control. After hatching and maturation of A. salina, 10-15 larvae were placed in each plate using glass capillary and incubated at 25-27°C for 24h under illumination. After 6 h and 24 h, the numbers of dead naupli in each plate were counted. The experiment was done in triplicate.

Lethality concentration determination and statistical analysis:
The percentage lethality was determined by comparison of mean surviving larvae of the test and control Plates. The percentage of mortality in each concentration was determined and LC_{50} values were obtained from best fit line plotted concentration verses percentage lethality using Microsoft excel 2007. The Statistical analysis was analysed out using GraphPad Prism software version 4.01.

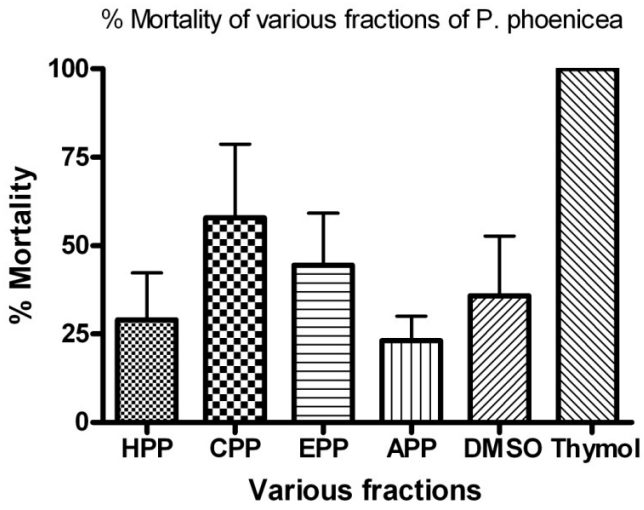
RESULTS

Table 1 shows the results of brine shrimp lethality after 24 hours of exposure to all the samples and the positive control, Thymol. The positive control compared to the negative control (DMSO + Sea water) was highly lethal,

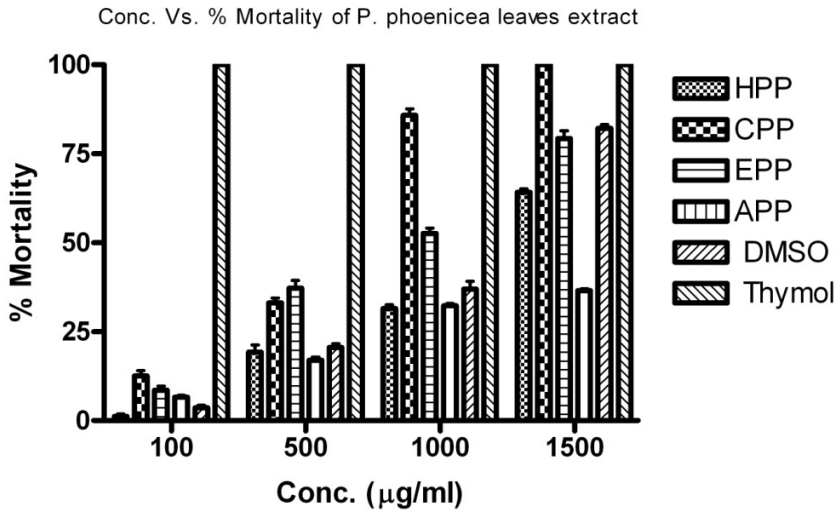
giving significant lethality to the shrimps. The lethal concentration (LC_{50}) of the test samples was obtained by plot of percentage of mortality versus the sample concentration and the best fit was obtained from the curve data by means of regression analysis and with software GraphPad Prism version 4.01. It was observed that the percentage mortality increases with the increase in concentration of test samples (Figure 1a,1b & 2a, 2b). The criterion of toxicity for fractions was established by following the findings established as LC_{50} values >1000µg/ml (non toxic), ≥ 500 ≤ 1000 µg/ml (weakly toxic) and < 500 (toxic).[12] In the brine shrimp test, among the evaluated extracts of plant *P. Phoenicea*, chloroform and ethyl acetate extract were found to be weakly toxic with LC_{50} 659.8 and 928.85 µg/ml, hexane and aqueous extract were non toxic with LC_{50} 1293.6, 1929.21 µg/ml. Among the evaluated extracts of plant *I. Carnea*, hexane (LC_{50} 111.11 µg/ml), chloroform (LC_{50} 211.28 µg/ml), ethyl acetate (LC_{50} 307.28 µg/ml) and aqueous (764.46 µg/ml) fractions showed significant toxicity. In the assay the positive control showed the LC_{50} less than 100 µg/ml.

Table1: Brine shrimp bioassay results of plant extracts

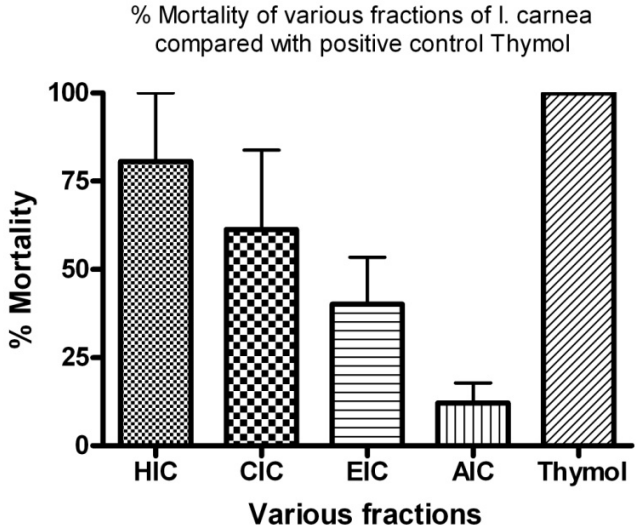
Test materials	LC50 (µg/ml) after 24 hr.
Pentapetes phoenicea	
Hexane (HPP)	1093.6
Chloroform (CPP)	659.8
Ethyl acetate (EPP)	928.5
Aqueous (APP)	1929.21
Ipomoea carnea	
Hexane (HIC)	111.11
Chloroform (CIC)	211.28
Ethyl acetate (EIC)	307.28
Aqueous (AIC)	764.46
DMSO (- Control)	1060.3
Thymol (+ Control)	<100µg/ml



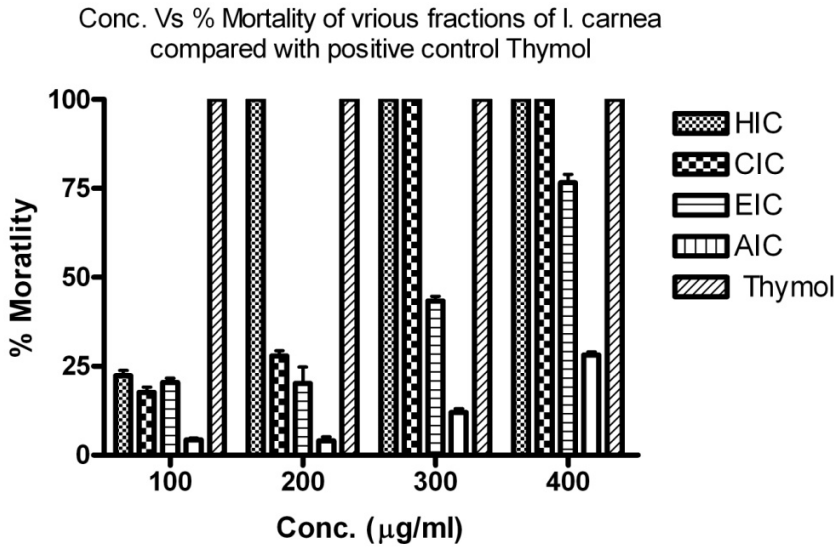
"Figure 1(a): Bar graph for various fractions of P. Phoenicea leaf extract, negative control (DMSO) and positive control (Thymol)"



"Figure 1(b): Dose dependent bar graph for various fractions of P. Phoenicea leaf extract, negative control (DMSO) and positive control (Thymol)"



"Figure 2(a): Bar graph for various fractions of I. Carnea leaf extract and positive control (Thymol)"



" Figure 2(b): Dose dependent Bar graph for various fractions of I. Carnea leaf extract and positive control" (Thymol)

DISCUSSION

Several studies have shown that brine shrimp assay has been an excellent method for preliminary investigations of toxicity, to screen medicinal plants popularly used for several purposes and for monitoring the isolation a great variety of biologically active compounds. Since its introduction, this in vivo test has been successively used for bioassay guide fractionation of active cytotoxic and antitumor agents. A positive correlation between the lethality to brine shrimp and the corresponding oral lethal dose in mice of medicinal plants has also been demonstrated.[13] .

The brine shrimp lethality bioassay is used as a screening tool for the determination of bioactivity of different extracts, fractions and pure compounds. This test is an indication of cytotoxicity, anticancer, antiviral, pesticidal, antimicrobial and other different pharmacological activities.[11] The brine shrimp lethality bio assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which in most cases correlates reasonably well with cytotoxic and antitumor properties. The assay is considered to be a very useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, toxicity of plant extracts, heavy metals, and cytotoxicity testing of dental materials. The brine shrimp toxicity assay have been carried out for the first time for the *P. Phoenicea* and *I. carnea.* The results obtained suggest the plant; *P. Phoenicea* can be used safely as per the traditional claims and can be tested further for acute toxicity on animal model to correlate the two methods of toxicity evaluation. The plant *I. carnea* as claimed traditionally toxic, can be further explored for cytotoxic activity.

Acknowledgements
The authors are thankful to Prof. Ashok Kumar, Vice-Chancellor, C.S.J.M. University, Kanpur for providing the necessary facilities at University Institute of Pharmacy. The authors also greatly acknowledge M/S Aquatic Enterprise CO. Malaysia for providing gift sample of Artemia (Brine shrimp eggs).

REFERENCES

[1] Sharma N, Gupta PC, Singh A, Rao ChV. *Asian Pac j Trop Biomed*. **2012;** (in press).
[2] Khalid MS, Singh RK, Reddy IVN, Kumar SJ, Kumar BS et al. *Pharmacologyonline*. **2011**; 1: 326-31.
[3] Meira M, Silva EPD, David JM, David JP. *Rev. bras. farmacogn*. **2012**; 22(3): 682-713.
[4] Barahoma MV, Sanchez FS. *Environ. Pollut*. **1999**; 104: 469-76.
[5] Pelka M, Danzl C, Distler W, Petschelt A. *J Dentology*. **2000**; 28: 341-45.

[6] Sam TW. Toxicity testing using the brine shrimp (*Artemia salina*). In: Colegate, S.M and Molyneux, R.J (Eds), Bioactive natural product Detection, Isolation and structural Determination. CRC press, Boca Raton, FL: **1993;** pp. 422-56.
[7] Meyer BN, Ferrigni NR, Putnam JE, Jacobsen LB, Nichols DE, McLaughlin JL. *Planta Med*. **1982**; 45(5): 31-4.
[8] Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. *Int. J Appl. Sci. Engg*. **2006**; 4(2): 115-125.
[9] Pisutthanan S, Plianbangchang P, Pisutthanan N, Ruanruay S, Muanrit O. Naresuan *University Journal*. **2004**; 12(2): 13-8.
[10] Olaleye, Tolulope M, *J Med Plants Res*. **2007**; 1(1): 9-13.
[11] Bastos MLA, Lima MRF, Conserva LM, Andrade VS, Rocha EMM, Lemos RPL. *Ann of Clin Microbiol Antimicrob*. **2009**; 8(16): 1-6.
[12] Deciga CM, Rivero CI, Arriaga AM, Castaneda CG, Angeles LGE, Navarrete A, et al. *J Ethnopharmacol*. **2007**; 110:334-42.
[13] Hossain MA, Ferdous T, Salehuddin SM, Das AK. As. *J. Food Ag-Ind*. **2009**; 2(3): 336-41.