Manipal Journal of Pharmaceutical Sciences

Volume 2 | Issue 1 Article 4

3-1-2016

Cytotoxic potential of medicinal plants – A comparative evaluation

Litty Joseph
littyjimmyalappatt@gmail.com

K K. Srinivasan

Sudheer Moorkoth

Jyoti Harindran

Original article

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Litty Joseph*, K K Srinivasan, Sudheer Moorkoth, Jyoti Harindran

Abstract

Objective: This study was carried out to evaluate the cytotoxic potential of petroleum ether and ethanolic extract of *Justicia simplex* (JSPE & JSOH), *Myxopyrum smilacifolium* (MSPE & MSOH), *Memecylon malabaricum* (MMPE & MSOH) and *Litzea quinqueflora* (LQPE & LQOH) were tested using Brine shrimp lethality bioassay. **Methods**: The extracts were prepared in different concentrations. LC_{50} was assessed by counting the number of surviving (larvae) shrimps after 12, 24 and 48 h. **Results**: The results obtained showed that the extracts of *Justicia simplex*, have the maximum lethality with significant LC_{50} values of 122 and 175 µg/mL, which were proportional to concentration and time. The order of cytotoxic potential was JSPE> JSOH> MSPE >MMPE> MSOH> LQPE> MMOH > LQOH. **Conclusion**: This study was concluded by affirming the cytotoxic potential of JSPE, JSOH and MSPE, whose LC_{50} values were below 250 µg/mL and were potential for further investigation.

Keywords: Brine shrimp lethality assay, Justicia simplex, Memecylon malabaricum, Litzea lingustrina, Myxopyrum smilacifolium.

Introduction

The Brine Shrimp Lethality Bioassay (BSLB) was suggested by Michael [1] and further established by Vanhaecke [2] and Sleet and Brendel [3]. The ability to kill laboratory-cultured *Artemia* nauplii is the basis of this assay and it is considered a valuable tool for preliminary evaluation of cytotoxicity[4]. It has been used for the screening of plant extract toxicity, cytotoxicity testing [5], pesticide toxins [6], cyanobacteria toxins [7], fungal toxins [8] and heavy metal poisoning. BSLB represents a rapid (24 hours), simple (e.g., no aseptic techniques are required), and inexpensive method among the cytotoxicity screening techniques. Furthermore, it needs lesser amounts of test material (2–20mg or less) [9-11].

The selected medicinal plants for study have been traditionally known to be anticancer agents [12]. *Justicia simplex D.* Don, *Memecylon malabaricum Cogn. Litsea quinqueflora* (Dennst.) [13-15] and *Myxopyrum smilacifolium* (Wall Blume) [16] were selected for the study. They also have been reported to possess anti fatigue, anti-stress, anti-spermicidal, anti-inflammatory and antimicrobial activity [17]. These plants are stimulating and have an effect on whooping cough, chikungunya, fever, and dysentery.

Materials and Methods

collection and identification: The aerial part of *Justicia simplex*, leaves of *Memecylon malabaricum*, *Litzea quinqueflora and Myxopyrum smilacifolium* were collected from Kottayam (Dist.) of Kerala

Litty Joseph
Dept. of Pharmaceutical Sciences, Mahatma Gandhi University, Cheruvandoor Campus, Kottayam, Kerala, India 686631
Email: littyjimmyalappatt@gmail.com

K K Srinivasan
Dept. of Chemistry, Shri Madhwa Vadiraja Institute of Technology and Management, Bantakal, Karnataka, India, 574115

* Corresponding Author

Sudheer Moorkoth
Manipal College of Pharmaceutical Sciences, Manipal University, Manipal, Karnataka, India. 576104

Jyoti Harindran
Dept. of Pharmaceutical Sciences, Mahatma Gandhi University, Cheruvandoor Campus, Kottayam, Kerala, India 686631

How to cite this article: Joseph L, Srinivasan K K, Moorkoth S, Harindran J. Cytotoxic potential of medicinal plants. *MJPS* 2016; 2(1): 21-25.

during the month of September - October 2010 in and around Cheruvandoor. Plants were identified and authenticated by Dr Jomy Augustine, Head of Department, Department of Botany, St. Thomas College, Palai, Kerala. The voucher specimens of these plants have been preserved in the Department of Pharmaceutical Sciences, MG University, Kottayam.

Preparation of petroleum ether extract: All the plants (20kg) were shade dried, milled into coarse powder and soaked in petroleum ether (60-80°C) for one day. The extractions were carried out in a batch of 150g in 5L soxhlet apparatus. The combined extracts were dried over sodium sulphate (anhydrous) and the solvent was completely removed by distilling under reduced pressure.

Preparation of alcoholic extracts: The marc obtained from the above extraction was kept soaked in ethanol (95%) for a day and then extracted exhaustively in a soxhlet apparatus. Under reduced pressure, the collective extracts were concentrated and evaporated to a semi-solid consistency. It was then kept in a desiccator for two days [18].

Test sample preparation for Brine shrimp bioassay: A stock solution with a concentration of 1mg/mL was prepared by dissolving the test samples in DMSO (Dimethyl sulfoxide). In order to prevent the possible error from DMSO toxicity, the final concentration of DMSO in the assay volume was kept at 2%. Negative control for the cytotoxicity assay were artificial sea water and DMSO.

Hatching of Brine shrimp cysts*:* Fresh cysts were obtained from CMFRI (Cochin Marine and Fisheries Research Institute) Cochin, India. Brine shrimp eggs (*Artemia salina* Leach) were permitted to hatch and mature as nauplii (Larvae) in seawater for 48 h at 25-28ºC, under a continuous light. The experiment was carried out by following the method described by Meyer. Artificial seawater was prepared by dissolving sea salt 38g in 1Lwater and the pH was adjusted to 8.5 with NaOH. To this, 70 mg of brine shrimp Leach was sprinkled and set aside under continuous aeration and light ray for 48 h in a hatching chamber. Active nauplii, which were free from eggshells, were collected from the brighter portion of the hatching chamber by using a dropping pipette. Petroleum ether and ethanolic extracts of *Justicia simplex* (JSPE, JSOH), *Myxopyrum smilacifolium* (MSPE, MSOH), *Memecylon malabaricum* (MMPE, MMOH) and *Litzea quinqueflora* (LQPE, LQOH), were used for the study. The toxicity of extracts was tested at various concentrations, 1, 10, 50, 100, 250, 500, 750 and 1000µg/mL in seawater containing 2% DMSO (v/v). About 4.5mL of seawater was added to each test tube. Ten nauplii were used in each test tube. A parallel series of tests and the blank control were included. In each test tube, 0.5ml of different concentration of the extracts was added. The toxicity was determined after 12, 24, and 48 h exposure. The procedure was done in triplicate. If the larvae did not unveil any movement during the observation, they were considered dead and the percentage of deaths was calculated from the number of survivors. In order to confirm that the mortality detected is due to the bioactive compounds and not due to undernourishment, we compared the dead larvae in the treatment group with the control group. Emerged brine shrimp nauplii can survive for up to 48 h without food [19] since they still nourish on their yolk sac. The percentage of mortality was calculated by:

% Mortality = (Average of survival in the control group – Average of survival in the treatment group) /Average of survival in the control group

Statistical Analysis: A graph of log concentration tested versus percentage of mortality of the brine shrimps was plotted. To determine the lethal concentration that was capable of killing 50 % of the brine shrimps (LC_{50} value) treated with respective extract, a linear regression analysis using best-fit line method was done.

Results and Discussion

The petroleum ether and ethanolic extracts of all four plants were evaluated for their cytotoxic potential by BSLB. The extracts exhibited low toxicity after the first 12 h exposure, even at the maximum concentration (1000µg/mL). A small relationship existed among the brine shrimp lethality and cytotoxicity after 12 h exposure. However, in general, many of the invertebrate exhibit toxicity

in most of the bioassays at 1000µg/mL after 24 h exposure [10, 20], and the result was consistent with other cytotoxic screening tests.

JSPE and JSOH showed maximum lethality which was proportional to the time of exposure. After 48 h exposure, JSPE and JSOH had the LC_{50} values of 122.22 and 175.46µg/mL, respectively. On the other hand MSPE and MSOH exhibited LC_{50} values of 196 and 496 µg/mL. MMPE and MMOH showed LC_{50} values of 322.63 and 804.79 µg/mL. LQPE and LQOH caused the least toxicity (849.43 and 969 µg/mL). In this study, there was a dose-dependent relationship existing between the percentage mortality and concentration of the extracts, and were in direct correlation (Table.1 & 2).

Table 1: Percentage mortality and LC_{50} of JSPE, LQPE, MSPE and MMPE obtained in BSLB *after 12, 24, and 48h of exposure*

Concentration µg/ml.	% Mortality of JSPE			% Mortality of LQPE			% Mortality of MSPE			% Mortality of MMPE		
	12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h
1000	80	100	100	46.67	58.62	65.51	66.67	82.14	96.64	60	72.41	93.1
750	70	89.65	89.65	33.33	44.82	55.17	53.33	57.14	92.86	46.67	51.72	89.65
500	53.33	72.41	72.41	16.67	31.03	44.82	20	46.42	82.14	30	41.37	75.86
250	26.67	37.93	37.93	13.33	24.13	37.93	13.33	35.71	71.43	13.33	24.13	65.51
100	20	24.14	24.14	3.33	17.24	31.03	0	28.57	50	0	20.68	41.37
50	10	17.24	17.24	0	10.34	17.24	0	17.86	32.14	0	10.34	27.58
10	10	13.79	13.79	0	3.44	6.89	0	10.71	17.86	0	3.44	10.34
1	3.33	10.34	10.34	0	3.33	3.44	0	3.57	7.14	0	0	3.44
LC_{50} µg/mL	537.58	378.4	121.22	>1000	831.96	643.49	780.92	552.47	196.72	826.22	666.98	322.63

Table 2: Percentage mortality and LC_{50} of MSOH, LQPE, MMOH and LQOH obtained in BSLB *after 12, 24, and 48h of exposure*

Concentration. µg/mL	% Mortality of MSOH			% Mortality of JSOH			% Mortality of MMOH			% Mortality of LQOH		
	12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h	12 h	24 h	48 h
1000	53.33	58.62	89.65	76.67	89.65	100	33.33	44.83	55.17	30	41.37	44.83
750	46.67	48.27	79.1	56.67	65.51	100	26.67	41.38	44.83	26.67	27.58	41.38
500	23.33	37.93	62.06	30	41.37	96.55	16.67	31.03	41.38	20	20.69	34.48
250	13	24.13	41.37	20	31.03	75.86	10	24.14	31.03	10	13.79	24.14
100	0	17.24	31.03	3.33	20.68	55.17	3.33	13.79	17.24	3.33	13	13.79
50	0	10.34	24.13	0	13.79	37.93	0	3.33	3.35	0	3.4	3.45
10	0	0	13.79	0	6.89	27.58	0	0	0	0	0	0
1	0	0	3.44	0	3.44	10.34	0	0	0	0	0	0
LC_{50} µg/mL	902.6	783.07	419.18	678.14	533.67	175.46	>1000	987	804.79	>1000	>1000	969

The crude extracts, having LC_{50} values of less than 250µg/mL were considered as significant and seemed to be potential for further investigation [21]. According to our results JSPE, JSOH and MSPE had LC_{50} value below 250µg/mL and were potential for further investigation.

The outcome of this study also showed the effect at the time of exposure of the extracts to the brine shrimp. The LC_{50} value decreased with prolonged exposure to the extracts. The order of cytotoxic activity was JSPE> JSOH> MSPE>MMPE > MSOH> LQPE> MMOH > LQOH with LC_{50} value 121.22, 175.46, 196.72, 322.63, 419.18, 643.49, 804.79, and 969 µg/mL. After 24 h exposure, the LC_{50} values were 378.4, 533.67, 552.47, 666.98, 783.07, 831.96, 987 and 1000 µg/mL. While after 12 h exposure, the LC_{50} values were 537.5, 678.14, 780.92, 826.221, 902.6µg/mL, respectively and the remaining showed more than1000µg/mL. After

24 h exposure, the percentage mortality increased slightly more than that observed after 12 h and there was a significant increase in the mortality of the shrimp on further exposure. On the other hand, high concentration of the extract showed high lethality at 12 h especially for JSPE, JSOH and MSPE (Table 1) and the activity increased considerably up to 48 h exposure.

In the BSLB, maximum sensitivity was obtained after 48 h exposure. The nauplii show the greatest sensitivity to test compounds when they reach second and third instar in their lifecycle [19].

The LC_{50} values decrease with increase of exposure time, which was similar to the observations reported by Elmer-Rico [22].

Conclusion

The BSLB is reflected as a useful tool in the primary assessment of cytotoxicity and thereby for the isolation of bioactive compounds from plant extracts. According to our study JSPE, JSOH, and MSPE showed the LC_{50} value below 250µg/mL, capable of producing toxicity and have potential for further investigation. Since JSPE and JSOH have exhibited promising cytotoxicity, antitumour activity in *albino mice* is in progress.

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