Available online on 15.07.2018 at http://jddtonline.info

Journal of Drug Delivery and Therapeutics

Open Access to Pharmaceutical and Medical Research

Open Access Research Article

BRINE SHRIMP LETHALITY BIOASSAY OF *BOUGAINVILLEA GLABRA*

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ABSTRACT

The crude methanolic extract of *Bougainvillea glabra* leaves has been investigated for the evaluation of the cytotoxic activity. All the extracts of the plant were screened for their cytotoxicity by using brine shrimp nauplii (*Artemia salina*) lethality bioassay. The toxicity was assessed in terms of LC_{50} (lethality concentration), 10 nauplii were taken into three replicates of each concentration of the methanolic leaf extract. Brine shrimps were checked for the mortality during 24 hrs period, surviving brine shrimps were counted and LC_{50} was evaluated. The results showed that all the extracts were showing potent toxicity to the nauplii. The LC_{50} values were compared to the standard potassium dichromate. It indicates that the extracts are toxic even at low doses. Further investigation is needed to study the acute and subacute toxicity of the extracts for its safe application to the humans.

Keywords: *Artemia salina*, cytotoxicity, *Bougainvillea glabra*, mortality

Article Info: Received 07 May, 2018; Review Completed 27 June 2018; Accepted 01 July 2018; Available online 15 July 2018

Cite this article as:

Dokuparthi SK, Lakshmi G, Anjana A, Fatima SF, Ashwini P, Kandagatla S, Raj S, Brine shrimp lethality bioassay of *Bougainvillea glabra*, Journal of Drug Delivery and Therapeutics. 2018; 8(4):244-246

DOI: http://dx.doi.org/10.22270/jddt.v8i4.1780

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INTRODUCTION

The brine shrimp lethality bioassay is a simple and rapid method to evaluate the cytotoxic activity. It inexpensive and requires small amounts of test materials[1]. The bioassay has a good correlation with pesticidal activity and with cytotoxic activity in solid tumors. This *invivo* lethality assay can be successively applied as a primary screening that can be backed up by more sophisticated and specific evaluation bioassays[2].

Bougainvillea glabra is an ornamental shrub also called as a paper flower[3]. It is native to many tropical and subtropical areas like Middle East, North America, Brazil, India etc. The genus, *Bougainvillea* belongs to the family Nyctaginaceae[4]. Out of 18 species of the genus *Bougainvillea glabra* is one of the famous species. The leaves are generally having small hairs. The flowers are in very attractive combinations colors like white, red yellow, orange, violet and purple. The stems are thin, with repeated prickles[5]. Traditionally, the plant is used in a variety of ailments like ulcer, cough, diarrhea, hepatitis and as an expectorant. The leaves are having more extensive uses like anti diabetic[6], anti viral[7], anti inflammatory[5], anti microbial[8], antifertility properties[3].

Our, present study is to investigate the toxicity of the plant leaf by brine shrimp lethality bioassay. The methanolic leaf extract was prepared by Soxhlet method and this extract was used for the further studies.

MATERIALS AND METHODS

Collection of plant material

The fresh plant was collected during the month of September, 2017 from Hayathnagar rural area, Hyderabad. The plants were authenticated and specimens have been deposited in the college museum. The fresh leaves were shade dried at room temperature. The dried seeds then subjected to size reduction by the cutter type electric mill to get a coarse powder.

Extraction of plant materials

The coarse powder of the plant material was then subjected to hot exhaustive percolation method by using Soxhlet apparatus. The Soxhlet extraction was performed by the use of different solvents starting with

nonpolar to polar solvents (n-Hexane, Ethyl acetate, and methanol respectively). The extraction was progressed around 4-6 cycles per hour for 24 hrs. Then each of the extracts was filtered using cotton plugs followed by Whatmann No. 1 filter paper. The filtrates were then concentrated and dried under reduced pressure in the rotary evaporator. The percentage yield was found to be 10.5%. The extracts were stored in airtight container in the refrigerator for future analysis.

Preliminary phytochemical screening

Preliminary phytochemical screening of the plant extracts was done by the standard procedures[9, 10]. The results are illustrated in Table 1.

Hatching of brine shrimp

The eggs of brine shrimp were procured from the local market and hatched in a glass compartment with sea water. The glass compartment is having two partitions, one is illuminated. After 24 hrs of hatching at room temperature, the eggs hatch into larvae (nauplii), which will swim towards the illumination chamber by leaving their shells in the dark chamber. The nauplii were collected carefully by pipette for the bioassay.

Preparation of test samples

32 mg of each of the test samples were taken and dissolved in 200 µl of pure dimethyl sulphoxide (DMSO). The final volume was made to 20ml with sea water. The concentration of the stock solution is 1600 µg/ml. Eight samples of volume 5ml having different concentration 1600, 1000, 800, 500,250,100, 50, 25 µg/ml were prepared by diluting the stock solution with sea water.

Control group

This group is used to validate the test method and ensure that the cytotoxicity is only due to the activity of the test compounds. Two types of control groups were used in this experiment- Potassium dichromate (200µg/ml) as the positive control and 50 µg/ml of DMSO in 4.95ml of sea water as the negative control.

Brine shrimp assay

To the pre-marked vials containing above-prepared concentrations, 10 nauplii were added. After 24hrs, the nauplii in each vial were counted with the aid of a 3x magnifying glass for the surviving brine shrimp. The mortality endpoint of this bioassay is the absence of controlled forward motion during the 30s of observation. These results were depicted in Table 2.

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening

The preliminary phytochemical screening of the methanol leaf extract of *Bougainvillea glabra* revealed the presence of various secondary metabolites which are illustrated in Table 1.

Table 1: Preliminary phytochemical screening of various extracts of *Bougainvillea glabra* leaf

Phytochemicals	n-Hexane extract	Ethylacetate extract	Methanol extract
Alkaloids	+	-	+
Glycosides	+	+	+
Terpenoids	+	+	+
Steroids	+	+	+
Saponins	+	+	+
Flavonoids	+	+	+
Tannins	-	+	+
Carbohydrates	-	-	-
Lipids	-	-	-
Proteins	-	-	-

Brine shrimp lethality assay:

Brine shrimp nauplii are simple zoological organisms which can be used to evaluate the lethality[11]. It is a simple and very useful tool to screen diverse compounds. This bioassay is also used to screen plant extracts. It is a safe, economical method for determination of the cytotoxicity of synthetic and plant products[12]. The bioassay can be correlated significantly to the inhibition of human solid tumor cell lines. It is the primary tool to detect the anti-tumor properties[13]. The toxicity of plant extracts can be evaluated by their LC_{50} values. If LC_{50} values are lower than 1000 µg/ml is considered as cytotoxic[11]. This bioassay can also be used for the evaluation of antifungal, teratogenic effect, pesticidal effect and environmental toxicity[14]. The brine shrimp bioassay results illustrated in Table 2. In this study, it was observed that n-Hexane, Ethyl acetate, and methanolic extracts were toxic to the brine shrimps nauplii. They exhibit potent toxicity, compared to the standard potassium dichromate. For n-Hexane, Ethyl acetate, and methanolic extracts respectively. The relation of toxicity of the extract to the nauplii was found to be directly proportional from low to high (25 µg/ml to 1600 µg/ml). Concentrations of the extracts ranging from lowest concentration (25 µg/ml) to highest concentration (1600 µg/ml). The presences of components like alkaloids, steroids, saponins, and tannins which are already reported to have the cytotoxic property are responsible for the reported activity in a dose dependant manner.

Table 2: Mean percentage of mortality of *Artemia salina* brine shrimp after 24 hrs at different concentrations of the *Bougainvillea glabra* leaf extracts.

Extract	Mean % of mortality at concentration (µg/ml)								
	Negative control	25	50	100	250	500	1000	1600	Positive control
n-Hexane	**0**	**21**	**40**	**60**	**70**	**85**	**99**	**100**	**100**
Ethyl acetate	**0**	**25**	**45**	**62**	**74**	**89**	**97**	**100**	**100**
Methanol	**0**	**28**	**47**	**68**	**77**	**81**	**100**	**100**	**100**

CONCLUSION

From the above study, it is observed that the methanolic leaf extract of *Bougainvillea glabra* exhibited cytotoxicity against the brine shrimp nauplii. The LC_{50} values are found to be less than 1000mg/ml. It may be due to the presence of bioactive components. This bioassay can only give information that it is having very potent biologically active components. But, this assay is inadequate to understand the mechanism of action of the phytochemicals present in it. Further investigations are needed in isolation, structure elucidations of the phytochemicals present in the leaf of *Bougainvillea glabra* and toxicity towards the cell lines will help us to understand its molecular mechanism of their bioactivity.

ACKNOWLEDGMENT

Authors are thankful to the management of Vijaya College of Pharmacy, for providing necessary facilities.

CONFLICT OF INTEREST

Authors do not have any conflict of interest

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