World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 5.088

BRINE SHRIMP LETHALITY BIOASSAY OF GARCINIA BRASILIENSIS LEAVES EXTRACTS

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Article Received on 10/07/2018 **Article Revised on 01/08/2018** **Article Accepted on 22/08/2018**

ABSTRACT

The total ethanolic extract of leaves of Garcinia brasiliensis was fractioned using solvents in increasing order of polarity. All the extracts of the plant and the isolated fraction obtained from ethyl acetate fraction were screened for their cytotoxicity by using brine shrimp nauplii (*Artemia nauplii*) lethality bioassay. The toxicity was assessed in terms of LC_{50} (lethality concentration). 10 nauplii were taken in each solution of varying concentration for the leaf extracts. Brine shrimps were checked for the mortality after 24 hrs period, surviving brine shrimps were counted and LC_{50} was determined using probit analysis method, as the measure of toxicity of the plant extract. The ethyl acetate fraction GE has the least toxicity to the brine shrimp with the high LC_{50} value of 223.87mcg/ml compared to the other fractions. The isolated fraction GEA from the ethylaceate fraction shows an LC_{50} value of 275.42mcg/ml, making it less cytotoxic than the ethyl acetate fraction GE. The results show that both ethyl acetate fraction and the isolated fraction GEA are safer. Further investigation is needed to study the acute and subacute toxicity of the extracts for its safe application to the humans.

KEYWORDS: Artemia nauplii, cytotoxicity, Garcinia brasiliensis, lethality, probit analysis.

INTRODUCTION

Bioactive compounds are often toxic. In order to study the toxicity of medicinal plants, the brine shrimp lethality bioassay is performed which is based on the ability to kill laboratory cultured brine shrimp *(Artemia nauplii)*. Cytotoxic activity of the plant extracts was determined by brine shrimp lethality bioassay. Bioactive compounds are often toxic to brine shrimp. Hence, lethality to brine shrimp can be used as a rapid and simple preliminary monitor for bioactive compounds during the isolation of natural products. The assay is considered as a useful tool for preliminary assessment of toxicity and it has been used for the detection of fungal toxins, plant extract toxicity, heavy metals, cyanobacterial toxins, pesticides and cytotoxicity testing of dental materials.[1]

Garcinia brasiliensis Mart. is a plant native to the Amazon region and is cultivated throughout Brazil. In Brazil, it is popularly known as 'bacuri' or 'bacupari'.[2] The great biodiversity of plant found in Brazil might serve as an important source of new pharmacological agents.[3] The leaves are short – petioled, ovate, narrowed at the base, blunt or slightly pointed at the apex and leathery.

It is used by the population for anti-inflammatory, anti-nociceptive, antioxidant and antitumor therapies. In Thailand, Srilanka, Malaysia, Philippines and India, ripe fruits are used in traditional medicine to treat abdominal pain, diarrhoea and dysentery. Leaves are in folkloric use to treat tumours, urinary tract infection and arthiritis.[4,5]

Several members of the Guttiferae family are used in Brazilian traditional medicines to cure various ailments. They contain wide variety of biologically active metabolites such as anthraquinones, flavonoids, xanthones, benzophenones and phloroglucinols,[3] which have proven activity against diseases, such as peptic ulcer, urinary tract infections and tumors.[6]

Our present study was to investigate the toxicity of the various extracts of plant leaf by brine shrimp lethality bioassay. The total ethanolic leaf extract was prepared by Soxhlet method which was fractioned using polarity gradient solvents and these extracts were used for the further studies.

MATERIALS AND METHODS

Collection of plant material

The fresh plant was collected from the SD convent Chunanganvely, Aluva, Ernakulam in the month of February 2012. Leaves were identified, authenticated and a voucher specimen (SES.M.G.UTY NO.1449) is

preserved at the Herbarium of School of Environmental Sciences, M.G University, Kottayam.

Extraction of *Garcinia brasiliensis* leaves

The collected leaves were dried under shade for two weeks. Shade dried leaves were coarsely powdered. The powdered material was stored in an air tight container and kept for the extraction process.

Extraction of the leaves of *Garcinia brasiliensis* were carried out using ethanol by hot extraction using the reflux condensation.[7] The total ethanolic extract (TE) was then fractionated using solvents in the increasing order of polarity to obtain the n-Hexane fraction (GH), Chloroform fraction (GC) and Ethyl acetate fraction (GE). The residue obtained after partitioning with ethylacetate was the Alcoholic fraction (GAL). Isocratic elution of the ethylactetate fraction was carried out using solvent system n-butanol: ammonium hydroxide which resulted in a GEA fraction. Isocratic elution of the ethylactetate fraction was carried out using solvent system n-butanol: ammonium hydroxide Isocratic elution of the ethylactetate fraction was carried out using solvent system n-butanol: ammonium hydroxide.

Preliminary phytochemical screening

Preliminary phytochemical screening of the plant extracts was done by the standard procedures[8,9] and antibacterial activity studies were conducted for various extracts.

Preparation of artificial sea water

Artificial sea water was prepared by dissolving 38.0 g commercial sea salt per liter of water (38g/L) and filtered. The pH was adjusted to 8.5 using 1N sodium hydroxide.

Hatching Of Brine Shrimp Eggs

Brine shrimp eggs were hatched in sterile artificial sea water 38 g/L under constant aeration for 48hrs .The eggs were sprinkled into the apparatus and were suitably illuminated and incubated at room temperature (30-37°C) for 48 hrs. Newly hatched free-swimming pink-coloured nauplii (larvae) were collected from the brighter side of the apparatus using a Pasteur pipette. The freshly hatched free-swimming nauplii were used for the bioassay.

Brine Shrimp Lethality Bioassay[10]

20 mg of various extracts of *Garcinia brasiliensis* leaves were dissolved in 1ml of DMSO and the volume was adjusted up to 20ml with artificial sea water. Then the solutions of varying concentration (10, 50, 100, 250 and 500µg/ml) were prepared by serial dilution. Ten nauplii were drawn using Pasteur pipette and placed in test tube containing 4.0 ml of brine solution. 1.0 ml of the plant extract was added to the test tube to adjust the final volume to 5.0 ml. The test tubes were kept at room temperature for 24 hours under the light. Test was performed in triplicate. The numbers of dead (non motile) nauplii in each test tubes were counted after 24hours. The probit values for each fraction were found using the probit table and were tabulated. The lethal concentration (LC_{50}) was determined using probit analysis method, as the measure of toxicity of the plant extract.

Calculation

$$\text{Percentage mortality} = \frac{\text{No: of dead brine shrimp X 100}}{\text{No: of brine shrimp taken}}$$

Corrected % Formula for 0 and 100% mortality
For 0% dead: 100(0.25/n)
For 100% dead: 100(n-0.25/n)
Where n = number of brine shrimp in each test tube.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

The preliminary phytochemical screening of ethanolic leaf extract of *Gracinia brasiliensis* revealed the presence of various secondary metabolites which are illustrated in Table 1.

Table 1: Results of the Chemical Test of Various Fractions.

Sl. No.	Phytoconstituents	GH	GC	GE	GAL
1.	**Phenolics**				
a)	Ferric chloride test	-	-	+++	++
b)	Lead acetate test	+	+	+++	+++
c)	Potassium permanganate test	+	+	+++	+++
2.	**Flavonoids**				
a)	Shinoda test	+	+	++	++
b)	Aqueous NaOH test	+	+	+	+
3.	**Terpenoids**	++	++	+	+
4.	**Steroids**				
a)	Salkowski's test	++	++	+	+
5.	**Carbohyrates**				
a)	Molisch's test	-	-	+	++
b)	Fehling's test	-	-	+	+
c)	Benedict's test	-	-	+	+

6.	**Glycosides**				
a)	Borntrager's test	-	-	-	-
b)	Legal's test	-	-	+	+
7.	**Proteins and amino acid**				
a)	Biuret test	-	+	-	-
b)	Millon's test	-	+	++	++
c)	Xanthoproteic test	-	-	-	-
d)	Ninhydrin test	-	-	-	-
8.	**Alkaloids**	-	-	-	-

Table 2: No: of Non Motile Nauplii After 24 HRS.

Extracts	no: of brine shrimp		No: of brine shrimp dead out of 10 after 24hrs				
		Control	10 µg/ml	50 µg/ml	100 µg/ml	250 µg/ml	500 µg/ml
GH	10	0	1	2.3	5.6	8.0	9.6
GC	10	0	1	3.6	6.3	8.3	9.6
GE	10	0	0	0.66	2.3	4.3	8.3
GAL	10	0	0	1.66	3.6	6.0	8.6
GEA	10	0	0	0.33	2.0	3.3	8.0

Table 3: Probit Values of the Various Fractions.

Conc (µg/ml)	Log conc.	GH		GC		GE		GAL		GEA	
		% mortality (corrected)	Probit value	% mortality (corrected)	Probit value	% mortality (corrected)	Probit value	% mortality (corrected)	Probit value	% mortality (corrected)	Probit value
10	1	10	3.72	10	3.72	0.83	2.22	0.83	2.22	0.83	2.22
50	1.69	23	4.26	36	4.64	6.6	3.49	6.6	4.03	3.3	3.12
100	2	56	5.15	63	5.33	23	4.26	36	4.64	20	4.16
250	2.39	80	5.89	83	5.95	43	4.82	60	5.25	33	4.56
500	2.69	96	6.75	96	6.75	83	5.95	86	6.08	80	5.84

Table 4: LC_{50} Values for the Various Fractions.

Fractions	LC_{50} (µg/ml)
GH	74.98
GC	62.67
GE	223.87
GAL	160.65
GEA	275.42

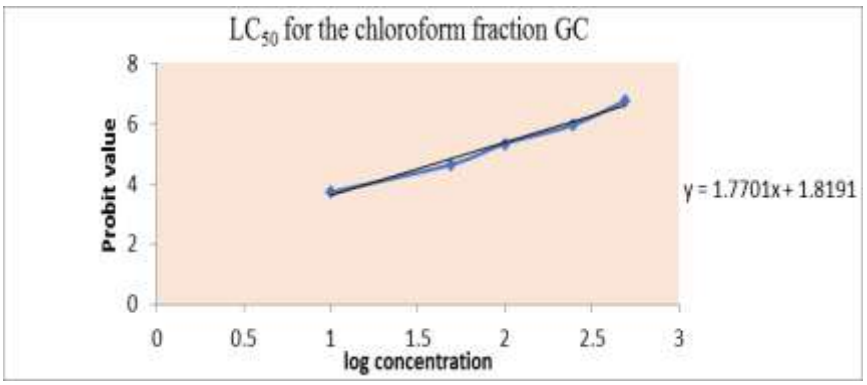

Fig. 1a: Calculation of the LC_{50} of the n-hexane fraction GH.

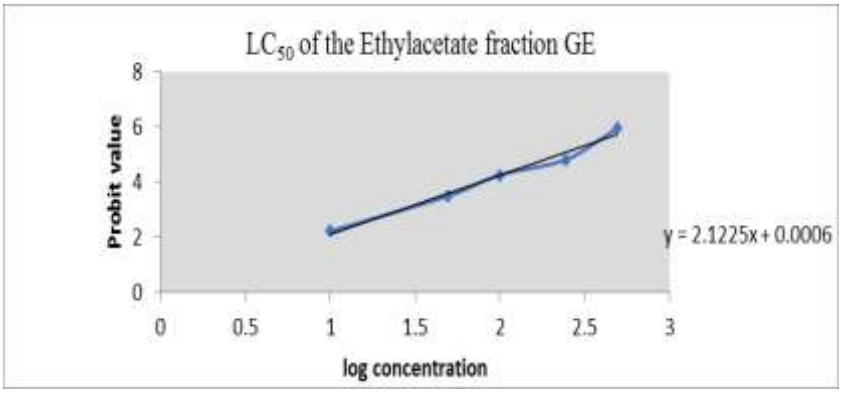

Fig. 1b: Calculation of the LC_{50} of the chloroform fraction GC.

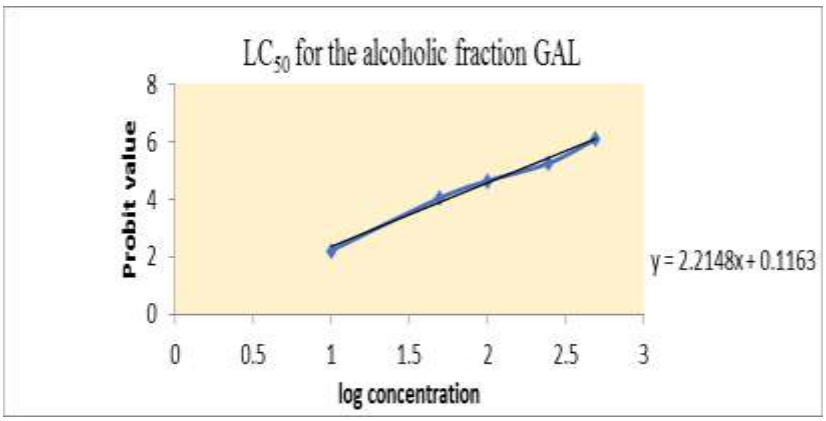

Fig. 1c: Calculation of the LC_{50} of the ethylacetate fraction GE.

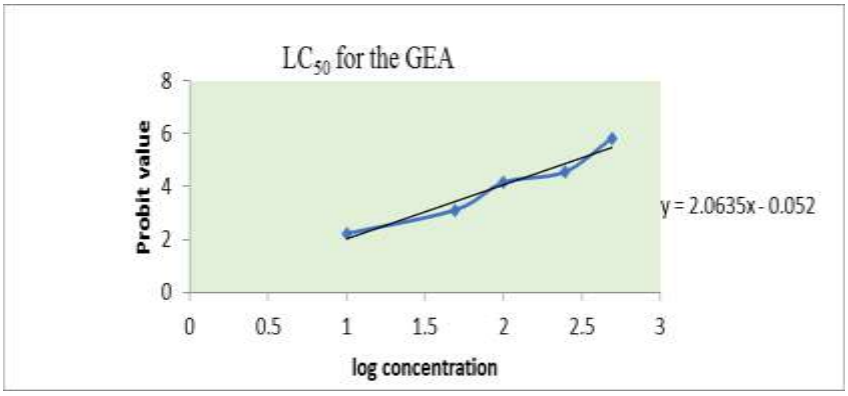

Fig.1d: Calculation of the LC_{50} of the alcoholic fraction GAL

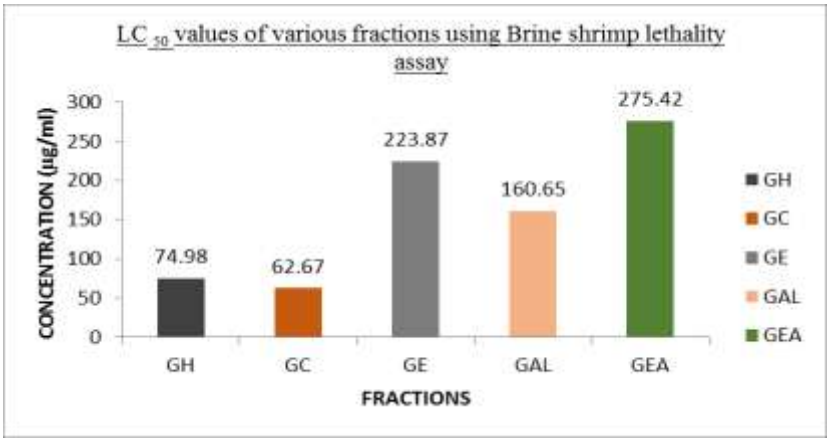

Fig. 1e: Calculation of the LC_{50} of the GEA.

Brine Shrimp Bioassay

The numbers of dead (non motile) nauplii in each test tubes were counted after 24hours and was tabulated in table 2. The probit values for each fractions were found using the probit table and are tabulated in table 3. The lethal concentration (LC_{50}) of the various fractions were determined by plotting a graph between the log concentration and the probit value as given in the fig. 1(a-e), which is the measure of toxicity of the plant extract.

LC_{50} values for the various fractions and the isolated GEA were calculated from the graphs above and the results are tabulated as in table 4 depicted in fig 2.

The ethyl acetate fraction GE has the least toxicity to the brine shrimp with the high LC_{50} value of 223.87µg/ml compared to the other fractions. Hence the ethyl acetate fraction is less cytotoxic in nature. However the isolated fraction GEA from the ethylaceate fraction shows an LC_{50} value of 275.42µg/ml, making it less cytotoxic than the ethyl acetate fraction GE. The results show that both ethyl acetate fraction and the isolated fraction GEA are safer when compared to others.

From the brine shrimp lethality assay it can be well said that the ethylacetate fraction and alcoholic fraction were less toxic to brine shrimp larvae (LC_{50} above 100µg/ml) whereas the other two fractions, i.e. n-hexane fraction and the chloroform fraction showed significant cytotoxicity (LC_{50} below 100µg/ml). The possible reason for the cytotoxicity of the n-hexane fraction and the chloroform fraction against brine shrimp may be due to the presence of cytotoxic compounds in these fractions. Cytotoxicity assay showed that the lethal concentration LC_{50} value for the ethylacetate fraction and the isolated mixture compound was 223.87µg/ml and 275.42µg/ml. The antibacterial activity concentration and antioxidant activity concentration [11] shown by them was far too less than the lethal concentration, thus both of them are safe and free from toxicity. Alcoholic fraction is found to have moderate cytotoxicity with an LC_{50}= 160.65µg/ml. The LC_{50} value for n-hexane fraction and chloroform fraction being less, 74.98µg/ml and 62.67µg/ml respectively, they show considerable cytotoxicity at their antibacterial activity concentration. Study of the cytotoxic potential of the chloroform and n-hexane fractions can be further investigated.

ACKNOWLEDGEMENTS

The author thanks local vendors Kottayam for Brine shrimp eggs for this work. The author also thanks Prof. Krishnakumar K, Department of Pharmaceutical Chemistry, M.G University to carry out this research work.

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