

CYTOTOXICITY STUDY OF BASELLA ALBA LEAVES EXTRACT ON BRINE SHRIMP LETHALITY BIOASSAY AND MTT ASSAY

A. K. Azad¹; M. A. Islam^{2*}; N. K. Sunzida³

¹Department of Pharmacy, Faculty of Pharmacy, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia.

²Department of CTS, Faculty of Science, International Islamic University Malaysia, 25200 Kuantan, Pahang, Malaysia.

³Gonoshasthaya Samaj Vittik Medical College, Mirzanagar, Savar, Dhaka - Aricha Hwy, Dhaka 1344, Bangladesh.

Email address: aminulislam@iium.edu.my

Abstract

Introduction: The main objective of the present study is to investigate the cytotoxicity of crude extracts of *Basella alba* L. leaves. It has been reported to show various bioactivities, but the toxicity and cytotoxicity effects have not been conducted yet. **Materials and Methods:** The phytochemical screening of the plants extract has been carried qualitatively. The cytotoxic effect was determined through in vitro MTT assay using MCF-7 cell line, and brine shrimp lethality bioassay. **Results:** Brine shrimp lethality bioassay showed the percentage of survival rate of nauplii which were 72, 88, 88 and 96 % at the concentration of 200, 100, 50 and 25 $\mu\text{L}/\text{mL}$ respectively. The mortality of brine shrimp nauplii was from 44% at the concentration of 800 $\mu\text{L}/\text{mL}$, while the negative control (Aluminium chloride) showed 80% mortality at the concentration of 20 $\mu\text{L}/\text{mL}$. However, the IC_{50} was found with the concentration of 617.31 $\mu\text{L}/\text{mL}$. In MTT assay, more than 90% cell viability showed from the concentration of 31.25 $\mu\text{L}/\text{mL}$ to 250 $\mu\text{L}/\text{mL}$, while the vehicle control (DMSO; 1% v/v) was 63.71%. Moreover, the cytotoxicity assay on MCF-7 cell line also concluded that the extract of *Basella alba* L. leaves is nontoxic even with very high concentration as well. **Conclusion:** Since it is a popular vegetable which is consumed by the people of Asia every day, therefore, it was very important to study the toxicity of this plant. It has been clearly proven by this study that this vegetable has no toxic effect.

Keywords: *Borreria articularis* (Linn.), cytotoxicity; MCF-7 cell line; brine shrimp nauplii.

Introduction

Kingdom : Plantae

Phylum : Magnoliophyta

Class : Magnoliopsida

Order : Caryophyllales

Family : Basellaceae

Genus : Basella

Species : alba

(Fig. 1) In India, due to its geographical and environmental positioning has traditionally been a good source for such products among the Asian countries. In Ayurveda, it is used for hemorrhages, skin diseases, sexual weakness, ulcers and as laxative in children. and pregnant women. The plant is febrifuge, its juice is a safe aperient for pregnant women and a decoction has been used to alleviate labour. It is also an astringent and the cooked roots are used in the treatment of diarrhea. The leaf juice is a demulcent, used in cases of dysentery. In India, it has been used for antipruritis and burn and has been used in Bangladesh for acne and freckle treatment (3). The Ayurvedic treatment in India has been used B. alba leaves and stem for anticancer such as melanoma, leukemia and oral cancer (4) Root and leaves has been used for the removal of after birth, stomach pains and increase milk production (5). Basella alba is administered orally for the treatment of anal prolapsed or hernia. Ground leaves of Basella alba are rubbed on the human hand to introduce the whole preparation into the animal vagina every morning for the treatment of sterility (6) The leaf juice is used in Nepal to treat dysentery, catarrh and applied externally to treat boils. The mucilaginous qualities of the plant make it an excellent thickening agent in soups, stews, etc. The purplish sap from fruits is used as a colouring agent in pasteries and sweets (7) Basella alba has been used for the treatment of Anemia in women, coughs, cold (leaf with stem), cold related infections (8) Maceration is taken orally for infertility, pelvic inflammatory disease, orchitis, epididymitis, threatened abortion, spurious labour (9). Leaves are used in constipation, poultice for sores, urticaria and gonorrhoea. It is also used in poultice local swellings, intestinal complaints etc (10). The mucilaginous liquid obtained from the

leaves and tender stalks of plants is popular remedy for headaches (11). In the present investigation an attempt was made to screen the cytotoxicity activity of Basella alba leaf in invitro MTT assay and brine shrimp lethality bioassay model.

Methods

Collection and Identification of plant material

The new Borreria articularis (Linn.) plants were gathered from Indera Mahkota-8, 25200 Kuantan, Pahang, Malaysia. The collection period was from February to March 2017. The plant material was deposited to the herbarium of Pharmacy Faculty at International Islamic University Malaysia.

Preparation of ethanol extract

The new Borreria articularis (Linn.) plants were gathered for the minuscule examination. For the extraction procedure, developed Borreria articularis (Linn.) are recognized, gathered and shade evaporated to 7-12 days; care taken to maintain a strategic distance from direct daylight contact. At that point, the plants were smashed and pounded into fine powder by a blender. The cold maceration was performed using 70% ethanol for completed twelve hours by ordinary shaking. A rotary vacuum evaporator (BUCHI R-205) was employed to separate or evaporate solvent from the extract (at 45 °C, 175mbar & 80-85rpm). Then the concentrated extract was placed into freezer at -70 °C. After 3 days the extract was shifted instantly for consecutive freeze drying by bench top freeze dryer at -50 °C (12).

Percentage of yield determination

The final extract was put in the refrigerator maintained at 4 °C temperature and aliquot for further experimental use. The yield of final amount of extract was dictated with the last concentrate load over the dried plant powder.

% of yield = (Extract weight)/(Powdered leaves weight) X100

Determination of cytotoxicity through Brine shrimp lethality bioassay

It is very fast, economical, environment friendly and extensive bioassay to decide cytotoxicity or toxicity of plant extract or chemically synthesis molecules of starting point. 12 Common traditional

herbs extract, isolated or fractioned bioactive lead molecules or extract can be tried for their bioactivity and LD50/ED50. It uses in vivo lethality in a straightforward natural life form (salt water nauplii) as a helpful technique for deciding the similarity of new biologically active molecules (12).

Preparation of seawater

For the preparation of seawater, 38g of salt was completely dissolved in one liter distilled water. The solution was mixed gently and 1 mol/L NaOH was added slowly to adjust the pH to pH-8.5. Then the mixture was filtered using cotton to get a clear solution (12).

Brine shrimp hatching for the experiment

Brine shrimp (*Artemia salina*) eggs were incubated in counterfeit ocean water arranged by normal commercially available ocean salt. One thousand mL hatching media (ocean water) was taken into a 2000 mL measuring beaker, then the eggs were placed into hatching media. Incubate for 48hrs to develop from eggs to nauplii. After 48hrs, freshly prepared media was taken into 24-well plate. Then, the targeted numbers (20) of nauplii were transferred from hatching chamber to 24-well plate using a micropipette (12).

Test sample preparation

Since the sample was not water soluble, 200 µl/ml of pure dimethyl sulfoxide (0.1% DMSO) was used to dissolve extract to make it soluble. Then, the extract solution was applied with different concentration. A two-fold serial dilution was employed with freshly prepared media to gain a test sample at the range of 800, 400, 200, 100, 50 and 25 µg/mL. The sample solution (2.5 mL) was taken into freshly prepared media (2.5 mL) and added 20 nauplii in each well. Through the colony counter, the number of experimental nauplii was precisely confirmed (12).

Positive control group preparation

In this study, 20% aluminium chloride was used with 100 µg/ml as a positive control (12).

Procedure of nauplii Counting

Every 4hrs interval, the experimental well plate placed under the light microscope to observe and count the nauplii. It was counted to determine the

surviving number of nauplii, % of mortality rate and LC50 (12).

Procedure of MTT-assay

The MCF-7 cells were cultured and maintained in a 75 t-flask with the standard regular medium (Dulbecco's modified Eagle's) where 100 µg/mL of streptomycin, 100 IU/mL of penicillin and 10% of fetal bovine serum were added. The cultured cells in 75 t-flask were placed into incubator at 37°C with 5% CO₂, 95% air and complete humidity. The cells were separated using trypsin/ethylene diamine tetraacetic acid (0.05%). Then trypan blue was applied into the flask and the cells were counted using hemocytometer. The cultured flask was monitored on regular basis. When cultured cells reached ~90% confluence, then the concentration of cells/cm² was calculated and taken once it reached 4 × 10⁴ cells/cm². The cells were added into a 96- well plate (i.e., 250 µL/well) by using micropipette (14).

Statistical data analysis

As for the statistical data analysis, the Microsoft Excel (2010) spreadsheet was used to plot the percentage rate of mortality of the brine shrimp against the log concentrations, MS spread sheet was used to generate Y and R² values through the regression equation. Finally, the regression value was employed to calculate LC50 of the experimental concentrations.

Results

Percentage of yield determination of crude extract

According to extraction, the yield in percentage (%) obtained from 1kg dried powder of *Borreria articularis* (Linn.) using 75% of ethanol and 30% of water to extract polar and nonpolar compounds to ensure the best biological activity. It was almost 300g of concentrate extract which was 29.52 (w/w) in percentage as shown in table-1 bellow.

Brine Shrimp Lethality Bioassay

It is a traditional and environmentally friendly technique to determine cytotoxicity or toxicity level of the targeted extract or any chemical compound. The tested sample was dissolved with DMSO (0.1%) as it was not soluble into water. DMSO (0.1%) and aluminum chloride were selected for the vehicle control and positive control. 10 In the current study,

different concentrations of extract solution were employed to determine the safety level of tested sample. The rate of mortality was noticed with different sample concentrations as shown in the figure 2.

The LC₅₀ was calculated through the linear regression equation value. The LC₅₀ showed with the concentration of 402.48 µg/ml.

The percentage of mortality was calculated following the standard equation as bellow.

$$\% \text{ of mortality} = ((N_0 - N_1) / N_0) \times 100$$

Where,

N₀ = No. of nauplii taken

N₁ = No. of nauplii alive

The most noticeable survival rate ranged from 72 to 96 % at the concentration between 100 µg/ml to 25 µg/ml showed in table 2. In addition, there is no less than 50 % survival rate was observed at the tested concentrations. However, the lethal dose concentration (LC₅₀) of the tested sample was 617.31 µg/ml.

The MTT-assay of MCF-7 Cell line.

The study was performed by partial modification of previously described method. The result of MTT assay showed the maximum cell viability 98.03% at 3.905 µL/mL while the lowest was 82.03% at 500 µL/mL. However, the concentrations of 7.8 and 15.62 µL/mL exhibited 96.27% and 94.89% cell viability respectively. In addition, more than 90% cell viability showed from the concentration of 31.25 µL/mL to 250 µL/mL. While the vehicle control (DMSO; 1% v/v) was 63.71%. MTT assay exhibited no lethal toxicity with the concentrations range from 3.905 µL/mL to 500µL/mL when compared to both control groups as shown in figure 3.

Conclusion

A popular vegetable, which is kept in the food menu every day in Asian cuisine, because it is on the daily list of their food menu, it is very important to note that the main and important part of current research work was to find out the fundamental harmful aspects of this vegetable. And the researcher has done his research with discretion, and

it has come to concluded that the vegetables have virtually no side effects.

Disclosures Statement

No potential conflict of interest is available to declare by the authors.

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Table 1. showing the percentage (%) of yield determination of *Borreria articularis* (Linn.) extract.

Plant Sample (powder)	Ext. Process	Solvent	Rotary evaporator settings			Crude extract (g)	Yield (%)
			T(°C)	R (rpm)	P (mbar)		
1kg	Cold Maceration	Ethanol (1:3)	60 °C	85 rpm	500-175	300	29.52

Table 2. Showing the employed concentration, log concentration, % of mortality, % of survival and lethal concentration (LC50) of *Basella alba* leaves extract.

Concentration of Treated Sample (µg/ml)	LogC	No. of nauplii taken	No. of nauplii died	No. of nauplii alive	% of mortality	% of survival	LC50 (µg/ml)
800	2.90309	25	11	14	44	56	
400	2.60206	25	8	17	32	68	
200	2.30103	25	7	18	28	72	
100	2	25	3	22	12	88	617.31
50	1.69897	25	3	22	12	88	
25	1.39794	25	1	24	4	96	
Vehicle control							
		25	15	10	60	40	
20% Aluminium chloride (AlCl ₃)		25	20	5	80	20	
Norman control		25	0	25	0	100	

Figure: showing the *Basella alba* L. bark and leaves

Figure 2. The % of mortality of brine shrimp nauplii (*Artemia Salina*) at 24 hr, after being exposed to various concentrations of *Basella alba* leaves extract.

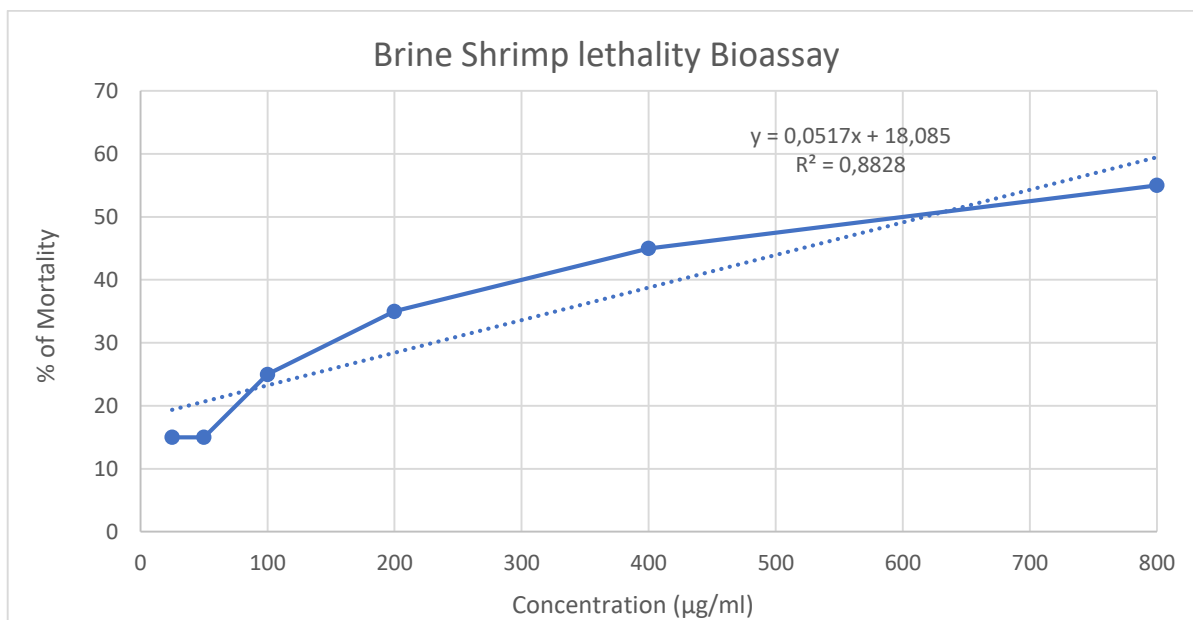


Figure 3. Percentage of cell viabilities at different concentration of *Borreria articularis* (Linn.) extract using MTT-assay.

