

## Phytochemical and Toxicity Evaluation of Traditional Herb: *Lagerstroemia speciosa* L. (Banaba) by MCF-7 Cell Line and Brine Shrimp Lethality Bioassay

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**Abstract:** The main objective of this study was to determine the cytotoxicity of crude extract of the leaves of *Lagerstroemia speciosa* (Banaba). *In vitro* MCF-7 cell line and *in vivo* brine shrimp lethality bioassay methods were followed to investigate the cytotoxicity of the selected sample. In this study, the qualitative phytochemical assessment demonstrated the presence of alkaloids, carbohydrate, glycosides, saponins, terpenes, steroids, phenols and flavonoids in Banaba extract. Moreover, the present findings revealed the non-cytotoxic nature of *L. speciosa* leaves extract on both MTT-assay and brine shrimp lethality bioassay.

**Key words:** Cytotoxicity, *Lagerstroemia speciosa*, MCF-7, Brine Shrimp Lethality, MTT.

### Introduction

Banaba (*Lagerstroemia speciosa* L., crepe myrtle) has been utilized to treat diabetes in different parts of the world, fundamentally Southeast Asia. Banaba, distinguished logically as *Lagerstroemia*, is a blooming tree that is local to the Malaysia, Philippines and India. The plant can be developed in any atmosphere (Azad *et al.*, 2015). It has been used as a folk medicine since ancient era among in the Philippines for the treatment of diabetes (Stohs *et al.*, 2012). The first published research study reported in 1940 indicated that the pure corosolic acid has been found to decrease blood sugar levels within 60 min in human subjects. Corosolic acid, isolated from Banaba, also exhibited anti-hyperlipidemic (Garcia *et al.*, 1940), anti-diarrhoeal, analgesic (Hossain Fahad, 2015), hepatoprotective (Lad *et al.*, 2011),

nephrotoxicity protective (Basha *et al.*, 2013), antioxidant (Saumya *et al.*, 2011), antidiabetic (Suzuki *et al.*, 2001) and alpha-amylase inhibitory activities (Hosoyama, 2003). Corosolic acid (CA), contained in the leaves of *L. speciosa*, is a pentacyclic triterpene, and has hypoglycemic effects. It has some direct effects on the cholesterol absorption process in the small intestine. It may inhibit the activity of cholesterol acyltransferase, which acts in the re-esterification of cholesterol in the small intestine, in type-2 diabetes (Stohs *et al.*, 2015). Based on previous reports and traditional claim, it has many potential therapeutic effects but unfortunately, no toxicity study was performed. Therefore, it was necessary to investigate the toxicity of this commonly consumed extract in Malaysia and rest of the countries. The present study investigated and found

its non-toxic activity in MCF-7 cell line and brine shrimp lethality bioassay methods.

## Materials and Methods

### *Collection and identification of plant material:*

The fresh and mature leaves of *L. speciosa* (15-20 cm) were collected from IIUM Kuantan Campus areas, Indera Mahkota, Kuantan-25200, Malaysia in the month of February-March, 2012. It was authenticated at the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, IIUM with a voucher specimen no. PIIUM: 0423.

*Preparation of ethanol extract:* For the extraction process, matured leaves of *L. speciosa* were identified, collected and shade dried up to 7-12 days taking care to avoid direct sunlight contact. Then, the leaves were crushed into fine powder by blender. The sieving was done repeatedly to remove the coarse parts. Defatting was done by immersing the leaf powder into petroleum ether for more than 12 hours with regular shaking and stirring. The defatted marc was used for extraction with ethanol. The ethanol extract was concentrated in vacuo (temperature at 45°C, 175 mbar and rotation 80-85 rpm) using a rotary vacuum evaporator (BUCHI R-205) to a final volume of 500 ml. This was further frozen at -70°C and shifted instantly to three weeks freeze drying at -50°C using bench top freeze dryer (ALPHA 1-4LD-2) to give a dry powder (Azad et al., 2015).

*Percentage of yield determination:* The extract was kept in the fridge (4°C) from which aliquots were withdrawn for the test. The yield of extract was determined by the final extract weight over the dried plant powder.

*Qualitative phytochemical screening of crude extract of L. speciosa:* The extract was evaluated by standard phytochemical screening of different constituents to detect the presence or absence of secondary metabolites, such as alkaloids, carbohydrates, saponins, amino acids, phytosterols, phenols and flavonoids (Azad et al., 2016; Trease and Evans, 2008).

*Brine shrimp lethality bioassay:* Thirty-eight gram sea salt was weighed, dissolved in 1 L of distilled water, adjusted to pH-8.5 using 1 mol/l NaOH and filtered off with cotton plug to get a clear solution (Asaduzzaman et al., 2015). Twenty milligrams of the test sample was dissolved in 200 µL pure dimethyl sulfoxide (DMSO) to give a crude extract concentration of 20 mg/ml and two fold serial dilution (250 - 1.95 µg/ml) was carried out with artificial sea water and 2.5 ml of the sample solution was added. After 24 hrs, the test tubes were inspected using a magnifying glass and the number of surviving nauplii was counted in each tube. Two types of control groups were used in the present study, distilled water as normal control and 0.1% DMSO as positive control.

*MTT-assay procedure:* MCF-7 cells were cultured in 25 t-flasks and were maintained in Dulbecco's modified Eagle's medium supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin, 10% fetal bovine serum at 37°C with 5% CO<sub>2</sub>, 95% air and complete humidity. They were detached using 0.05% trypsin/ethylene diamine tetraacetic acid and counted by means of trypan blue and hemocytometer when reached ~90% confluence and then suspended again at a concentration of 4 × 10<sup>4</sup> cells/cm<sup>2</sup> to add into a 96- well plate (i.e., 250 µl/well) via a channel pipette. Some wells were kept cell-free as blanks (i.e., controls) for background absorption and comparison (Azad et al., 2018)

*Statistical analysis:* The mean results of percentage mortality of the brine shrimp versus the log of concentrations were plotted using the Microsoft Excel (2010) spreadsheet application, which also formulated the regression equation. Then, it was used to calculate the LC<sub>50</sub> values for the test samples.

## Results and Discussion

The ethanol extract of *L. speciosa* leaves extract (Banaba) gave 34.23% yield from 520 g of raw fruits and finally it became 178 g of dry crude extract. The preliminary qualitative phytochemical tests exhibited the presence of various phytochemical groups like

alkaloids, carbohydrates, glycosides, saponins, terpenes, steroids, phenols and flavonoids (Table 1).

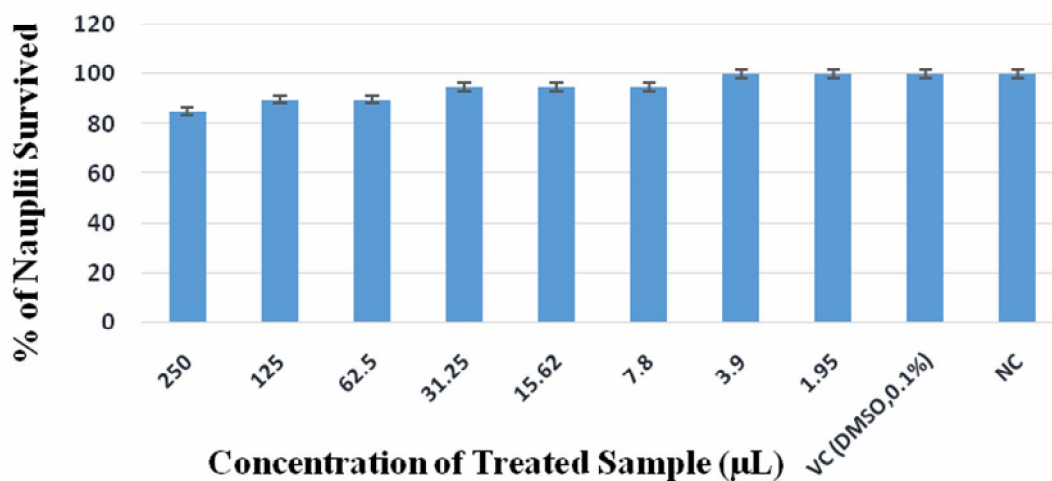
Cytotoxicity test on brine shrimp nauplii (*Artemia salina*) is considered as an in vivo toxicity experiment. The sample extract was dissolved in

0.1% DMSO. Therefore, 0.1% DMSO was used as a vehicle control and potassium dichromate was used as a positive control throughout the experiment. The extract revealed LC<sub>50</sub> as shown figure 1.

**Table 1. Qualitative phytochemical analysis of the *L. speciosa* leaf extract.**

Compound types	Test	Positive indication	Results
Alkaloids	Meyer's test	White/creamy ppt.	++
Carbohydrates	Fehling's test	Red precipitate	+
Glycosides	Benedict's test	Precipitate	+
Saponins	Foam test	Foam	+
Terpenoids	Libermann-Burchard's test	Pink-purple colour	++
Steroids	Salkowski Tests	Red colour	+
Phenols	FeCl <sub>3</sub> test	Dark-green	++
Protein & amino acids	Ninhydrin solution	Purple colour	+
Flavonoids	Magnesium and hydrochloric acid reduction test	Crimson colour	++

(+) indicated the presence/intensity of the phytochemicals group in tested sample.



\*VC=Vehicle Control, NC= Normal Control.

Figure 1. The mortality rate % of brine shrimp nauplii (*Artemia salina*) at 24 hr, after being exposed to various concentrations of *L. speciosa* leaf extract.

In the cytotoxic activity study, the different mortality rate of the naupli was observed in experimental groups, where the survival rate was more than 90 to 95% at the concentration range of from 7.8 to 125 µl and the mortality rate was very negligible until the concentration of 250 µl. The

tested sample concentration of 7.8 and 15.62 µl exhibited the same percentage of cell viability with equal standard bar as well. Interestingly, the concentration of 3.9 µl and 1.95 µl showed 100% survival rate which was really noticeable (Figure 1). This result indicated that the extract is nontoxic.

The MTT-assay results showed that the highest percentages of cell viability was 96.0% at concentration of 1.95  $\mu\text{l}$  and the lowest percentage was 88.0% at concentration of 250  $\mu\text{l}$ . However, rest of the concentration (3.9, 7.8 and 15.62  $\mu\text{l}$ ) showed almost 95% of cell viability. The overall results from

*in vitro* MCF-7 cell line showed that the ethanol extract was non-toxic at the range of the concentration of from 1.95 to 250  $\mu\text{l}$  when compared with normal control and vehicle control (1% DMSO, Figure 2).

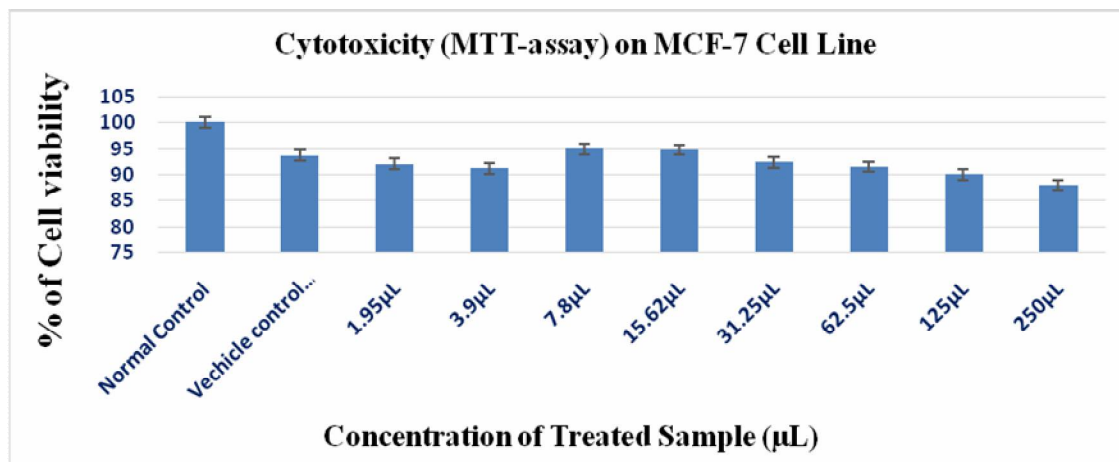


Figure 2. Percentage of cell viability at different concentration of *L. speciosa* leaf extract using MTT-assay.

## Conclusion

The present study suggested that the crude ethanol extract of Banaba is non-toxic and well tolerated at the tested dose levels. It could be used for further experiment in animal or cell-line studies to determine its level of extended.

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