



SHORT COMMUNICATION

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Preliminary cytotoxic activity of different extracts of *Averrhoa bilimbi* (fruits)

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ABSTRACT

The crude methanolic extract of *Averrhoa bilimbi* Linn. (Oxalidiaceae) fruits and its different fractions have been investigated for the evaluation of *in vitro* cytotoxic potential. The dried and powder fruits were extracted with methanol at room temperature and the concentrated methanolic extract was fractionated by the modified Kupchan partitioning method to provide pet-ether, carbon tetrachloride, chloroform and aqueous soluble fractions. Brine shrimp (*Artemia salina*) lethality bioassay was used to investigate the cytotoxic potential of *A. bilimbi*. Compared to vincristine sulfate (with LC₅₀ of 0.839 µg/ml) methanolic extract, carbon tetrachloride and pet-ether soluble fractions demonstrated a significant cytotoxic potential (having LC₅₀ of 0.005µg/ml, 1.198µg/ml and 0.781µg/ml, respectively). The LC₅₀ values of chloroform and aqueous soluble fractions were 5.691µg/ml and 6.123µg/ml, respectively. This study reveals that *A. bilimbi* possesses effective cytotoxic properties and hence can be a potential source for the isolation of active principle(s) for cancer therapy.

Key Words: *In vitro*, methanolic extract, brine shrimp lethality bioassay, *Artemia salina*, vincristine sulphate, LC₅₀.

From the beginning of human civilization, plants have beneficial activity in the treatment of human diseases. World Health Organization (WHO) survey reveals that about 80% of the world's inhabitant's problem is treated by medicinal herbal drug for their primary health care (Etkin and Nina, 1993). Plants have long antiquity used in the treatment of malignancy. Active constituents of *Catharanthus roseus*, *Angelica gigas*, *Podophyllum peltatum*, *Taxus brevifolia*, *Podophyllum emodii*, *Ocrosia elliptica* and *Campototheca acuminata* have been used in the management of progressive stages of various malignancies. There are various medicinal plants that convey anti-cancer activity in the Ayurvedic system of medicine. *Averrhoa bilimbi* is one of them with established anti-cancer activity (Rahman *et al.*, 2008).

Averrhoa bilimbi Linn. belongs to the family Oxalidiaceae. Some common name of *A. bilimbi* include Creole: bimbling plum, blimblin; English: bilimbi, cucumber tree, tree sorrel; Filipino: kamias; French: blimblin, blinblin, carambolier bilimbi. This is attractive, long-lived tropical tree, reaches 16 to 33 ft. (5-10 m) in height; has a short trunk soon dividing into a number of upright branches. *A. Bilimbi* is native of Moluccas in Indonesia. This plant is also found semi-wild throughout, Brazil, Cuba, Philippines, Sri Lanka, Bangladesh, Myanmar (Burma) and Malaysia. *A. bilimbi* is used as traditional medicine for treating cough, cold, itches, boils, rheumatism, syphilis, diabetes, whooping cough, and hypertension in Asia (Goh

et al., 1995; Mackeen *et al.*, 1997). *A. bilimbi* is also used in the treatment of children's cough (syrup of flowers), stomach ache (fruits) and as a cooling drink (juice of preserved fruits). Earlier studies showed that ethanolic leaf extract of *A. bilimbi* and its semi-purified fractions possesses hypoglycemic and hypolipidemic properties in Type I diabetic rats when administered both intraperitoneally (Tan *et al.*, 1996) as well as orally (Pushparaj *et al.*, 2000; Pushparaj *et al.*, 2001).

A survey of the published literature shows that there is a number of research works for the assessment of cytotoxic potential of *Averrhoa bilimbi* fruits using its crude extracts; however there is no research work for the assessment of cytotoxic potentials of *Averrhoa bilimbi* fruits using its different fractions. So our present study is aimed to investigate cytotoxic potential of methanolic extract of *Averrhoa bilimbi* fruits with its different organic solvent soluble fractions.

The fruits of *Averrhoa bilimbi* were collected from daudkandi, Comilla, Bangladesh on July, 2012. After collection, fruits were thoroughly washed with water, sliced with a knife and dried under sun. The plant was identified and authenticated by Taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka (Accession number: DACB 37752).

Cold maceration technique was used for extraction. The dried and powdered fruits (500g) were soaked in 2500ml of methanol for about 15 days at room temperature with occasional stirring. After 15 days the solution was filtered using filter cloth and Whatman's filter paper. The filtrate (methanol extract) obtained was evaporated under ceiling fan and in a water-bath until dried. It

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Table 1: Cytotoxic potential of methanolic extract of *A. bilimbi* fruits and its different fractions along with Vincristine Sulphate.

Sample	LC ₅₀ (µg/ml)	Regression Equation	R ²
VS	0.839	y = 34.02x + 52.58	R ² = 0.9521
ME	0.005	y = 9.0599x+79.669	R ² = 0.8893
CSF	5.691	y = 22.348x+33.123	R ² =0.9129
CTSF	1.198	y = 20.536x+48.383	R ² =0.9731
PSF	0.781	y = 22.146x+52.374	R ² =0.9167
AQSF	6.123	y=21.744x+32.877	R ² =0.9301

VS= Vincristine sulphate, ME=Methanolic extract, PSF=Pet-ether soluble fraction, CSF=Chloroform soluble fraction, CTSF=Carbon tetrachloride soluble fraction, AQSF=Aqueous soluble fraction

rendered a brown granular compound. The brown granular was designated as crude methanol extract.

The concentrated methanol extract was separately partitioned by the modified Kupchan method (Vanwagenen *et al.*, 1993) using pet-ether, carbon tetrachloride, and chloroform. The aqueous methanolic fraction was preserved as aqueous fraction. All the four fractions were evaporated to dry by keeping 7 days in room temperature.

The brine shrimp lethality bioassay was used to predict the cytotoxic potential (Meyer *et al.*, 1982; McLaughlin *et al.*, 1999) of the methanolic crude extract, pet-ether, carbon tetrachloride, chloroform, and aqueous methanolic fractions. For the experiment, 4 mg of each of the extract was dissolved in dimethylsulfoxide (DMSO) and solutions of varying concentrations (400, 200, 100, 50, 25, 12.5, 6.25, 3.13, 1.56, 0.78 µg/ml) were obtained by the serial dilution technique using simulated sea water. The solutions were then added to the pre-marked vials containing 10 live brine shrimp nauplii in 5 ml simulated seawater. After 24 hour the vials were inspected using a magnifying glass and the number of survived nauplii in each vial was counted. From this data, the percent of lethality of the brine shrimp nauplii for each concentration and control was calculated. An approximate linear correlation was observed when logarithm of concentration versus percentage of mortality was plotted and the values of LC₅₀ were calculated using Microsoft Excel 2010. Vincristine Sulphate was used as positive control.

Methanolic extract of *Averrhoa bilimbi* fruits and its different fractions were assessed for cytotoxic potential using brine shrimp lethality bioassay (Meyer *et al.*, 1982), which is a well-accepted assay for the primary screening of plant extracts, fractions or purified compounds for potential anticancer and antitumor activity.

In the present study, methanolic extract and its four fractions showed significant cytotoxic potential demonstrating that samples are biologically active. The lethal concentration (LC₅₀) of the test samples after 24 hours were found by a plot of percentage of the shrimps died in contrast to the logarithm of the sample concentration (toxicant concentration) and the

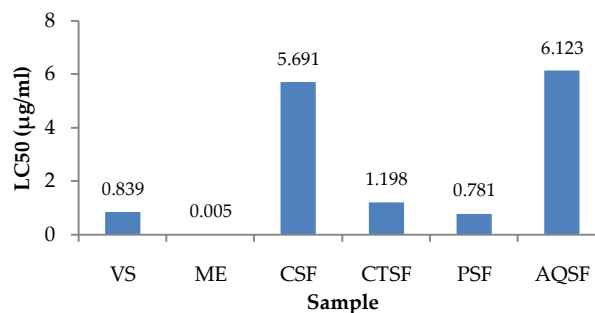


Figure 1: Comparative cytotoxic potential of *A. bilimbi* fruits along with vincristine sulfate.

best-fit line was obtained from the curve data by means of regression analysis (table 1 and figure 1). Vincristine Sulphate (VS) was used as positive control and the LC₅₀ value was found as 0.839µg/ml.

The LC₅₀ values of crude methanolic extract, chloroform, carbon tetrachloride, pet-ether and aqueous soluble fractions of *Averrhoa bilimbi* fruits were found to be 0.005, 5.691, 1.198, 0.781 and 6.123µg/ml, respectively. Therefore, the obtained result tends to suggest that plant extract of *Averrhoa bilimbi* fruits may be candidate for anticancer therapy.

In light of the results of the present study, it can be concluded that the plant extract and its fractions possesses cytotoxic potential. Positive result of methanolic extract and its different organic solvent soluble fractions led us to the inference that the plant extract may contain bioactive compounds which may aid ongoing anticancer drug discovery. Hence, further studies are recommended to be undertaken to isolate the exact compound(s) and to better recognize the mechanism of such actions scientifically.

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