



ORIGINAL RESEARCH ARTICLE

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## Evaluation of cytotoxic and antioxidant activity of different fractions of methanolic extract of *Baccaurea ramiflora* (Lour.) fruits

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### ABSTRACT

Chloroform and petroleum ether soluble fractions of the crude methanolic extract of the fruits of *Baccaurea ramiflora* (Lour.) have been investigated for the evaluation of cytotoxic and antioxidant activity. Brine shrimp lethality bioassay was used to evaluate the cytotoxic activity and free radical scavenging activity method was used to evaluate antioxidant activity. In brine shrimp lethality bioassay, the chloroform soluble fraction showed greater cytotoxic activity ( $LC_{50}$  value 74.2  $\mu\text{g/ml}$ ) than petroleum ether soluble fraction ( $LC_{50}$  value 129  $\mu\text{g/ml}$ ) compared to the standard drug vincristine sulfate ( $LC_{50}$  value 0.54  $\mu\text{g/ml}$ ). In free radical scavenging activity method (DPPH assay method), the chloroform soluble fraction showed greater antioxidant activity ( $IC_{50}$  value 49.78  $\mu\text{g/ml}$ ) than petroleum ether soluble fraction ( $IC_{50}$  value 75.31  $\mu\text{g/ml}$ ) compared to the standard antioxidant butylated hydroxytoluene ( $IC_{50}$  value 24.51  $\mu\text{g/ml}$ ). This study reveals that *Baccaurea ramiflora* fruit possesses cytotoxic and antioxidant activity hence can be a potential source for the isolation of active principle(s) for different therapies.

**Key Words:** *Baccaurea ramiflora*, cytotoxic activity, brine shrimp lethality bioassay, antioxidant activity, DPPH free radical scavenging assay.

### INTRODUCTION

Plants have been a source of medicinal substances for thousands of years. Plants and phytoproducts continue to play a vital role in the treatment of various diseases including cancer. Drug discovery from plants is a multi-disciplinary approach which combines various botanical, ethno-botanicals, phytochemical and biological and chemical separation techniques. However, despite these observations, it is significant that over 60% of currently used anti-cancer agents are derived from natural sources (Asokan and Thangavel, 2014) and about 80% of the world's inhabitants' problems are treated by medicinal herbal drug for their primary health care (Ali *et al.*, 2013). Moreover, most of the antioxidant compounds in a typical diet are derived from plant sources and the main characteristic of an antioxidant is its ability to trap free radicals (Sharma *et al.*, 2013) which are constantly formed in the human body by normal metabolic action and exert oxidative damaging effects by reacting with nearly every molecule found in living cells including nucleic acids, proteins, lipids or DNA and may involve in several chronic and degenerative diseases including gastritis, reperfusion injury of many tissues, atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others (Lakshmanashetty *et al.*, 2010; Vyas *et al.*, 2010).

*Baccaurea ramiflora* Lour. syn. *B. sapida* (Roxb.) Muell.-Arg. (Family: Euphorbiaceae) is native to South-east Asia region (Goyal *et al.*, 2013) growing wild as well as under cultivation in Nepal, India, Myanmar, Bangladesh, South China, Indo-China, Thailand, , the Andaman island and Peninsular Malaysia (Abdullah *et al.*, 2005) and its fruit is locally known as 'Lotkon'. It is a semi-evergreen tree reaching a height of about 5-10 m. Fruit is yellowish and velvety, 2-3 cm in diameter with leathery pericarp,

three seeded arillus embedded in pinkish white pulp. *B. ramiflora* fruit finds its importance as a novel food additive because of its high content of vitamin C, protein and iron. The fruit juice is mainly used for the treatment of constipation, whereas different parts of the plant are used to treat arthritis, abscesses and injuries. They are also stewed or made into wine (Goyal *et al.*, 2013).

Fruits are important sources of minerals, fiber and vitamins, which provides essential nutrients for the human health. Increased consumption of fruit and vegetables significantly reduce the incidence of chronic diseases, such as cancer, cardiovascular diseases and offer protection against free radicals that damage lipids, proteins, and nucleic acids (Prakash *et al.*, 2012). So our present study is aimed to investigate cytotoxic and antioxidant potential of chloroform and petroleum ether soluble fractions of methanolic extract of *B. ramiflora* fruits.

### MATERIALS AND METHODS

#### Plant material and preparation of extracts

The fresh lotkon fruits were collected from Dhaka, Bangladesh in the year 2013 and identified. It was ensured that the fruits were free from contamination, sand and microbial growth.

The fruits of the plant were sun-dried and then, dried in an oven at reduced temperature (not more than 50°C) to make suitable for grinding purpose. Dried powdered fruits (200g) were successively extracted using methanol as solvent in a soxhlet apparatus for continuous extraction till exhaustion. The solvent was completely removed and the crude extract thus obtained was used for investigation.

#### Fractionation

The concentrated methanolic extract of the plant material was partitioned sequentially by modified Kupchan method (Kupchan and Tsou, 1973; Vanwagenen *et al.*, 1993) and the resultant partitionates *i.e.*, chloroform and petroleum ether soluble fractions were evaporated to dryness.

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### Brine shrimp lethality bioassay

Cytotoxicity of the plant extract was determined by brine shrimp lethality bioassay technique (McLaughlin *et al.*, 1998; Meyer *et al.*, 1982) using brine shrimp eggs *i.e.*, *Artemia salina*. Vincristine sulfate was used as standard in this bioassay. Shrimp eggs were added to one side of a small tank containing 3.8% NaCl and then this side was covered. Two days were allowed to hatch the shrimp and to be matured as nauplii. Constant oxygen supply was carried out through the hatching time. The hatched shrimps were attracted to the lamp through the perforated dam and they were taken for experiment.

Measured amount of each of the samples was dissolved in dimethyl sulfoxide (DMSO) and solutions of varying concentrations such as 500, 200, 100, 50, 20, 10, 5 and 1 µg/ml were obtained by serial dilution technique. Then, the solutions were added to the pre-marked vials containing ten (10) live brine shrimp nauplii in 5 ml simulated sea water. After 24 hours, the vials were inspected using a magnifying glass and the number of survivors were counted. The mortality in percent (%) was calculated for each dilution by following equation:

$$(\%)Mortality = \left[ \frac{(Total-Alive)}{Total} \right] \times 100$$

The percentage of mortality was plotted against the logarithm of concentration and the median lethal concentration LC<sub>50</sub> of the test sample after 24 hours was calculated and compared with reference standard.

### Evaluation of free radical scavenging activity

The determination of the free radical scavenging activity of the methanolic extract was carried out using the DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay method (Brand – Williams *et al.*, 1995). 2.0 ml of a methanol solution of the sample (Control / extractives) at different concentration from 500.0 to 0.977 µg/ml were mixed with 3.0 ml of a DPPH methanol solution (20 µg/ml). After 30 minutes reaction period at room temperature in dark place the absorbance was measured at 517 nm against methanol as blank by UV spectrophotometer. Inhibition of free radical DPPH in percent (%) was calculated as follows-

$$(\%)inhibition = \left( \frac{1 - A_{sample}}{A_{blank}} \right) \times 100$$

where A<sub>blank</sub> is the absorbance of control reaction (containing all reagents except the test material). Extract concentration providing 50% inhibition (IC<sub>50</sub>) was calculated from the graph plotted inhibition percentage against extract concentration.

## RESULTS AND DISCUSSION

### Cytotoxic study

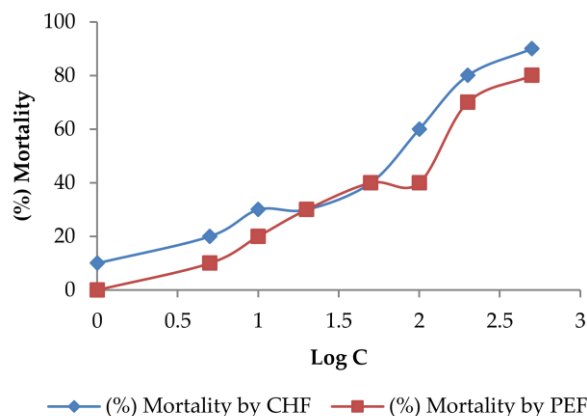
In this study, chloroform and petroleum ether soluble fractions of *B. ramiflora* fruits found to be toxic to brine shrimp nauplii, with LC<sub>50</sub> values of 74.2 µg/ml and 129 µg/ml respectively (table 1) whereas anticancer drug vincristine sulphate showed LC<sub>50</sub> value of 0.54 µg/ml (table 2). They might have antitumor activity as the cytotoxicity of plant material is considered to be caused by the presence of antitumor compounds (Rahman and Ahmed, 2013). The brine shrimp lethality bioassay also indicates antifungal effects, pesticidal effects, teratogenic effects, toxicity to environment and many more (Rahman *et al.*, 2014). Chloroform soluble fraction showed higher cytotoxic potential than petroleum ether soluble fraction. The degree of lethality shown by the fractions of methanolic extract was found to be directly proportional to the concentration of the extractives ranging from the lowest concentration (1 µg/ml) to the highest concentration

**Table 1: Effect of chloroform soluble fraction (CHF) and petroleum ether soluble fraction (PEF) of the methanolic extract of *B. ramiflora* fruits on brine shrimp nauplii.**

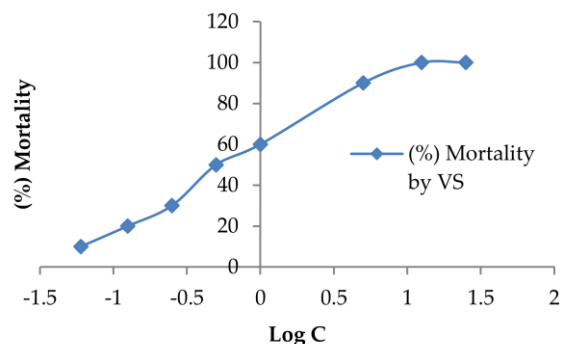
Conc. (µg/ml)	Log Conc.	Mortality (%)		LC <sub>50</sub> (µg/ml)		Regression equation		R <sup>2</sup>	
		CHF	PEF	CHF	PEF	CHF	PEF	CHF	PEF
1	0	10	0						
5	0.699	20	10						
10	1	30	20						
20	1.301	30	30	74.2	129				
50	1.699	40	40			y = 30.96x - 0.282	y = 30.13x - 7.811	0.915	0.933
100	2	60	40						
200	2.301	80	70						
500	2.699	90	80						

**Table 2: Effect of vincristine sulfate on brine shrimp nauplii.**

Test sample	Conc. (µg/ml)	Log Conc.	Mortality (%)	LC <sub>50</sub> (µg/ml)	Regression equation	R <sup>2</sup>
	0.125	-0.9030	20			
	0.25	-0.6020	30			
	0.5	-0.3010	50	0.54		
	1	0	60			
	5	0.69897	90			
	12.5	1.09691	100			
	25	1.39794	100			



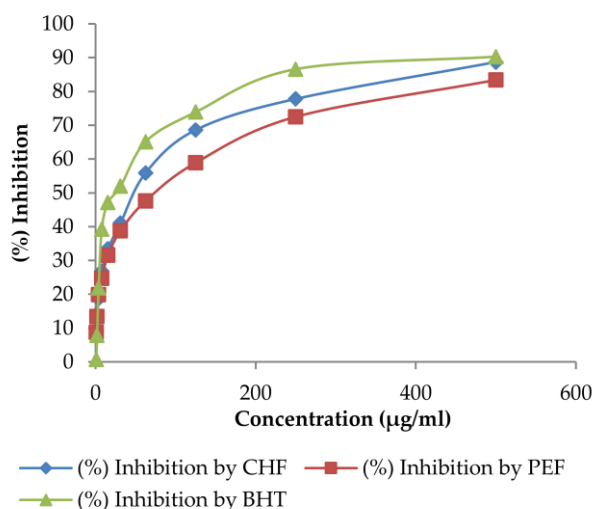
**Figure 1: Effect of chloroform soluble fraction (CHF) and petroleum ether soluble fraction (PEF) of methanolic extract of *B. ramiflora* fruits on brine shrimp nauplii.**



**Figure 2: Effect of vincristine sulfate (VS) on brine shrimp nauplii.**

**Table 3: Antioxidant activity of chloroform soluble fraction (CHF), petroleum ether soluble fraction (PEF) of methanolic extract of *B. ramiflora* fruits and butylated hydroxytoluene (BHT).**

Absorbance of blank	Conc. (µg/ml)	Absorbance of extract			(% Inhibition)		
		CHF	PEF	BHT	CHF	PEF	BHT
0.433	500	0.049	0.072	0.042	88.7	83.4	90.3
	250	0.096	0.119	0.058	77.8	72.5	86.6
	125	0.136	0.178	0.113	68.6	58.9	73.9
	62.5	0.191	0.227	0.151	55.9	47.6	65.12
	31.25	0.255	0.265	0.208	41.1	38.8	51.96
	15.625	0.288	0.296	0.229	33.5	31.6	47.11
	7.813	0.317	0.326	0.263	26.8	24.7	39.26
	3.906	0.352	0.347	0.338	18.7	19.9	21.94
	1.953	0.372	0.375	0.399	14.1	13.4	7.85
	0.977	0.394	0.395	0.43	9.01	8.8	0.69



**Figure 3: Antioxidant activity of chloroform soluble fraction (CHF), petroleum ether soluble fraction (PEF) of methanolic extract of *B. ramiflora* fruits and butylated hydroxytoluene (BHT).**

(500 µg/ml). This concentration dependent increment in percent mortality of brine shrimp nauplii produced by the *B. ramiflora* fruits indicates the presence of cytotoxic principles.

#### Antioxidant study

In this study, the chloroform soluble fraction of the methanolic extract of *B. ramiflora* fruits showed strong antioxidant activity with IC<sub>50</sub> value of 49.78 µg/ml and petroleum ether soluble fraction showed good antioxidant activity with IC<sub>50</sub> value of 75.31 µg/ml whereas standard antioxidant butylated hydroxytoluene showed IC<sub>50</sub> value of 24.51 µg/ml (table 3 and 4). As fruits of *B. ramiflora* produce significant amount of antioxidants to prevent the oxidative stress caused by reactive oxygen species (ROS), it represent a potential source of new compounds with antioxidant activity. Further study will be aimed at isolating and identifying the substances responsible for the antioxidant and cytotoxic activity of extract, which may be further exploited in herbal formulations.

#### CONCLUSION

The results from the experiment confirmed that chloroform and petroleum ether soluble fractions of methanolic extract of *Baccaurea ramiflora* (Lour.) fruit in general,

**Table 4: IC<sub>50</sub> values of chloroform soluble fraction (CHF), petroleum ether soluble fraction (PEF) of methanolic extract of *B. ramiflora* fruits and butylated hydroxytoluene (BHT).**

Sample	IC <sub>50</sub> (µg/ml)	Regression equation	R <sup>2</sup>
CHF	49.78	y = 0.148x + 28.61	0.727
PEF	75.31	y = 0.138x + 26.16	0.775
BHT	24.51	y = 0.147x + 33.73	0.573

possess cytotoxic and antioxidant activity. Therefore consumption of the fruit could offer health benefits in terms of prevention of diseases caused by oxidative stress and cancer. However, further studies are suggested to be undertaken to understand the underlying mechanism of the observed activities and to isolate, identify and purify the cytotoxic and antioxidant components present in it.

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