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CYTOTOXIC ACTIVITY OF *N*-HEXANE, CHLOROFORM AND CARBON TETRACHLORIDE FRACTIONS OF THE ETHANOLIC EXTRACT OF LEAVES AND STEMS OF *BACCAUREA RAMIFLORA* (LOUR.).

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ABSTRACT

The purpose of the study was to find out the cytotoxic activity of the *n*-hexane, chloroform and carbon tetrachloride fractions of the ethanolic extracts of the leaves and stems of *Baccaurea ramiflora* (Lour.). Ethanolic extracts of the leaves and stems of *Baccaurea ramiflora* were subjected to solvent-solvent partitioning using *n*-hexane, chloroform and carbon tetrachloride to obtain *n*-hexane leaves fraction, chloroform leaves fraction, *n*-hexane stems fraction, chloroform stems fraction and carbon tetrachloride stems fraction. Each fraction was assayed for their cytotoxic effect using brine shrimp lethality bioassay. Among the fractions, the *n*-hexane fractions of leaves and stems showed significant cytotoxic effects having LC_{50} values of 7.79 µg/ml (95% confidence interval 6.48-9.37) and 5.78 µg/ml (95% confidence interval 4.76-6.99) respectively as compared to vincristine sulfate (LC_{50}= 2.81 µg/ml (95% confidence interval 1.97-4.01) which was used as positive control. The results support the traditional uses of *B. ramiflora* for various medicinal purposes and thus demand the isolation and identification of active principles and thorough bioassay.

INTRODUCTION: *Baccaurea ramiflora* (Lour.), (family: Euphorbiaceae) is a slow-growing evergreen tree growing to 25 m, with a spreading crown and thin bark. The fruit of *B. ramiflora* is 1-2″ around and yellow to red in color. This fruit tree is native to the Southeast Asian region and found growing wild [1, 2] as well as under cultivation in Nepal, India, Myanmar, South China, Indo-China, Thailand, the Andaman Islands and Peninsular Malaysia.

It grows in evergreen forests on a wide range of soils. The common names include Latkan or Bhubi (Bengali), Leteku (Hindi), Mafai (Thai) and Burmese grape (English) [1, 2].

In Chinese Dai medicine, the whole plant of *B. ramiflora* is utilized as an antiphlogistic and anodyne against rheumatoid arthritis, cellulitis, abscesses and to treat injuries [3]. The plant is also used as medicine by hill-tribes in Northern Thailand [4]. Young leaves of *B. ramiflora* are used as vegetable, flavoring agent with curries and minced meat in Bangladesh [5]. In India, fresh bark is chewed or juice is used orally for constipation [1].

The hydromethanol extract of the fruit pericarp of *B. ramiflora* showed significant DPPH scavenging activity with IC_{50} of 31.38 µg/ml [5] which indicates presence of phenolic compounds such as flavonoids, polyphenols, tannins and phenolic terpenes [6].

Phytochemical researchers focused on essential oil isolation from *B*. *ramiflora* root, leaf & fruit. The results showed that 10 essential oils from leaves and roots of *B. ramiflora* were the same, whose relative contents were 76.66%. Seven essential oils from fruits and roots of *B. ramiflora* were the same, whose relative contents were 69.82% [7]. Epidihydrotutin is a new sesquiterpene lactone isolated from root of *Baccaurea ramiflora* [8].

Previous phytochemical investigations showed that two new phenols, 6'-O-vanilloylisotachioside and 6'-O-vanilloyltachioside, together with nine known compounds were isolated from the leaves of this plant. Seven compounds revealed potent antioxidant activities against H_2O_2-induced impairment in PC12 cells, and exhibited significant DPPH radical-scavenging activities [4].

The methanolic extract of the bark of *B. ramiflora* showed significant hypoglycemic activity compared to control (P<0.01) with a significant 24.89% and 29.19% inhibition at 200 mg/kg and 400 mg/kg body weight respectively [9].

From the stems of *Baccaurea ramiflora,* three new and four known compounds were isolated. The new compounds were identified as 4'-O-(6-O-vanilloyl)-β-D-glucopyranosyl tachioside D, 6'-O-vanilloylpicra-quassioside D, and 6'-O-vanilloylicariside B_5. One of the compounds exhibited significant DPPH radical-scavenging activity with an IC_{50} value of 36.9 µM, while another compound revealed weak antioxidant activity against H_2O_2-induced impairment in PC12 cells [10].

The objective of this research work was to investigate the cytotoxic activity of the different fractions of the ethanolic extract of the leaves and stems of *Baccaurea ramiflora* (Lour.) [9].

MATERIALS AND METHODS:

Plant Collection: The fresh leaves and stems of the plant *Baccaurea ramiflora* were collected from Hotapara, Gazipur of Bangladesh during December-January (2008-2009). The experiment was carried out during the period of February 2009.

Extraction: The air dried and powedered leaves and stems of *Baccaurea ramiflora* plant materials were extracted with ethanol through occasional shaking and stirring for 7 days. The extracts were then filtered through a cotton plug and finally with Whatman no. 1 filter papers. Rotary evaporator (RV 10 Basic, IKA, Germany) was used to reduce the volume of filtrate at low temperature and pressure. Then the ethanol extract was suspended in deionized water and partitioned sequentially with *n*-hexane, chloroform and carbon tetrachloride using solvent-solvent partitioning technique designed by Kupchan and Tsou [11] and modified version of Wagenen [12]. Thus, the *n*-hexane leaves fraction (NHLF), chloroform leaves fraction (CHLF), *n*-hexane stems fraction (NHSF), chloroform stems fraction (CHSF) and carbon-tetrachloride stems fraction (CTCSF) were obtained.

Bioassay: The cytotoxic property of the plant extractives was determined by applying *in vitro* lethality test- Brine shrimp lethality bioassay technique [13] using brine shrimp nauplii eggs i.e. *Artemia salina*. Vincristine sulphate and DMSO were used as positive and negative control, respectively. Eggs were placed in one side of a small tank divided by a net containing 3.8% NaCl solution for hatching and the pH of the hatching solution was maintained at 8.4 [14]. In the other side of the tank, a light source was placed to attract the nauplii. After 2 days of hatching period, the nauplii were ready for the experiment.

Four mg of each of the extractives were dissolved in Dimethyl sulfoxide (DMSO) and solutions of varying concentrations such as 400, 200, 100, 50, 25, 12.50, 6.25, 3.125, 1.563 and 0.781 µg/ml were obtained by serial dilution technique. Then, the solutions were added to the pre-marked vials containing thirty (30) live brine shrimp nauplii in 5 ml simulated sea water. After 24 hours, the vials were inspected using a magnifying glass.

The number of survived nauplii in each vial was counted. Mortality was corrected using Abbott's formula [15]. From this data, the percentage (%) of lethality of the brine shrimp nauplii were calculated for different fractions for each of their respective concentration. The mortality data were subjected to 'Probit analysis' according to Finney [16] and Busvine [17]. The LC_{50} values were calculated (at the confidence interval level of 95%) using Microsoft Excel 2007 by a plot of percentage Probit mortality against the logarithm of the sample concentrations.

RESULT AND DISCUSSION: In case of Brine Shrimp Lethality Bioassay, the lethality of the *n*-hexane leaves fraction (NHLF), chloroform leaves fraction (CHLF), *n*-hexane stems fraction (NHSF), chloroform stems fraction (CHSF) and carbon-tetrachloride stems fraction (CTCSF) of *Baccaurea ramiflora* were evaluated against *A. salina*. **Table 1** shows the results of the brine shrimp lethality testing after 24 hours of exposure to the samples and the positive control, vincristine sulphate (VS). The LC_{50} values were found to be 7.79 µg/ml (95% CI; 6.48-9.37), 29.94 µg/ml (95% CI; 27.98-32.04), 5.78 µg/ml (95% CI; 4.76-6.99), 58.98 µg/ml (95% CI; 53.39-65.18), 87.99 µg/ml (95% CI; 79.44-97.46) and 2.81 µg/ml (95% CI; 1.97-4.01) for NHLF, CHLF, NHSF, CHSF, CTCSF and VS respectively (Table 1).

TABLE 1: EFFECT OF *N*-HEXANE LEAVES FRACTION (NHLF), CHLOROFORM LEAVES FRACTION (CHLF), *N*-HEXANE STEMS FRACTION (NHSF), CHLOROFORM STEMS FRACTION (CHSF), CARBON TETRACHLORIDE STEMS FRACTION (CTCSF) OF ETHANOLIC EXTRACT OF THE LEAVES AND STEMS OF *B. RAMIFLORA* AND POSITIVE CONTROL VINCRISTINE SULPHATE (VS) ON BRINE SHRIMP

Sample	Regression Equation R^2 value	LC_{50} (µg/ml)	95% Confidence interval	
			Upper limit	Lower limit
NHLF	y = 0.685x + 4.389 R^2=0.960	7.79	9.37	6.48
CHLF	y=0.867x+3.720 R^2= 0.885	29.94	32.04	27.98
NHSF	y = 0.763x + 4.419 R^2= 0.875	5.78	6.99	4.76
CHSF	y =0 .602x + 3.934 R^2= 0.907	58.98	65.18	53.39
CTCSF	y = 0.54x + 3.95 R^2= 0. 837	87.99	97.46	79.44
VS	y = 1.0221x + 4.54 R^2= 0.950	2.81	4.01	1.97

The above results (LC_{50} values of different fractions of leaves and stems extracts of the studied plant) clearly indicate the presence of potent bioactive principles in these extractives, which might be very useful as antiproliferative, antitumor, pesticidal and other bioactive agents. The isolations of the active biochemical compounds are required for further study and it is also necessary to isolate compounds responsible for exerting cytotoxic effect.

Brine shrimp lethality bioassay is an elementary experiment to assay for potential cytotoxic activity. Many scientists have reported cytotoxic activity of plant materials using brine shrimp activity as zoological specimen [18, 19]. The cytotoxicity of plant material is considered to be caused by the presence of antitumor compounds [20].

In this study, the non-polar fractions of *n*-hexane of the ethanolic extract showed the most cytotoxic activity, which indicates that the responsible cytotoxic agent could be non-polar in nature.

CONCLUSION: The different fractions of ethanolic extracts of leaves and stems of plant *Baccaurea ramiflora* (Lour.) showed promising cytotoxic activity. Further, bioactivity-guided study should be done to isolate the active chemical compound and measure its safety *in vivo*.

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