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In vitro Cytotoxic Activity of Methanolic Extract of M. pachycarpa (Benth) Leaves

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Millettia *pachycarpa* belongs to the family Fabaceae, a perennial climbing shrub belonging to the genus *Millettia*. The present investigation evaluates the cytotoxic activity of methanolic extract of M. *pachycarpa* Benth leaf. Brine shrimp lethality bioassay method is used to assess the cytotoxic activity of M. *pachycarpa* Benth. The laboratory studies show that brine shrimp bioassay method the LC50 value of methanolic extract of M. *pachycarpa* leaf is1.7493 µg/ml with a 95% confidence limit[1] of 1.3679-2.2370 µg/ml.

Keyword: Millettia *pachycarpa*, bioassay, cytotoxic, LC50, 95% CL.

1. INTRODUCTION:

From the very beginning of the civilization there is an extreme relationship between human beings and plants. In ancient period the system of treatment was not enriched like today. The ancient people used to utilize several parts of plants in different treatment purposes. Plants are used not only as medicine but also in a number of their daily jobs (e.g. fishing, hunting etc.) eventually plants are the ultimate caretaker of environment in a sense. A single part of plant may consists of numerous medicinal values, but it has been proved that direct intake of crude plant is not good; even it may causes toxicity more cases. However plants are used as the major sources in modern and traditional system of medicine. Bangladesh has a lot of medicinal plants which has been using for a period of times locally as well as in ayurvedic & herbal medicine system; has been developing day by day. Though the plants are using unconsciously and in an improper dose, a chronic adverse effect may develop after a long run. So the proper use of plant with appropriate dose can be ensured by surveying its effect with scientific methodology. Today's medical science is employing a great concern over cancer

disease, which may be defined as the abnormal cell division within different organs of the body. The major causes of cancer have been reported is either by microbial infection or by free radicals. Drugs that used in treatment of cancer are mostly cytotoxic. Plant having cytotoxic activity can be referred for further laboratory process to isolate chemicals that can be used in treating cancer.

M. pachycarpa is a climbing shrub [2]. Its pod contains 1-5 seeds. The flowers are lilac-colored and the leaves have 13-17 papery leaflets. It is endemic to south-east Asian region including Bangladesh, Bhutan, China, India, Myanmar, Nepal, Taiwan, Thailand and Vietnam. It is one of the most well known species of *Millettia*, as it is widely used in traditional practices, such as fish poisoning(so as it is called ' fish poison climber')[3-4], agricultural pesticide[5], blood tonic, and treatments of cancer and infertility

2. Experimental section:

2.1 Plant collection:

The plant was collected from Bandarban, the hilly region of Chittagong, Bangladesh in the month of October 2012. Then the plant has been identified by Dr. Shaikh Bokhtear Uddin, associate Professor, Department of Botany, University of Chittagong.

2.2 Extraction:

Extraction of plant leaves was done by using organic solvent [6].The fresh leaves of *M. pachycarpa* Benth were cut, washed and air dried at room temperature ($24^0 \pm 2^0$ C) for about 10 days. Dried leaves were macerated into coarse powder. Dried powder (500 gm) was then extracted using Methanol. Then methanolic extract was shaken by rotary shaking apparatus for 7 days. The extract was collected using Buckner funnel. The Methanol was evaporated at a temperature below 45^0 C and concentrated extract was weighed 29 gm, stored at 4^0C.

2.3 Hatching of Brine Shrimp:

Cytotoxic activity of plant extract was determined by Brine Shrimp lethality bioassay as described by Meyer et al[7]. Shrimp eggs were added to the simulated "sea water" (38 g sea salt pure NaCl was weighed, dissolved in 1 litre of distilled water adjusted to pH 8.5 using 1N NaOH and was filtered off to get clear solution) in the larger compartment of an unequally dividend tank. The chamber was kept under illumination using a table lamp for 48 h for the eggs to hatch into shrimp larvae. The illuminated compartment attracts shrimp larvae (nauplii) through perforations in the dam.

2.4 Preparation of solution:

Mother solution was prepared using DMSO (dimethyl sulfoxide) and sea water as solvent (20% DMSO with rest portion water to make 1:1 solution). From the stock solution 8 other concentration was prepared as, 10, 50, 80, 100, 200, 300, 400 and 500µg/ml. 8 test tube was taken with 10 brine shrimp in each of them, filled with different conc. and sea water to make total volume 5ml. A test tube of DMSO was made as control. After 24 hour each test tubes were checked. From the % of morbidity of brine shrimp LC_{50} (lethal concentration) was calculated by plotting against logarithm of concentration. Computer software "BioStat-2009" was used to calculate.

3. Result and discussion:

Percentage of lethality of brine shrimp at eight different concentration (10 to 500µg/ml) using methanolic extract of *Mellittea pachycarpa* Benth presented in Table 1. Plant extract shows lethality as their concentration got increased. More

specifically, from 10% to 100% mortality, it's a clear dose dependency manner.

3.1 Statistical analysis:[8]

From the % of lethality probability unit for each concentration was calculated using "BioStat-2009", then by plotting %response or %lethality against log concentration of corresponding LC_{50} was obtained 1.7493 µg/ml (Table 2 and fig-1).

Table 1: log concentration and % of lethality of M. pachycarpa . By the increase of concentration, rate of mortality increases.

Dose (µg/ml)	Log dose	Total (n)	Survived	Death	% of lethality	Actual %
10	1	10	9	1	10	0.1
50	1.698	10	8	2	20	0.2
80	1.903	10	7	3	30	0.3
100	2	10	6	4	40	0.4
200	2.301	10	5	5	50	0.5
300	2.477	10	4	6	60	0.6
400	2.602	10	4	6	60	0.6
500	2.698	10	0	10	100	0.975

Table2: **LC_{50}**, 95% confidence limit, chi-square.

Log_{10} LC_{50}	LC_{50}µg/ml	95% confidence limit	Chi-square
0.243	1.75	1.3679-2.2370	0.8734

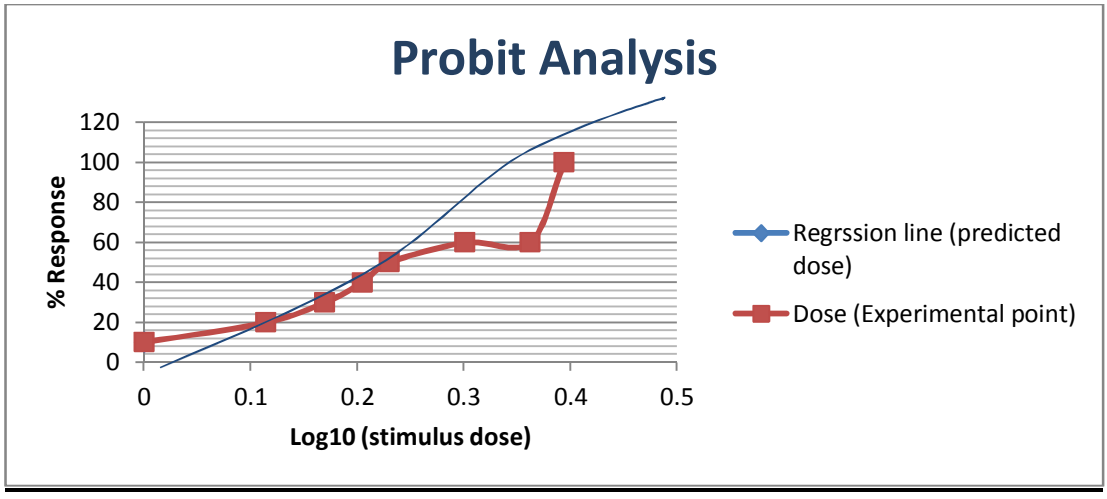

Fig-1: Probit Analysis graph for determination of LC_{50} of methanolic extract of *M. pachycarpa.*

4. Conclusion:

This laboratory study shows that, *Milletia pachycarpa* has a significant cytotoxic effect. This report in a sense may authorize the local use of this plant as fishing poison, insecticides etc. However a further Phytochemical analysis may be done to screen the possible constituent(s) responsible for this pharmacologic effect.

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