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Toxicological Evaluation of Crude Alkaloid Fraction Isolated From Indian Folklore Plant *Telosma Pallida* (Roxb) wg Craib Root Using Probit Value Analysis

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Abstract: Present study first time reported the toxicological profile of *Telosma pallida* (TP) crude alkaloid fraction (CAF) isolated from the root of the climber plant by brine shrimp lethality test (BLT). *Telosma pallida* is a perennial herb found throughout the Junagadh district and surrounding. Previous studies showed the use of these alkaloids in the inhibition of thymidylate synthetase enzymes and cell growth. Brine shrimp toxicity study was carried out with *Artemia salina* Leach. In this assay, brine shrimp was hatched in sea salt water and allowed to contact with various concentrations of the crude alkaloid fractions. At 500µg/ml, highest mortality was found 74.44±0.35%whereas in the case of positive control, at the dose of 50µg/ml, 80.00±0.609% mortality was found. LD_{50} values for CAF and test control was found to be 89.12µg/ml and 12.59µg/ml, respectively. Further *in-vitro* and *in-vivo* studies may testify the anticancer potential of this plant.

Kewords: *Pergularia pallida*, alkaloids, anticancer, brine shrimp, toxicity

1. INTRODUCTION

Medicinal plant based chemicals are gaining more and more importance now a days. More than 70% of the world population still relies on the plant or plant based medicines (Shah *et al.,* 2011). Diseases like cancer, coronary heart diseases has been well studied by the researcher and academicians and also studied the

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role of natural products in the treatment and prevention of the diseases (Bhatt and Pandya, 2010). Apart from this, the second approach behind the BLT is to identify new plant base herbicides. Due to the toxic effect of synthetic herbicides, plant based natural herbicides are being more famous due to less or no toxic effects on the crops and related plant varieties (Shah *et al.,* 2014).

Alkaloids, saponins, and some other phytochemicals like flavonoids; terpenes have been assessed for their cytotoxic effects, as well other pharmacological effects like anti-inflammatory, anticancer etc (Odebiyi and Sofowora, 1979).

Telosma pallida (Roxb) Craib (also known as *Pergularia pallida*) is perennial climber plant is found throughout the Junagadh region of Gujarat, India. Flowers and leaf of the plant are commonly used as a vegetable in this region (Indraji, 1998).

Phytochemical screening of the plant revealed the presence of alkaloids in the root, triterpene in the leaf and flavanones as well chlorogenic acid in the flowers (Rajgure & Bhogaonkar, 2012).

Previous reports have claimed the efficacy of phenanthroindolizidine namely tylophorine, tylophorinidine, pergularinine in thymidylate synthase enzyme (Mulchandani and Venkatachalam, 1976).

These alkaloids have also been claimed to have different pharmacological activities like anti-inflammatory, anti-asthmatic etc (Govindachari *et al.,* 1973).

Brine shrimp Lethality assay is one of the most common as well, easiest tests to evaluate toxic effect of any compound or plant extracts (Wu, 2014).

Various *Artemia* species like *salina, monica, sinica, urmiana* etc are available. *Artemia salina* Leach is most commonly used species in this assay. Adult shrimp have three eyes and eleven legs. They can grow up to 15 mm in size. Upon reaching adult age, they found to have blood in their body which contains haemoglobin, found in vertebrates. Their eggs are hatched into nauplii which have almost 0.5 mm size. Eggs and organism itself can't survive in fresh salt-free water. In nature, green algae are the main source of food for them (Anonymous, 2016).

Present study elaborates the effect of cytotoxic CAF of *T. pallida* on *Artemia salina*. The results from this research may found useful for cancer research as well designing of new herbicide from the herbal origin. This is the first report on BLT for *T. pallida* alkaloids.

2. MATERIALS AND METHODS

2.1 Chemicals

All the solvents were of analytical grade purchased from Merck and SD fine chemicals, India. Etoposide was obtained from Sigma-Aldrich, India. Milli-Q® water was used throughout the experiment.

2.2 Collection and authentication of plant material

The root of *Telosma pallida* was collected during the July to September months of 2014 and 2015 from Joshipura area (21.55711ºN, 70.44772ºE) of Junagadh district, Gujarat, India.

Plant specimen was identified by Dr H. B. Singh, Chief Scientist and Head of Department of Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources, New Delhi, India.

A voucher specimen (No. SSPC/DPC/VS/01) was submitted in the Department of Pharmacognosy, Shree Swaminarayan Pharmacy College, Kevadia Colony, Dist. Narmada, Gujarat, India, for future reference. Roots were washed with distilled water and dried in an oven at 45^0C constantly for 6 days. It was Powdered and stored in an airtight container until further use.

Phytochemical screening of root extracts showed the presence of alkaloids in it.

2.3 Extraction and isolation

Powdered root (100g) moistened with 25% ammonia solution (25ml) in 1000ml Erlenmeyer flask and mixed thoroughly with a plastic rod until uniform mixing. Then plant material was extracted with Chloroform: Methanol (4:1) 500ml three times at room temperature by maceration only. Combined solvent extracts were reduced under pressure and brownish mass (12g) was dried in the air overnight.

This mass was suspended in 3% HCl (500ml) and shaken with first chloroform (100ml, 3 times). The chloroform layer was removed and pH of aqueous layer was adjusted to 9 by NH_4OH solution. The basic solution was shaken with again chloroform (30ml, 6 times) until clear chloroform layer obtained.

The chloroform layer was removed and dried under reduced pressure. Dark brownish yellow mass (0.8g) so obtained was named a crude alkaloid fraction.

2.4 Brine shrimp lethality test

Brine shrimp lethality test (BLT) was performed as per standard protocol reported previously with minor modifications (Carballo *et al.,* 2002, Musa, 2012).

Eggs of *Artemia salina* L. were firstly hatched with sea salt water prepared by dissolving 38g sea salt in 1000 ml distilled water by continuous agitations. Eggs were left for hatching for overnight with aeration to supply oxygen. Next day, nauplii were observed in the container.

30 nauplii were transferred in previously sterile glass vials of 10 ml capacity. 5ml of fresh sea salt water was added. Each concentration of test and standard were tested in triplicate.

Crude alkaloid fraction was dissolved in DMSO/water to prepare final concentrations at 25, 50, 100, 200 and 500µg/ml. Etoposide was used in the concentration 10, 25 and 50µg/ml as a positive control. All the vials were incubated for 24 hours at the temperature range 27 ± 1°C under illuminations.

After 24 hours, all the vials were observed for the survival of brine shrimps. % Mortality or death was calculated by the following formula;

$$\% \text{ Mortality or death} = \frac{\text{No.of dead nauplii}}{\text{Total no.of nauplii took initially}} \times 100$$

2.5 Calculation of standard error of LD_{50}

The standard error (SE) for LD_{50} value was calculated by the method (Ghosh, 1984). Following equation was used to calculate standard error;

$$\text{Approx SE of } LD50 = \frac{(\log\log LD84 - \log\log LD16)}{\sqrt{2N}}$$

2.6 Statistical analysis

All the data were represented as mean (±SD). The calculation of the LD_{50} was done as per standard method mentioned in the literature. This involved the calculation of percentage death (mortality) as per equation mentioned above. Transformation of % mortalities to probit values has been taken from standard probit table. A log dose verse probit was plotted in Microsoft Excel 2007. Extrapolation of probit value 5 on X-axis gave the value of log dose required. Antilog of the same gave LD_{50} value in µg/ml (Randhawa, 2009). The percentage death data were analyzed by one-way analysis of variance followed by bonferroni's multiple comparison test.

3. RESULTS

3.1 Crude alkaloid fraction

The percentage yield for CAF was found to be 0.8%.

3.2 Brine shrimp lethality test

Crude alkaloid fraction of *Telosma pallida* at the dose range of 25, 50, 100, 200, and 500µg/ml produced significant mortality of *Artemia salina* L. Highest

death 74.44±0.35% was found at the dose of 500µg/ml (Table 1). At this dose, highest probit was found to be 5.64 from probit table. Plotting of log dose versus probit graph gave the value of half Lethal dose (or LD_{50}) 89.12µg for test concentrations (Fig 1).

Etoposide was used as positive control in the dose range of 10, 25, and 50µg/ml. More than 80% mortality was found at the dose of 50µg/ml with probit value 5.84 (Fig 2). The LD_{50} value for the etoposide was found to be 12.59µg in this concentration range.

4. DISCUSSION

Phytochemical like alkaloids, saponins are played a vital role in the various pharmacological evaluations. Brine shrimp lethality assay is a first step to

Table 1: Dose vs. percent death for test and standard compounds.

TEST (*Telosma pallida* crude alkaloid fraction)						
Dose (µg/ml)	**Log Dose**	**Death**	**% Death**	**Probits**	**LD_{50} (µg)**	**Standard Error**
25	1.398	8.33 ± 0.58	27.78 ± 0.35***	4.39		
50	1.699	12.33 ± 1.15	41.11 ± 0.70***	4.77		
100	2.000	16.67 ± 1.53	55.56 ± 0.93***	5.13	89.12	9.7
200	2.301	20.00 ± 1.00	66.67 ± 0.61***	5.41		
500	2.699	22.33 ± 0.58	74.44 ± 0.35^{ns}	5.64		
STANDARD (Etoposide)						
10	1.000	13.67 ± 1.53	45.56 ± 0.930	4.87		
25	1.398	19.67 ± 1.53	65.56 ± 0.930	5.39	12.59	9.7
50	1.699	24.00 ± 1.00	80.00 ± 0.609	5.84		

All the data were expressed as mean (±SD) (n=30). Probit values were obtained from standard probit table given in the literature. Data were analyzed by one-way analysis of variance followed by Bonferroni's Multiple Comparison Test. Comparison was made by considering 50 µg/ml Etoposide as a control where ****p*-value<0.0001 was considered as highly significant and ***p*-value<0.001

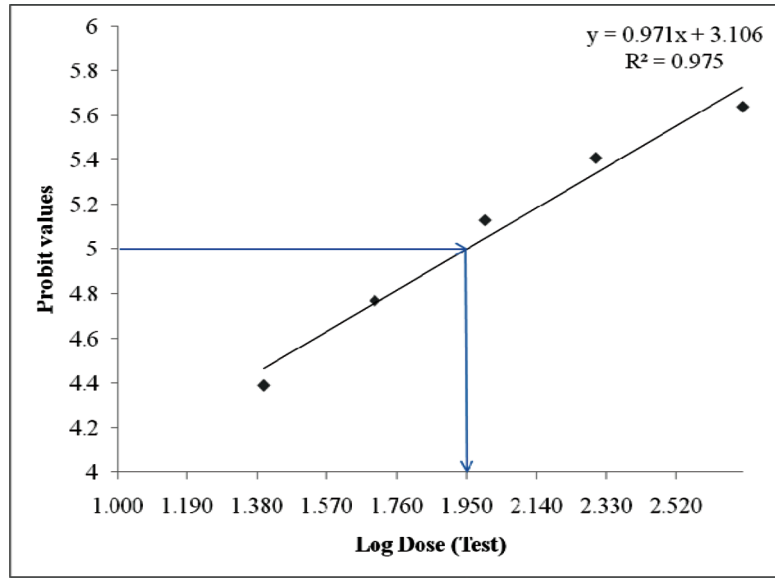

Figure 1: Log dose vs. Probit value of crude alkaloid fraction from *T. pallida*.

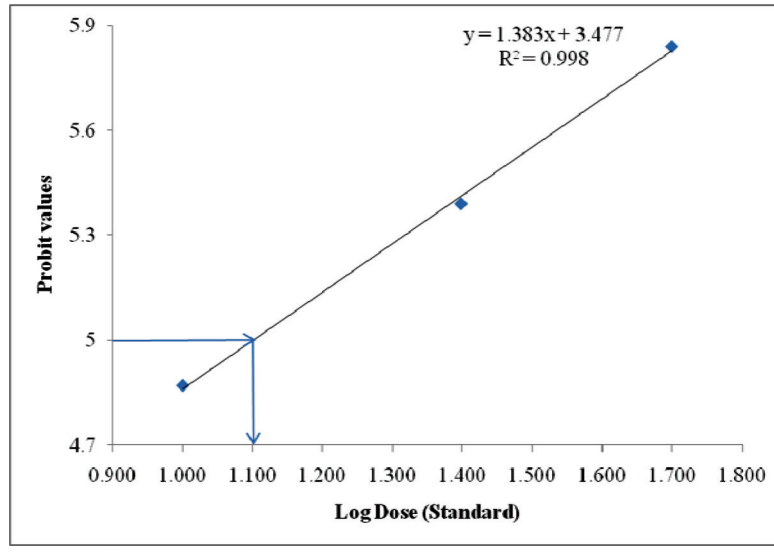

Figure 2: Log dose vs. Probit value of etoposide.

evaluate the anticancer activity of any compound or phytochemical (Kerwin, 2004).

In the present study, the crude alkaloid fraction (CAF) from *Telosma pallida* roots shown very good cytotoxicity i.e. LD_{50} value was found to be 89.12µg/ml as compared to standard anticancer drug etoposide.

The SE values for LD_{50} for both test and standard was found to be 9.7 i.e. crude alkaloid fractions are 89.12 ± 9.7 μg and 12.59 ± 9.7 μg. This can be explained at 95% confidence level LD_{50} value of CAF between 79.42 to 98.82 μg.

The root contains phenanthroimidazolidine alkaloids namely, pergulardidine and tylophoridine. Structurally, these alkaloids are phenolic alkaloids and found to have -OCH_3 groups and phenanthrene ring structure (Mulchandani and Venkatachalam, 1976). Etoposide is the semi-synthetic derivative of the podophyllotoxin isolated from *Podophyllum peltatum* root. Etoposide inhibits the topoisomerase-II enzyme which damages the DNA and finally cell division is blocked (Kutzang, 2006).

This model provides a simple but effective correlation between the anticancer activity and insecticidal action simultaneously. Simple zoological creature *Artemia salina* has been well studied for development of this assay (Urmi *et al.,* 2013).

Further study is going on for the isolation of target compounds and evaluation of the toxic effect on *Artemia salina* L.

5. CONCLUSION

The present study revealed the cytotoxic action of crude alkaloid fraction from folklore plant. This study can be useful in the development of anticancer herbal formulation. This study may also be useful in agricultural research for the development of new herbicide.

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