

Evaluation of Total Phenolic Compound and Cytotoxic Activity of *Clinacanthus nutans*

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Clinacanthus nutans leaves were extracted in distilled water at 0.5, 1, 3, 5 and 24 h. The plant extracts were determined the highest amount of total phenolic compound and used for evaluating the cytotoxicity test. The median (LC50) and 90% lethal concentration (LC90) against *Artemia salina* at varied concentrations of this plant extractions as 0, 400, 800, 1600, 3200, 6400 and 12800 µg/ml were evaluated within 24 h exposure. The result revealed that the total phenolic compound measurements in each time extraction were 34.68±3.17, 46.71±9.31, 36.13±7.06, 26.53±8.83 and 30.15±3.09 mg gallic acid equivalent per g of extract, respectively. Due to the highest amount of total phenolic compound, the 1 h aqueous extract of *C. nutans* leaf expressed the 24 h LC50 and LC90 values against *A. salina* were 7,151.1 and 12,008.7 µg/ml, respectively.

Key words: Antioxidant, *Artemia salina*, brine shrimp, *Clinacanthus nutans*, total phenolic compound

The famous saying “there is good and bad in everything” implies to the plants too. The good side is that it has antioxidant substances and the bad side is the toxicity. Medicinal plant parts including roots, leaves, stems, barks, flowers and fruit have been used in virtually all cultures as a source of medicine because of their pharmacological activities and antioxidant properties^[1]. They are commonly rich in phenolic compounds such as flavonoids, phenolic acids, tannins, coumarins, and lignins^[2]. *Clinacanthus nutans* has been widely used as a traditional medicinal plant in Asia for treating skin rashes, bugs and snake chomps, sores brought on by herpes simplex infection, diabetes mellitus, fever and diuretics^[3].

C. nutans is a well-known antsnake venom and antiviral agent^[4]. It belongs to the family Acanthaceae which is a large family, comprised of about 250 genera and 2,500 species distributed in the world^[5]. The genus *Clinacanthus* comprises two species, *C. nutans* (Burm. f.) Lindau and *C. spirei* Benoist, and synonymous with *Justicia nutans*, *J. fulgida*, *C. burmanni*, *C. siamensis*^[4]. *C. nutans* is also known as *giro de flores*, *cocodrilo flor*, *e zui hua* (Chinese), *yudunsou* (Japanese), *yōuduncho* (Korean), *ki tajam*, *dandang gendis* (Indonesian), *belalai gajah*, *sabah snake grass* (Malay), *ki tajam* (Sunda), *saled pangpon tua mea*, *phaya yor*, *phak man kai*, *phak lin khiat* (Thai), and *mánh cōng; lá cằm; bìm bịp; xươg khi* (Vietnamese)^[6-8].

C. nutans is a shrub of 1-3 m height with pubescent branches. Leaves are pale green, simple, opposite, narrowly elliptic oblong with acute apex size 2.5-13.0 cm long and 0.5-1.5 cm wide. The leaf base are obtuse rounded or truncate and often oblique. In addition, it has about 6-7 pairs side of veins. The stem is stiffly straight along with internodes and vertical strips along the stem. Flowers are dense at the peak of the branch, has glandular-pubescent calyx around 3.5 cm with dull red, green base and yellow streaks. As the flowers are in dense cymes at the top of the branches and often terminating drooping horizontal^[4]. Stamens are exert from the corolla. The ovary is packed into two cells and each cell contains two ovules^[9]. Phytochemical analysis has shown that *C. nutans* contains several kinds of clinamide derivatives^[10], alkaloids^[11], chlorophyll^[12], flavonoids^[13], phenolic compounds^[14].

Medicinal plants have gained huge interests from researchers around the world because of their positive biological activity^[15]. However, there is still not much data available about the toxicity of medical plants. For this reason, this experiment is set out to observe the cytotoxic effect of *Clinacanthus nutans* extract against *Artemia salina*. The brine shrimp lethality assay is a widely used method used to indicate general toxicity because of its simplicity^[16-18]. The findings from this study would give basic contributions for the development of new treatments for health providers.

Fresh, mature, green leaves of *C. nutans* were randomly collected in Surat Thani Province, Southern part of Thailand. The voucher specimen was numbered and kept in our research laboratory

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for the further reference. The leaves were washed with tap water and air dried in shade for 24 h and dried in a hot air oven at 70° for 6 h, and crushed with a blender (fig.1). The extraction procedure was determined by the method of Pavananundt, *et al.*^[19] with modifications. Five grams of leaf powder was extracted with 100 ml of distilled water on a shaker at 180 rpm for 0.5, 1, 3, 5, and 24 h at room temperature. The whole mixture was then filtered through a fresh gauge plug, and centrifuged at 4000 rpm for 10 min. Finally, supernatant was filtered with a Whatman number 1 filter paper, the clear filtrate used as a stock solution for total phenolic compound measurement and bioassay experiment.

Total phenolic compound was determined using Folin–Ciocalteu reagent according to methods of Jiraungkoorskul^[20] and McDonald, *et al.*^[21] with modifications. Briefly, the 50 µl of the extraction in each time (0.5, 1, 3, 5 and 24 h) was mixed with 250 µl of 10% Folin-Ciocalteus and 200 µl of 0.7 M sodium carbonate then add distilled water until 5 ml and incubated at room temperature for 2 h in the dark room. The mixture was measured at 724 nm by using a spectrophotometer. Quantification was based on the standard curve of the gallic acid and expressed as gallic acid equivalent (GAE) using the following linear equation based on the calibration curve as shown in this equation ($OD=8314.1C^2-2631.6C+75.261$), where OD was the absorbance and C was concentration as GAE.

The brine shrimp lethality assay was assigned to determine the cytotoxic effect of plant extract. It followed the method by Meyer, *et al.*^[16]. Due to the highest amount of total phenolic compound, the required concentrations (0, 400, 800, 1600, 3200, 6400 and 12800 µg/ml) were prepared through mixing up of the 1 h extraction with variable amounts of 2.5% NaCl. Ten *A. salina* were added into five replicates of each concentration of the leaf extract. The bioassay was maintained at 26±1° throughout the test. The mortality was recorded for a maximum of 24 h of exposure. They were considered dead or moribund if they stopped moving for a prolonged



Fig. 1: Pictures of *Clinacanthus nutans* leaves. *Clinacanthus nutans* in fresh (A) and dry (B) leaves

period even after gentle probing with a small spatula. The LC₅₀ was analyzed by the probit method of Finney^[22] using the SPSS 18.0 (Statistical Package of Social Sciences) software. It estimated the lethal concentration and the slope of the regression line with its confidence interval ($P \leq 0.05$).

The total phenolic compound from leaves of *C. nutans* measurement in each time extraction 0.5, 1, 3, 5 and 24 h were 34.68±3.17, 46.71±9.31, 36.13±7.06, 26.53±8.83 and 30.15±3.09 mg/g GAE, respectively (fig.2). The result of brine shrimp assay was expressed in percentage of mortality. The dose dependent mortality was observed, as the rate of mortality (y) was positively correlated with the concentration (x) of the leaf extract as evident from established regression equations ($y=121.44x+1079.1$). The percentage mortality increased as the concentration of aqueous extract of *C. nutans* increased. The 1 h aqueous extract of *C. nutans* leaf expressed the 24-h LC₅₀ and LC₉₀ values in *A. salina* were 7151.1 and 12008.7 µg/ml, respectively. *C. nutans* showed a significant effect against brine shrimp. The correlation (R²) between concentration and mortality was 0.9767 (fig.3).

There is an increasing interest in the supplementation of antioxidants from a natural plant^[23]. To avoid any solvent effect, the aqueous solvent was used to extract *C. nutans* in the present study. Literature

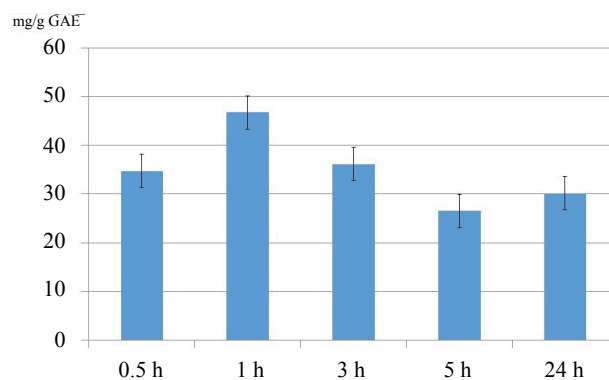


Fig. 2: Total phenolic compounds of *Clinacanthus nutans* leaf extract

Concentration of total phenolic compounds in the leaf extract of *Clinacanthus nutans* expressed as mg/g GAE at each time interval

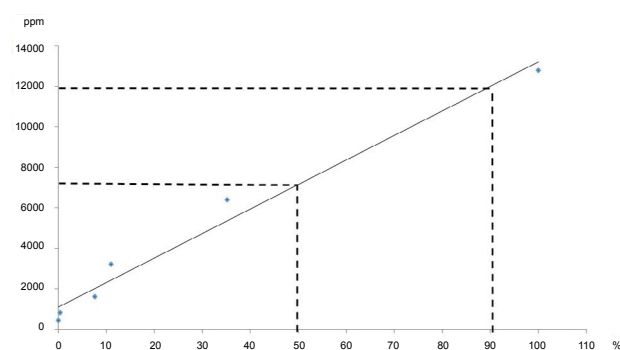


Fig. 3: The correlation between concentration and mortality. The relationship between the percentage of *Artemia salina* dead (X-axis) and *Clinacanthus nutans* leaf extract concentration (Y-axis)

survey has revealed a direct relationship between antioxidant activity and total phenolic content^[24,25]. The present result revealed that the total phenolic compound measurements in 0.5, 1, 3, 5 and 24 h extraction were 34.68±3.17, 46.71±9.31, 36.13±7.06, 26.53±8.83 and 30.15±3.09 mg/g GAE, respectively. These results were in agreement with earlier reports. Thongrakard and Tencomnao^[26] in Thailand extracted *C. nutans* leaves with ethanol at 1:5 (w/v) for 48 h and reported the total phenolic compound measurement was 4.67 mg GAE/g of extract. Yuann, *et al.*^[27] in Taiwan extracted 1 g of *C. nutans* leaves with 5 ml 70% ethanol for 20 mins and reported the total phenolic compound measurement was 23.5 mg GAE/g of extract. Lee, *et al.*^[28] in Malaysia extracted 20 g of *C. nutans* leaves and stems with 500 ml absolute methanol for an hour and reported the total phenolic compound in stem and leaf were 0.12 and 2.68 mg GAE/g of extract, respectively. Wong, *et al.*^[29] in Malaysia extracted *C. nutans* leaves with distilled water at 1:19 (w/v) for an hour and reported the total phenolic compound measurement was 14.70 mg GAE/g of extract.

Using brine shrimp lethality bioassay the tested cytotoxic activity of the aqueous extract of leaves of *C. nutans* were found to show a little toxicity as expressed the 24 h LC₅₀ and LC₉₀ values in *A. salina* were 7151.1 and 12008.7 µg/ml, respectively. Each of the different concentrations samples showed different mortality rates. When graphed, the concentrations versus mortality percentage showed an approximate linear correlation. These results were not in agreement with earlier reports because most of the studies on *C. nutans* cytotoxicity have been done using crude extracts. Various researchers have reported the cytotoxicity of *C. nutans* in different doses, time and solvent extraction. Haetrakul, *et al.*^[30] found that *C. nutans* plant extract at 0.005 and 0.01% damaged Koi Fin cell line and for non-toxic concentration of this plant was 0.001%. Kunsorn, *et al.*^[4] reported the IC₅₀ values of *C. nutans* extracted with *n*-hexane, dichloromethane and methanol against herpes simplex virus-1 were 32.05±3.63, 44.50±2.66, and 64.93±7.00 µg/ml, respectively. Ping *et al.*^[31] reported that the repeatedly dosing of *C. nutans* extract at 0.3, 0.6 and 0.9 g/kg up to 14 days was proven safe in male Sprague Dawley rats without causing any adverse effects and organ damages in rats. Arullappan, *et al.*^[7] reported that the petroleum ether extracts of *C. nutans* demonstrated the strongest cytotoxic activity against HeLa and K-562 cell lines with IC₅₀ of 18.0 and 20.0 µg/ml, respectively. This activity could be explained by the phenols and

flavonoids present in the extract. There are the reports in the literature describing the antimicrobial activity correlated high content of phenolics and flavonoids in *C. nutans* leave extract^[7,32].

In conclusion, the aqueous extract of *C. nutans* can be alternatively used as the natural product. However, further studies are necessary to find out what the active substances are and how they perform or the mechanism of them in the target species.

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CONFLICT OF INTERESTS

None declared.

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