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Artemia salina Lethality and Histopathological Studies of Siam Weed, *Chromolaena odorata*

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Abstract

Siam weed, *Chromolaena odorata*, leaves, stem, and root were extracted with aqueous and ethanol solvents within 1, 3, 5 and 24 hours. The extractions were determined amount of total phenolic compound using Folin–Ciocalteu reagent. The extract that showed the highest amount of total phenolic compound was used for evaluating the cytotoxicity test against *Artemia salina* at varying concentrations as 0, 50, 500, 5,000 and 50,000 ppm. The cytotoxicity data were determined the median and 90% lethal concentration, LC_{50} and LC_{90}, respectively, within 24 hours. The result revealed that the highest amount of total phenolic compound was 198.02+3.96 mg of gallic acid equivalent per gram of aqueous leaf extraction in 24 h. Therefore, the 24-hour aqueous extract of *C. odorata* leaf expressed the 24-h LC_{50} and LC_{90} values in *A. salina* were 43, 551 and 78,391 ppm, respectively. The lesions were observed in intestinal parts such as edema, deformation or elongation of the enterocytes, blebbing cells, and pyknotic cells.

Keywords: *Artemia salina,* Brine Shrimp, *Chromolaena odorata,* Histopathology, Plant, Weed

1. Introduction

Invertebrates can be used to replace the most commonly used laboratory animals[1]. The genus *Artemia* is commonly known as the brine shrimp is a small crustacean, which has a short life cycle and can be studied in large number, a distinct advantage over the vertebrates. It is an important model organism in scientific research, such as toxicological evaluation of nanoparticles[2], heavy metal[3], and plant products[4]. It has been established as a safe, practical and economic method to determine the bioactivity of plant constituents, i.e., Brazilian[5]; Savannah[6]; Indian[7]; Sudanese[8]; and Tanzania[9] medicinal plants. But there are no information about the histopathological changes in this crustacean after exposure of the test substances. In recent years, *Chromolaena odorata* (Family: Asteraceae) formerly known as *Eupatorium odoratum*, has received much attention worldwide due to its wide spectrum of pharmacological activities. *C. odorata* or Siam weed is a scrambling perennial shrub which grows to 2-3 m in height with straight, pithy, brittle stems that branch readily. Phytochemical analysis has shown that *C. odorata* contains flavonoids[10], terpenoids[11], alkaloids[12], essential oils[13], tannins, and saponins[14]. The traditional uses or phytochemical properties of *C. odorata* are anticancer[15], antidiabetic[16], anti-inflammatory[17], antimicrobial[18], and antioxidant activities[19]. This research evaluated the *Artemia salina* lethality and histopathological studies on *Chromolaena odorata* extract.

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2. Materials and Methods

2.1 Plant Collection and Extraction

Fresh leaf, stem and root parts of *C. odorata* were collected from Amnat Charoen Province, Thailand. The plant was washed to remove dust and debris with tap water, dried in sunlight and then placed in 60 ºC hot air oven for 24 h, then ground to fine powder by a domestic grinder. The extraction procedure was determined by the method of Jiraungkoorskul[20]. Five grams of dry powder were extracted with each solvent, 100 ml of distilled water or ethanol on a shaker at 180 rpm for 1, 3, 5, and 24 hours at room temperature. The extract solution was filtered through a fresh gauge plug and centrifuged at 4,000 rpm for 10 minutes. Finally, supernatant was filtered through Whatman filter paper number 1, the clear filtrate used as a stock solution for total phenolic compound measurement and lethality bioassay.

2.2 Total Phenolic Compound Determination

The total phenolic compound was determined using Folin–Ciocalteu reagent according to Jiraungkoorskul[20] and Agbor *et al.*,[21] with modifications. Briefly, the test was done in triplicate, the 50 µl of the aqueous and ethanolic extract (2.5 mg/ml) in each time (1, 3, 5 and 24 hours) was mixed with 250 µl of 10% Folin-Ciocalteu phenol reagent in distilled water and 200 µl of 0.7 M sodium carbonate then added distilled water up to 5 ml and incubated at room temperature for 2 hours in the dark condition. The mixture was measured at 765 nm by using a spectrophotometer. Quantification was based on the standard curve of the gallic acid and expressed as mg of Gallic Acid Equivalent (GAE) per gram of extract.

2.3 Brine Shrimp Lethality Bioassay

The brine shrimp lethality assay was assigned to determine the cytotoxic effect of plant extract[22]. Due to the highest amount of total phenolic compound, the required concentrations (0, 50, 500, 5000 and 50,000 ppm) were prepared by mixing up of the 24-hour aqueous leaf extraction with variable amounts of 2.5% sodium chloride as salt water. *Artemia salina* (n=10) was added into five replicates of each concentration of the leaf extract. The bioassay was maintained at 26±1 ºC throughout the test. The mortality was recorded for a maximum of 24 hours of exposure. They were considered dead or moribund if they stopped moving for a prolonged period, even after gentle probing with a small spatula. The LC_{50} was analyzed by the Probit method[23] 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 ($p \leq 0.05$).

2.4 Specimen Preparation for Light Microscopic Study

The histology procedures were performed with following the methods[24]. Briefly, *A. salina* was fixed in 10% buffered formaldehyde for 24 h, dehydrated through a graded series of ethanol, and cleared with xylene solutions. It was embedded in a block using melted paraffin at the embedding station. The paraffin blocks were sectioned at 5 µm thickness using a rotary microtome and stained with Harris's hematoxylin and eosin. The glass slides were examined for abnormalities using the Olympus CX31 light microscope and photographed by a Canon EOS 1100D digital camera.

3. Results

The total phenolic compound from leaf, stem, and root of *C. odorata* aqueous and ethanol determined in each time extraction 1, 3, 5 and 24 hours are shown in Table 1. The highest amount of total phenolic compound was 198.02±3.96 mg of gallic acid equivalent per gram of aqueous leaf extraction in 24 h.

The result of the brine shrimp assay was expressed in percentage of mortality. The dose dependent mortality was observed, as the percent of mortality (x) was positively correlated with the concentration (y) of the leaf extract as evident from established regression equations (y=871.02x). The percentage mortality increased as the concentration of aqueous extract of *C. odorata* increased. The 24-hour aqueous extract of *C. odorata* leaf, expressed the 24-h LC_{50} and LC_{90} values in *A. salina* were 43,551 and 78,391 ppm, respectively.

An adult *A. salina* has an elongated body with big two eyes in front, a linear intestinal tract, and 11 pairs of appendages alongside the body (Fig. 1A). In the control group, the intestinal tract was a tubular structure which

Table 1: Total phenolic compound from leaves, stem, and root of *C. odorata* aqueous and ethanolic determine in each time extraction 1, 3, 5 and 24 hours

Plant part	Solvent	Total phenolic compound in each time (mg of gallic acid equivalent per gram of extract)			
		1h	3h	5h	24h
Leaf	aqueous	193.44±2.19	194.11±1.86	195.67±5.07	198.02±3.96
	ethanol	126.62±4.18	117.81±10.83	123.16±7.25	141.46±7.04
Stem	aqueous	41.72±1.27	43.17±2.92	49.09±2.02	53.21±2.41
	ethanol	1.34±0.89	2.23±1.84	2.90±1.51	4.91±0.51
Root	aqueous	38.38±2.23	38.82±2.09	42.84±2.61	45.29±4.56
	ethanol	6.92±5.32	3.79±0.51	7.92±3.55	10.15±1.08

is composed of a single columnar cell layer of enterocytes (Fig. 1B). The histopathological lesions after exposure to *C. odorata* were shown edema (Fig. 1C), deformation or elongation of the enterocytes (Fig. 1D), blebbing cells (Fig. 1E), and pyknotic cells (Fig. 1F).

4. Discussion

The interests of natural phenolic compounds are increasing because several researches have revealed a direct relationship between antioxidant activity and phenolic compounds[25,26]. The present study revealed that the 24 h extraction of *C. odorata* showed the highest amount of total phenolic compound in these following leaf > stem > root part, and the aqueous solvent showed higher amount than that of ethanol solvent. The highest amount of total phenolic compound was 198.02±3.96 mg of gallic acid equivalent per gram of aqueous leaf extraction in 24 h.

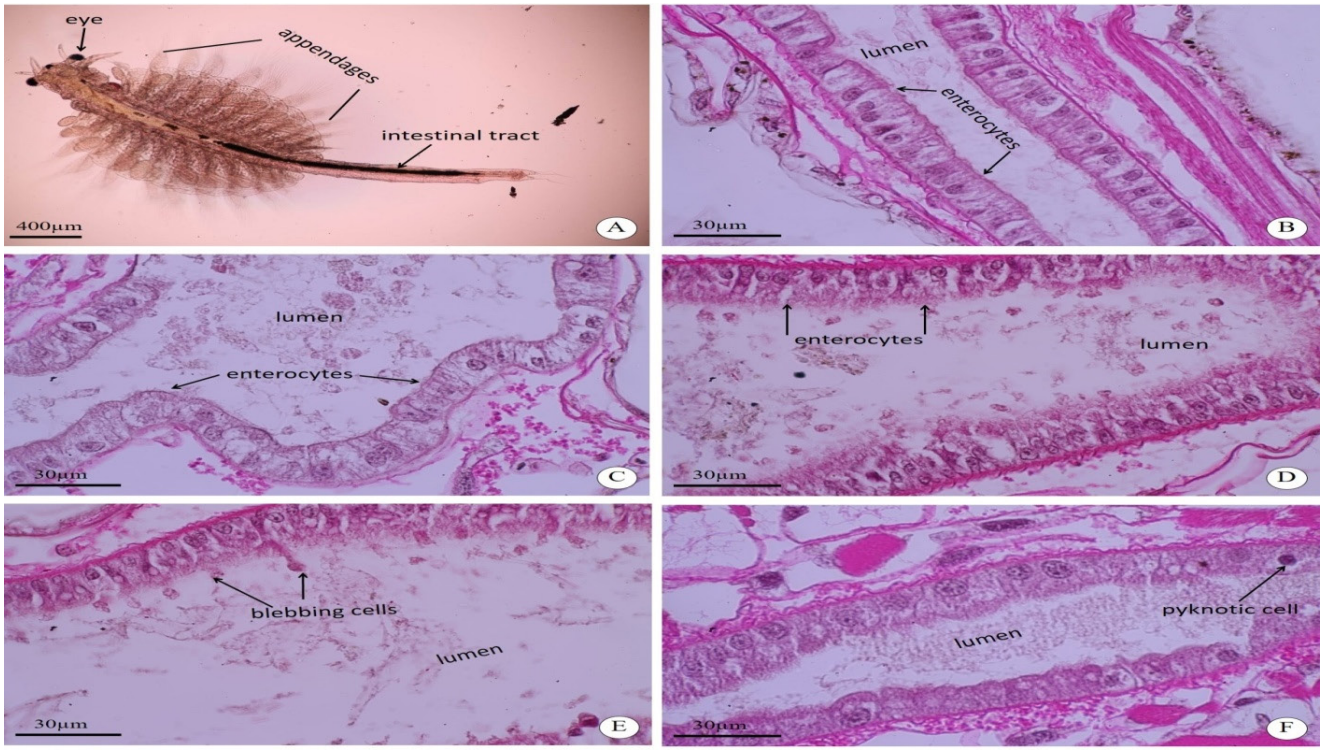

Fig. 1. *Artemia salina* (A) gross morphology showing an eye, appendages and intestinal tract; (B) longitudinal section of control midgut showing a single columnar cell layer of enterocytes; (C) histopathological studies of *C. odorata* aqueous leaf extract showing edema; (D) deformation or elongation of the enterocytes; (E) blebbing cells; and (F) pyknotic cells.

The phenolic compounds in *C. odorata* were earlier reported in different plant parts and different solvent extractions. Rao *et al*.,[26] extracted the 50 g of air-dried leaves of *C. odorata* from India with 500 ml of chloroform and reported the total phenolic compound determined was 242 mg GAE/g of extract. Krishanti *et al*.,[27] reported the total phenolic compound determined was 455.55 mg GAE/g of methanol extract. Balakrishna *et al*.[28] reported the total phenolic compound determined of root bark from India was 313.31±2.88 mg GAE/g of ethyl acetate extract, 246.16±1.05 mg GAE/g of methanol extract, and 125.00±2.18 mg GAE/g of aqueous extract. Hanphakphoom *et al*.,[29] extracted the 20 g of dried three plant parts (leaves, stem and root) from Thailand with 500 ml of four solvents (water, ethanol, methanol, and hexane). The maximum content of total phenols was found in the ethanol leaf extract, followed by water, methanol, and hexane. The phenolic compounds in different parts of *C. odorata* were proved to have powerful protection from oxidative damage[30]. The phenolic compounds were reported the antioxidant activity by different mechanisms such as reduction electrons, hydrogen donation and by quenching the singlet oxygen or decomposing peroxides.

Using brine shrimp lethality bioassay tested the cytotoxic activity of the aqueous extract of leaves of *C. odorata* were found to show a little toxicity as expressed the 24-h LC_{50} and LC_{90} values in *A. salina* were 43,551 and 78,391 ppm, respectively. The lethal concentration value of this plant suggested it is a relatively nontoxic plant. The classification of toxicity based on LC_{50} values stated by the Organization for Economic Co-operation and Development[31] is as follows: very toxic ≤ 5 mg/kg; 5 > toxic ≤ 50 mg/kg; 50 > harmful ≤ 500 mg/kg; and 500 > no label ≤ 2000 mg/kg. Therefore, an LC_{50} of more than 2000 mg/kg of plant extract is an indication that *C. odorata* leaf extract does not possess any toxic effects. Each of the different concentration samples showed different mortality rates. When graphed, the concentrations versus mortality percentage showed an approximate linear correlation.

There were the earlier reports on *C. odorata* cytotoxicity using crude extracts. Asomugha *et al*.,[32] extracted *C. odorata* leaves from Nigeria with water and ethanol (1:10 w/v) for 24 h. The cytotoxicity to brine shrimp showed LC_{50} values of 324 and 392 ppm for aqueous and ethanol extracts, respectively. Olowa and Nuneza[33] extracted 20 g of *C. odorata* leaves from the Philippines with 250 ml of ethanol for 48 h. The brine shrimp lethality concentration LC_{50} value was 10 ppm. This activity could be explained by the phytochemical components present in the extract like alkaloids, flavonoids, and phenolics. There are no report of histological changes after expose with *C. odorata* extract. The morphological characteristics of the digestive tract of *A. salina* in the present study were similarly with the earlier reports[34-36]. In this study, histopathological alterations were observed in the intestinal tract including edema, swelling, and the deformation or elongation of enterocytes. Moreover, cells protruding into the lumen or blebbing cells and pyknotic cells were also found in some areas. Because of a little data about brine shrimp histopathological analysis, these tissue changes were in similar appearance with our previous reports[37,38], suggested that its phytochemical compounds led to morphological damage in the enterocytes of the intestinal tract, which is likely where these substances are absorbed. Regardless of the type of substance used, the similarity of the detrimental changes in the organism indicates that these alterations are a common response to cellular toxicity. In conclusion, *Artemia* lethality and histopathological studies can be the alternative tool for evaluating the natural product or chemical substances.

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6. Conflict of Interests

The authors do not have any conflict of interest to declare.

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