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Acute and Cytotoxicity Studies of Aqueous and Ethanolic Leaf Extracts of *Chromolaena odorata*

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ABSTRACT

Chromolaena odorata, a commonly used traditional remedy for different ailments, believed to be quite safe in terms of toxicity was evaluated for acute toxicity and cytotoxic potentials. Acute toxicity was done on albino Wistar rats using the Lorke method while brine shrimps were used to test for cytotoxicity. The results showed that the estimated LD₅₀ for the aqueous and ethanolic extracts was 2154 and >5000 mg kg⁻¹ body weight, respectively. Cytotoxicity to brine shrimps showed LC₅₀ values of 324 and 392 ppm for aqueous and ethanolic extracts, respectively. These results indicate the relative non toxic nature of *Chromolaena odorata* extracts.

Key words: *Chromolaena odorata*, acute toxicity, cytotoxicity

INTRODUCTION

Fossil records date human use of plants as medicines to at least the middle Paleolithic age-about 60,000 years ago (Solecki, 1975). Sequel to an age-long efficacy demonstrated by quite a number of medicinal plants, almost 80% of the world's population have incorporated them into their primary modality of health care (Farnsworth *et al.*, 1985). Different portions of medicinal plants (root, stem, leaves, seeds and flowers) contain active constituents (Fett Neto and DiCosmo, 1992) and have been used as treatment remedies (Pala *et al.*, 2010).

Chromolaena odorata (Fig. 1) is abundant and widespread in nature. Mabberley (1997) reported that about 165 species of the plant *Chromolaena* are distributed in tropical and warm temperate regions. It is widely used in Nigeria in traditional medicine practice as an anti-malaria remedy, it can also be traditionally applied to wounds to stop bleeding. In Vietnam and other tropical countries, fresh leaves or decoction of the leaves are used for treatment of leech bites, soft tissue wounds, burns, dentoalveolitis and skin infections (Phan *et al.*, 2001). Studies carried out so far on the aqueous and ethanolic extracts of *Chromolaena odorata* have provided enough scientific justification for its use in traditional medicine. There are

reports from India, the Philippines and Togo showing that *Chromolaena odorata* leaf extracts exhibited anti-cancer activity on human and mouse cell lines (Vital and Rivera, 2009). Prabhu *et al.* (2011), isolated essential oil from fresh leaves of *Chromolaena odorata* which was screened for *in-vitro* cytotoxicity against human cervical cell line (HeLa), human laryngeal epithelial carcinoma cells (HEp-2) and (NIH-3T3), mouse embryonic fibroblast cancer cell lines; their reports showed that essential oil isolated from fresh leaves of *Chromolaena odorata* consisting mainly of 5, 6-diethenyl-1-methyl-cyclohexene (44.7%), β-guiane (11.9%), elemol (8.5%) and patchoulene (8.6%), had significant cytotoxic effect with IC₅₀ values of 60.3, 67.5 and 72.00 μg mL⁻¹ towards HeLa,

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**Chromolaena odorata* (Asteracea) has many common names: siam weed, christmas bush, devil weed...

Popularly known as "Abani diegwu" in the South East of Nigeria

*The leaves are used in traditional medicine for the treatment of malaria and cough



Fig. 1: A branch of *Chromolaena odorata* plant with flowers

HEp-2 and NIH 3T3 cancer cell lines, respectively. Earlier reports by Koba *et al.* (2009), on the cytotoxicity potential of essential oil of *Chromolaena odorata* on human epidermis cell line Hecate, showed that it had a moderate cytotoxicity potential with IC₅₀ of 700 µL mL⁻¹ mainly as a result of its neural component.

MATERIALS AND METHODS

Collection and identification of plant material: The leaves of the plant *Chromolaena odorata* were collected from a local farm in Otolu Nnewi, Nigeria. The plant material was identified by Prof. J. C. Okafor of Tree Crops and Tropical Ecology Center, No. 7 Dona Drive, Off Ihiala Street, Independence Layout, Enugu, Nigeria. Voucher specimen of the plant has been deposited at the Department of Biochemistry, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

Preparation of the plant material: The leaves of the plant were air-dried and ground into fine powder using a blender. Water extraction was done with deionized water (1:10 w/v) by boiling for 5 min after which it was allowed to cool. The ethanolic extract was obtained by cold maceration of the ground samples in 95% ethanol (1:10 w/v) for 24 h. The mixtures were thereafter filtered after each extraction, respectively and freeze dried. The samples were then placed in airtight containers and refrigerated.

Phytochemical screening: The aqueous and ethanolic extracts of *Chromolaena odorata* were subjected to phytochemical analysis (Harborne, 1984), to assess for the presence of phytochemical constituents. Quantitative analysis of the constituents was done using HP6890 Gas chromatography coupled with flame ionizing detector and powered by HP station Rev. A.09.01 [1206] software. Result is as shown in Table 1.

Acute toxicity study: Aqueous and ethanolic extracts of *Chromolaena odorata* leaves were tested orally for acute toxic effect (after phytochemical screening was done), using the method proposed by Lorke (1983). The method comprises two phases, phase 1 and 2.

In phase 1 nine Wistar albino rats were divided into groups 1, 2 and 3, of 3 animals per group and doses of 10, 100 and 1000 mg kg⁻¹ body weight of aqueous extracts were administered to the three groups of animals, respectively. The animals were then monitored for signs of toxicity and death within 24 h.

As there was no death recorded, the experiment proceeded to phase 2 where nine Wistar albino rats were also divided into groups 5-7, of three animals per group. The groups consequently received 1600, 2900 and 5000 mg kg⁻¹, respectively and again monitored for another 24 h for sign of toxicity and death.

Table 1: Quantitative phytochemical analysis of various fractions present in ethanolic and aqueous extracts of *Chromolaena odorata* (Asteraceae laeves) (mg/100 g)

Fraction	Ethanolic extract	Aqueous extract
Flavonoid	2314.47	2078.13
Terpenes	196.63	17.12
Tanins	8.93	7.77
Anthraquinone	23.11	19.39
Sterols	7.12	1.34
Saponins	466.62	1759.12
Glycosides	36.94	41.42
Protease inhibitors	30.92	51.75
Alkaloid	3.75	2.06
Allicin	2.01	0.54

The median lethal dose (LD₅₀) was estimated as the geometric mean of the least dose which did not kill any of the animals and that which killed all the animals.

Brine shrimp lethality bioassay: The LC₅₀ of the aqueous extract was determined using the method of Meyer *et al.* (1982).

Hatching: About 50 mg of *Artemisia salina* (Leach) eggs, (Interpet. Ltd. England) was added to about 150 mL solution of sea water in a beaker. The mixture was allowed to incubate for 48 h in a warm well ventilated room (22-29°C) under a light source. Larvae (nauplii) were collected with a Pasteur pipette after they had been attracted by the light source.

Brine shrimp assay: Twenty milligrams of the aqueous extract was dissolved in 1 mL of distilled water and dilutions were made to obtain concentrations corresponding to 10, 100 and 1000 ppm in 500 µL of each solution sample. Ten shrimps were transferred into each test-tube and the samples were added. The final volume was made up to 5 mL using sterilized sea water. Each sample was tested in triplicate and incubated for 24 h and finally, the tubes were examined under a magnifying glass and the number of dead shrimps was counted. The mortality percentage of each concentration was calculated as:

$$\text{Death (\%)} = \frac{\text{No. of dead shrimps}}{\text{No. of survival shrimps in control}} \times 100$$

This data was represented graphically by plotting log of concentration against percentage death (mortality) from which LC₅₀ for each sample was determined by drawing regression line calculated using regression analysis.

RESULTS

Phytochemical screening of *Chromolaena odorata* extracts: The relative abundance of the phytochemicals as shown in the aqueous extracts was in the following order: Flavonoids>Saponins>Protease inhibitors>Glycosides>

Table 2: Determination of the LD₅₀ of the aqueous and ethanolic extract of *Chromolaena odorata* in rats

Group of rats	Dose of extract (mg kg ⁻¹ body weight)	No. of deaths recorded for aqueous extract	No. of deaths recorded for ethanolic extract
Phase 1			
1	10	Nil	Nil
2	100	Nil	Nil
3	1000	Nil	Nil
Phase 2			
4	1600	Nil	Nil
5	2900	3	Nil
6	5000	3	Nil

Table 3: Toxicity study of ethanolic and aqueous leaf extracts of *Chromolaena odorata* against brine-shrimp nauplii

Sample and concentration (µg mL ⁻¹)	Log concentration	Lethality (%)	LC ₅₀ (µg mL ⁻¹)
Crude aqueous extract			
10	1	33.3	324***
100	2	56.7	
1000	3	66.7	
Crude ethanolic extract			
31.25	1.4949	16.7	392*
62.50	1.7959	25.0	
125	2.0969	34.8	

Anthraquinones>Terpenes>Tannin>Saponin >Alkaloids>Allicin. Ethanolic extracts also showed similar pattern but more terpenes and less alkaloid, are reflected in Table 1.

Acute toxicity study of *Chromolaena odorata*: The result of the acute toxicity study are presented in Table 2.

The result obtained in the study showed that high doses of oral administration of *Chromolaena odorata* aqueous extract produced symptoms of toxicity and behavioral changes. The animals manifested visible signs of toxicity and recorded mortalities of three animals each for the dose groups of 2900 and 5000 mg kg⁻¹ body weights, respectively in the phase 2 test. Some of the behavioral changes noticed included; loss of spinal reflex, raising of furs, diarrhea and gait disturbances. However, the phase 1 test did not record any mortality.

The ethanolic extract did not exhibit any visible toxic effect or behavioral changes. Hence, no death was recorded following the oral administration of the ethanolic extract as seen in Table 2. The median lethal doses (LD₅₀) of the aqueous and ethanolic extracts were 2154 and >5000 mg kg⁻¹, respectively in male Wistar albino rats.

Table 3 shows that the degree of lethality was most with the highest concentration. It also exhibited lethality pattern that was found to be directly proportional to the concentration of the extracts.

Aqueous and ethanolic leaf extracts of *Chromolaena odorata* were tested for toxic activities using brine shrimp lethality bioassay. The results are presented in Table 3 and Fig. 2. The result from the brine shrimp lethality bioassay show that the amount of *Chromolaena odorata* (aqueous and ethanolic extracts) needed for 50% cytotoxicity was 324 and 392 µg mL⁻¹, respectively.

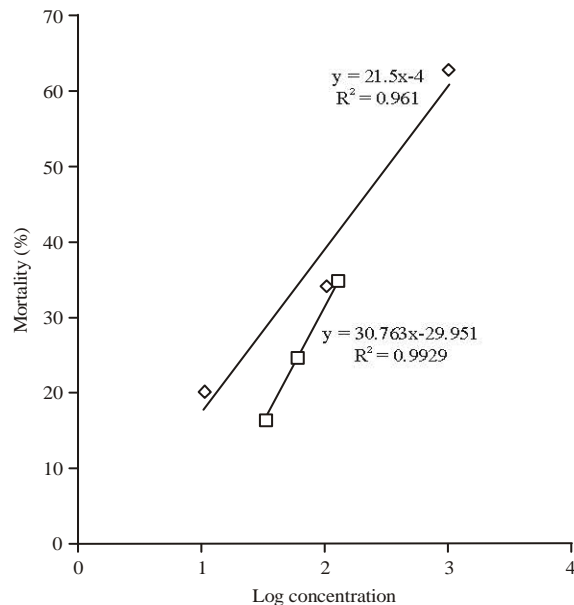


Fig. 2: Log concentration versus average percentage mortality of nauplii of aqueous and ethanolic extracts of *Chromolaena odorata* leaves

DISCUSSION

In the acute toxicity study, the LD₅₀ (median lethal dose) was found to be greater than 5000 mg kg⁻¹ for the ethanolic extract and 2154 mg kg⁻¹ for the aqueous extract. This difference appears quite significant and probably could be accounted for by the differential amounts of the phytochemical components of the fractional extracts. While the ethanolic extract had greater concentrations of alkaloids, glycosides, sterols, allicin, tannins, terpenes and flavonoids, the aqueous extract had a greater concentration of protease inhibitors and saponins. The greater concentrations of protease inhibitors and saponins that are well known potentially toxic substances may also contribute to this greater toxicity observed with the aqueous extract.

Leeuwen and van Vermeire (2007), suggested that LD₅₀ of chemical substances that are less than 5 mg kg⁻¹ as highly hazardous, <50 mg kg⁻¹ as hazardous, <500 mg kg⁻¹ are moderately toxic and <5000 mg kg⁻¹ as slightly toxic or having no significant toxic effect.

Based on this report, we suggest the aqueous and ethanol extracts of *Chromolaena odorata* are not significantly toxic. We therefore conclude that the extracts are relatively safe.

The brine shrimp lethality assay represents a rapid, inexpensive and simple bioassay for testing plant extracts bioactivity which correlates reasonably well with cytotoxic and anti-tumor properties (McLaughlin *et al.*, 1993). In this study, toxicity testing against brine shrimp was conducted using both the aqueous and ethanolic extracts of *Chromolaena odorata* plant species collected from Nnewi Nigeria. The extracts showed non-significant cytotoxic activities with

LC₅₀ values of 324 and 392 µg mL⁻¹ with aqueous and ethanolic extracts, respectively. Moshi *et al.* (2010) investigated the brine shrimp toxicity of 30 medicinal plants in Tanzania concluded that extracts with LC₅₀ of <500 µg mL⁻¹ can be safely said to be practically non-toxic while extracts with LC₅₀ >100 µg mL⁻¹ but <500 µg mL⁻¹ can be classified as non-toxic. Progressive percentage of toxic responses was observed in this study, being highest with the highest extract concentration of 1000 µg L⁻¹. Similar activities had been reported of *Vernonia Amygdalina* which is of the same family Asteraceae. This has been attributed to the presence of sesquiterpene lactone (Kupchan *et al.*, 1969). Also, Lagnika *et al.* (2011) reported in their studies that extracts giving LC₅₀ values higher than 100 µg mL⁻¹ are said to exhibit very low toxicity. All these tend to agree with the relative low toxicity associated with *Chromolaena odorata* in our study.

The finding in this study is suggestive that the extract may possess some cytotoxic activities at higher dose (1000 µg L⁻¹) and therefore, may have a potential for therapeutic investigation. We therefore conclude that the extract is relatively safe for consumption.

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