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Phytochemical and Oral Toxicity Studies of *Chromolaena odorata* L. (King and Robinson)Leaf Extract

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ABSTRACT

In this study, the ethanolic leaf extract of *Chromolaena odorata* was analyzed for qualitative and quantitative composition and evaluated for oral toxicity in Swiss Webster albino mice. The mice were grouped into two and tested for acute (fixed single dose of 2000 and 5000 mg/kg) and sub-acute (daily dose of 250 and 500mg/kg extract for 28 days) toxicity. Animal behavior, body weight, morbidity, and mortality were monitored for 14 days (acute) and 28 days (sub-acute), respectively. Hematologic and blood chemistry parameters (alanine transaminase (ALT), blood urea nitrogen (BUN), creatinine) were measured and analyzed. The mice were sacrificed and necropsied at the end of the study, and organ weights were analyzed. Based on the phytochemical analysis, *C. odorata* contained phenols, flavonoids, tannins, saponins, and anthraquinones. Total phenolic and flavonoids were 146.69±10.25 mg gallic acid equivalent (GAE/L) and 25.75±2.64 mg quercetin equivalent (QE/g). A single dose of the extract (2000-5000mg/kg) is non-lethal but causes temporary toxicity signs. Repeated doses (250-500 mg/kg) caused low ALT, mortality (500 mg/kg), tachypnea, dry hair coat, and alopecia. Both acute and sub-acute toxicity of the leaf extract hampers growth.

Keywords: Chromolaena odorata, ethanolic, phytochemicals, oral toxicity

INTRODUCTION

In rural areas where commercially prepared drugs appear to be scarce and costly, people often resort to herbal medicines to treat illnesses. One plant that is traditionally used to treat ailments is C. odorata. The plant is herbaceous to woody perennial with a bushy habit (Crutwell, 1989; Gautier, 1992). It is popularly known as "hagonoy" in the Philippines. The plant medicinal properties include relief of fever, lethal on mites and ticks, heart tonic, antimicrobial, expel worms, wound healing, relieve muscle spasms, astringent, anti-inflammatory, diuretic, antihypertensive and potential treatment for diabetes mellitus (Iwu et al., 1993; Bunyapraphatsara et al., 2000; Vital & Rivera, 2004; Panda et al., 2010; Kikiowo et al., 2020). Medicinal and toxicological actions are presumed to be produced by the phytochemical components of plants, which, depends upon the variety, growing conditions, cooking, and processing (Saxena et al., 2013). Putri et al. (2019) revealed several antioxidants contained in leaves including new flavonone odoratenin. C. odorata leaf can serve as the potential sources of fatty acids (Alara et al. (2019). Several authors cited plants component high on phenolic and flavonoid content is responsible for its medicinal value (Atmani, 2009; Khodammi, 2013; Tapas, 2008).

Several studies of C. odorata extract reported to decreased ALT, AST, and alkaline phosphatase levels in albino rats (Anyanmu et al., (2017) while Asomugha et al. (2013) and Yakubu et al. (2012) reported elevated liver enzymes. The presence of protease inhibitors and saponins (Asomugha et al., (2015) and pyrrolizidine alkaloids (Fu et al., 2002) makes the extract potentially toxic. Pyrrolizidine alkaloids are carcinogenic and hepatotoxic chemicals. Some authors noted poisonous effects of the plant, such as changes in physical behavior and deaths in rats and destruction of cells in brine shrimps (Ogbonia et al., 2010; Asomugha et al., 2015). Other researchers reported elevated BUN and creatinine and abnormal intestinal histology of albino rats (Anyanwu et al., 2017), and it induces cancer and destructive to liver cells (Fu et al., 2002). Asomugha et al. (2015) reported that C. odorata ethanolic extract is relatively non-toxic with an LD50 of >5000 mg/kg.

Existing studies on C. odorata suggest possible medical use, yet toxic effects are not well documented especially using the standard OECD guidelines. Hence, the present study determined C. odorata ethanolic leaf extract bioactive components and evaluated for acute and sub-acute oral toxicity in Swiss Webster albino mice.

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METHODOLOGY

Plant Collection and Preparation

Five (5) kilograms of *C. odorata* mature healthy leaves (dark-green coloration, fully expanded) were collected, clean with flowing water, and rinsed with distilled water. The leaves were air-dried for five days and were subjected to size reduction using a mechanical grinder until the coarse powder was obtained. Powdered leaves were soaked in 95% ethanol for three days. Extracts were then filtered, and filtrates were concentrated in a vacuum using a rotary evaporator. The leaves were selected and collected from Musuan, Maramag, Bukidnon (7.8706°N, 125.0691°E).

Animals

Swiss Webster albino mice (12 females and 15 males; 6-8 weeks;15-30g) were obtained from the Philippine Institute of Tropical Medicine (PITAHC). The animals were kept in plastic and wire cages with wood scrape as beddings, acclimatized for one week, and maintained at room temperature (22±2°C) and relative humidity (45-65%) under 12h night and 12h light cycle. All animals were regularly cleaned, fed with a pelleted diet, and provided with water ad libitum. The research complied with the requirements of the Institutional Animal Care and Use Committee of the College of Veterinary Medicine, Central Mindanao University, Philippines.

Phytochemical analysis

Test for Alkaloids

Three ml of the ethanolic extract was added in a test tube and mixed with 1 ml sulfuric acid, and allowed to stand. The mixture was added with 2-3 drops of Dragendorff's reagent. A yellow-orange precipitate indicates the presence of alkaloids.

Test for saponins

In a test tube, 1 ml of the extract was gradually added with six concentrated sulfuric acid drops. A yellow to a red color reaction within 30 minutes, followed by violet or blue-green, indicates saponins' presence.
Test for steroids and terpenoids

A mixture of 1 ml of the extract and 1 ml of acetic anhydride was made and put in an ice bath in a test tube. Then 1 ml of the concentrated sulfuric acid was gradually added. A red color reaction indicates the presence of terpenoids and bluish green for steroids.

Test for anthraquinones

A mixture of 1 ml of the extract was made in a test tube with 0.5 ml of 5% KOH in methanol. The orange color reaction indicates the presence of anthraquinones.
A mixture of 0.5 ml of the extract was made with 1 ml water in a test tube and added with 1-2 drops of 10% aqueous ferric chloride. The blue color indicates gallic tannins, and the green-black color indicates cathecholic tannins.

Determination of total flavonoid content

The aluminum chloride method using quercetin as standard was used for the determination of the total flavonoid content. A mixture of 4 ml distilled water, and 1 ml of the extract was initially mixed in a volumetric flask, and 0.5 ml of 5% $NaNO_2$ was added. After 5 minutes, the mixture was added with 0.3 ml of 10% $AlCl_3$. After 6 minutes, 2 ml of 1M NaOH was added, and distilled water was added to make 10 ml volume. Absorbance was measured against blank at 430 nm, and total flavonoid content is expressed as quercetin equivalent in mg/g extract (mg QE/g).

Determination of total phenolic content

The total phenolic content (TPC) was determined using the Folin-Ciocalteu assay (Singleton and Rossi, 1965). The extracts were dissolved in a DMSO (0.1%): methanol: water (15:5:2) solution at 2 mg/ml. A volume of 20 ml of the mixture was then added with Folin-Ciocalteu reagent (1:10) and was shaken. A 5% sodium carbonate solution was added after 30 minutes. The microplate was incubated at room temperature for 2 hours, then the absorbance at 750 nm using spectra Max250 was measured. Gallic acid was expressed as mg gallic acid equivalent (GAE) per liter.

All measurements were triplicated.

Acute oral toxicity test

The extract was dissolved with Dimethyl Sulfide (DMSO, 0.1%). The acute oral toxicity was conducted as per OECD 423 guidelines. Twelve (12) female Swiss Webster albino mice rats were randomly allocated into four (4) groups of three (3) mice each. Each mouse per treatment group was singly gavage with distilled water, DMSO (0.1%), 2000 mg/kg extract, and 5000 mg/kg extract, respectively. The mice were monitored daily for signs of toxicity and mortality for 14 days. Immediate observations for physical behavior changes and mortality were done, commencing at 30 minutes after dosing, every hour for the first day, and daily for two weeks. Animals were weighed twice, initially at the start and finally at the end of the study. At the study's termination, all surviving animals were sacrificed by cervical dislocation, necropsied, and gross examination of organs was performed.

Sub-acute oral toxicity test

The extract was dissolved with 10% Tween-20. Fifteen (15) male Swiss Webster albino mice were utilized for the 28-day oral toxicity test. The mice were randomly distributed into three treatment groups of 5 mice each. The treatment group was gavage daily with 10% tween-20 solution, 250 and 500 mg/kg extract, respectively, for 28 days.

The animals were weighed weekly and observed daily for toxicity signs, e.g., mortality, behavioral, and physical changes. On the 28th day of treatment, animals were given an anesthetic (Tiletamine+Zolazepam), and blood samples were collected via intracardiac puncture. Plasma and serum samples of each mouse were subjected

Table 1

Chromolaena odorata ethanolic leaf extracts bioactive components.

Components	Result
Alkaloids	-
Tannins	+
Saponins	+
Terpenoids and Steroids	-
Anthraquinones	+

Legend: (+) present; (-) absent

Table 2

Chromolaena odorata ethanolic leaf extracts phenolic and flavonoid content.

Components	Result
Total phenolic content	146.69+10.2 mg GAE/L
Total flavonoid content	25.75+2.64 mg QE/g

Legend: gallic acid equivalent (GAE/L); quercetin equivalent (QE/g)

to hematological and biochemical analysis, respectively. Animals were then subjected to euthanasia by cervical dislocation. Gross examination and weighing of the organs (liver, kidney, spleen, heart, and intestine) were done (OECD, 2008). The relative organ weight (ROW) of each animal was calculated as follows:

$$ROW = \frac{absolute\ organ\ w}{t./\ b.\ wt.\ of\ the\ animal\ on\ sacrifice\ day}\ x\ 10$$

Statistical analysis

Measurable data are expressed as mean±SD. In the acute toxicity test, initial and final body weight data were analyzed using a t-test. One way analysis of variance was used to test the difference between treatment groups in acute and sub-acute toxicity tests. Where significant differences were observed, the Tukey's Post Hoc test was used to identify and compare the differences between treatment means. Measured values were considered significant if $p<0.05$.

RESULTS AND DISCUSSION

Phytochemical Analysis

Analysis of the plant ethanolic leaf extract bioactive components revealed the presence of phenols, flavonoids, tannins, and saponins, while alkaloids, terpenoids, and steroids were absent (Table 1). The study demonstrated high levels of total flavonoids and total phenolics (Table 2). The finding on the absence of alkaloids contradicts Fu et al.'s (2002) report that the plant contains toxic pyrrolizidine alkaloid.

Demonstration of phytochemicals in the extract with high phenolic and flavonoid content confirms the plant's medicinal value (Atmani et al., 2009; Khodammi et al., 2013; Tapas et al., 2008). However, saponins' presence makes the extract potentially toxic, as reported by Asomugha et al. (2015).

Acute oral toxicity

Within 30 minutes after a single administration of the extract (2000 mg/kg and 5000 mg/kg), the mice exhibited temporary erection of hairs, tachypnea, lacrimation, excessive saliva, retching, unusual sound, lethargy, and lack of touch response, which disappeared within a day (Table 3). This finding agrees with the study of Ogbonia et al. (2010) and Asomugha et al. (2015) that a high dose of *C. odorata* extracts causes signs of toxicity. However, Ogbonia et al. (2010) toxicity observations were limited to 72 hrs. post treatment, which varied with the 14 days observation time recommended by the OECD.

At two weeks of observation, the mice showed no persistent signs of abnormality in behavior and physical attributes. Morbidity nor mortality was not observed; thus, the LD50 was estimated to be >5000 mg/kg, similar to the findings of Asomugha et al. (2015); study of Ogbonia et al. (2010) revealed LD50 of 16.50 g/kg body weight. Though the mice's body weight initially increased in all groups, it is less pronounced with the extract-treated groups versus the control (Table 4). However, the weight difference among treatment groups is not statistically significant ($p>0.05$). This finding indicates that the extract exhibited a negative effect on growth. Although gross organ lesions were not observed at necropsy and in the absence of histopathologic examination, it is premature to dismiss toxicity suspicion.

These findings indicate the relative safety of a single oral dose of the extract. Based on the rating of toxic chemicals, a single dose of *C. odorata* ethanolic leaf extract is slightly toxic (LD50, >5000 mg/kg).

Table 3

Behavioral and physical observations in mice given with a single oral dose of Chromolaena odorata ethanolic leaf extract.

Observations	DH^2O	DMSO (0.1%)	*C. odorata* ethanolic leaf extract	
			2000 mg/kg	5000 mg/kg
Skin color	n	n	n	n
Piloerection	-	-	+	+
Tachypnea	-	-	+	+
Stool	n	n	n	n
Lacrimation	-	-	+	+
Salivation	-	-	+	+
Retching	-	-	+	+
Vocalization	-	-	+	+
M. membrane	n	n	n	n
Palpebral opening	n	n	n	n
Tremor	-	-	-	-
Staggering gait	-	-	-	-
Grip	n	n	n	n
Opisthotonos	-	-	-	-
Lethargy	-	-	-	+
Touch response	n	n	++	++
Morbidity	-	-	-	-
Mortality	-	-	-	-

Legend: (n) normal; (++) abnormal (-) absent; + (present)

Table 4

Body weight of mice given a single dose of *C. odorata* ethanolic leaf extract.

Weight	DH^2O	DMSO (0.1%)	*C. odorata* ethanolic leaf extract	
			2000 mg/kg	5000 mg/kg
Initial b. wt	26.66 ±1.1	24±2.2	25.33 ±0.5	24.1 ±0.1
Final b. wt. ^{ns} (Pvalue=0.07)	29.67 ±2.1	26±2.3	26.33 ±0.6	24.33±4
T-test on body wt. ^{ns}	0.12	0.28	0.10	0.89
% Difference in body wt.	11.15	8.36	3.93	0.90

Legend: Values are expressed as mean ± SD; ns – not significant

Subacute oral toxicity

Initially, the mice were repeatedly dosed with the extract at 1000-2000 mg/kg; however, several morbidity and mortality were noted after a few days. Based on OECD guidelines, the fixed-dose was then reduced to the next lower level.

Repeated oral administration for 28 days of 250-500 mg/kg *C. odorata* ethanolic leaf extract to the mice showed no marked abnormality on most of the behavior and physical parameters. However, some signs of toxicity were observed (Table 5). After dosing, the mice showed heavy breathing for two days, persistent dry hair coat, and alopecia on the ventral region commencing on day 9 of treatment. One (1) mortality occurred at day 20 to the group that received 500 mg/kg extract, exhibiting liver congestion, and distension of intestines upon necropsy. This finding indicates that repeated administration of the extract caused significant signs of toxicity. It agrees with Asomugha et al. (2015) that *C. odorata* extract causes physical abnormality and mortality. However, no gross lesions were observed in all mice that survived the study's entire duration at necropsy.

The bodyweight of mice's dosed with the extract progressively improved, the increase is much lesser and declined at day 28 compared to the control group (Table 6). However, there was no statistical difference in body weight ($p>0.05$) among treatment groups. The finding indicates that repeated administration of the extract at 250-500mg/

Table 5

Behavioral and physical observations in mice given daily oral doses (28 days) of *C. odorata* ethanolic leaf extract.

Observations	Control	*C. odorata* ethanolic leaf extract	
		250 mg/kg	500 mg/kg
Skin color	n	n	n
Piloerection	-	-	-
Alopecia	-	-	+
Tachypnea	-	+	+
Stool	n	n	n
Lacrimation	-	-	-
Retching	-	-	-
Vocalization	-	-	-
M. membrane	n	n	n
Palpebral opening	n	n	n
Tremor	-	-	-
Staggering gait	-	-	-
Grip	n	n	n
Opisthotonus	-	-	-
Alertness	n	n	n
Touch response	n	n	n
Morbidity	-	-	-
Mortality	-	-	1/5

Legend: (n) normal; (-) absent; (+) present

Table 6

Mean body weights of mice given with repeated oral dose (28 days) of C. odorata ethanolic leaf extract.

Treatment	Mean Body Weight (grams, days)					% Difference in wt.
	0	7	14	21	28	
Control	26.20 ±1.69	28.32 ± 1.66	29.50 ± 1.03	30.15 ± 1.12	30.77 ± 1.18	15.3
250 mg/kg	25.56 ± 0.82	26.43 ± 1.64	27.66 ± 2.33	29.20 ± 2.70	28.43 ± 3.66	10.9
500 mg/kg	25.57 ± 0.89	27.28 ± 1.23	27.56 ± 2.09	27.83 ± 3.77	27.18 ± 3.86	5.89
p-value^{s}	0.642	0.185	0.233	0.448	0.242	

Legend: Values are expressed as mean ± SD; ns – not significant, p>0.05

Table 7

Relative organ weights of mice given with repeated oral dose C. odorata ethanolic leaf extract for 28 days.

Organ	Control	C. odorata ethanolic leaf extract		*p*-value^{ns}
		250 mg/kg	500 mg/kg	
Liver	4.72 ± 0.24	5.05 ± 1.05	4.90 ± 1.55	0.877
Kidney	1.57 ± 0.27	1.71 ± 0.28	1.55 ± 0.22	0.568
Spleen	0.54 ± 0.16	0.57 ± 0.16	0.53 ± 0.42	0.966
Heart	0.60 ± 0.12	0.54 ± 0.13	0.52 ± 0.16	0.621
Intestines	9.71 ± 1.05	10.85 ± 1.17	11.38 ± 0.84	0.087

Note. Values are expressed as mean (grams) ± SD; ns – not significant, p>0.05

Table 8

Serum ALT, BUN, and creatinine values of mice were given with oral doses of *C. odorata* ethanolic leaf extract for 28 days.

Parameter	Control	*C. odorata* ethanolic leaf extract		*p-value*
		250 mg/kg	500 mg/kg	
Creatinine (mg/dL)	0.42 ±0.08	0.43 ± 0.10	0.43 ± 0.06	0.983^{ns}
Urea/BUN (mg/dL)	27.14 ± 6.06	23.32 ± 3.72	28.38± 4.04	0.283 ^{ns}
ALT (IU/L)	70.9 ±13.4a	54.9 ±13.4 ^{ab}	42.78 ±5.4 ^{b}	0.009 **

Note: Values are expressed as mean ± SD; ns – not significant; ** highly significant, p<0.01

Table 9

Blood values of mice given with an oral dose of *C. odorata* ethanolic leaf extract for 28 days.

Parameter	Control	*C. odorata* ethanolic leaf extract		*p*-value^{ns}
		250 mg/kg	500 mg/kg	
RBC (10^6/ uL)	8.60±0.9	8.04±1	7.9±1.2	0.572
Hemoglobin (g/L)	13.8±1.6	14.50±3.7	13.8±2.3	0.891
Hematocrit(%)	46.4±4.4	41.75±7.6	41.8±6.6	0.533
WBC (10^9/L)	7.5±1.8	6.25 ±1.8	4.50±1.1	0.054
Neutrophil (%)	27.4±12	54.40±7.8	31.75±8.5	0.623
Lymphocyte (%)	63.6±9.7	72.60±5.8	63.75±8.9	0.196
Monocyte (%)	1.2±0.5	1.00±0.4	2.00±0.9	0.067
Eosinophils (%)	1.2±0.6	1.50±1.1	0.88±0.5	0.546
Basophils (%)	0.14±0.1	0.22±0.1	0.45±0.4	0.144
Platelet (10^3/uL)	1045.6± 147.87	933.4± 102.86	928.5± 158.40	0.358

Legend: Values are expressed as mean ± SD; ns – not significant, p>0.05

kg for four weeks harmed animals' growth, similar to acute toxicity.

Relative organ weight of the liver, kidney, spleen, heart, and intestines in mice given multiple doses of the extract vary with the control group (Table 7). An increase in the liver and intestines' weights in mice given with the extract was noted and may indicate inflammation. However, the organ weight difference among treatment groups was not significant (p>0.05). Though there is no statistical difference, this finding indicates that the extract induces damage to the liver and intestines. This finding agrees with Anyanwu et al. (2017) study that the extract causes damage to rat intestine histology.

Serum chemistry analysis showed no significant difference in creatinine and BUN values; however, a highly significant decrease in ALT values compared to the control group was observed (Table 8). This finding was corroborated by the study of Anyanmu et al. (2017) that the extract decreased ALT, AST, and alkaline phosphatase levels in albino rats. However, this contradicts the findings of Fu et al. (2002) and Asomugha et al. (2013), who reported elevated liver enzymes in their studies. Ramaty et al. (2014) explained that low ALT values might serve as an independent predictive marker for long-term mortality.

Hematological values (red blood cells (RBC), hemoglobin, hematocrit, white blood cells (WBC), and platelets) of mice given with the extract showed no significant differences than the control group (Table 9). This observation is similar to the oral administration of Carica papaya and Euphorbia hirta extracts in mice, as reported by Ping et al. (2013). However, slight variations in some parameters were observed: RBC, hematocrit, WBC, and platelets slightly decline. This result may indicate minimal toxicity of the extract to the blood cells.

CONCLUSION

Chromolaena ethanolic leaf extract contains bioactive components with potential toxic properties. The absence of morbidity, mortality, and persistent alteration on physical parameters and behavior makes the single oral dose (5000mg/kg) relatively safe. However, caution should be taken on repeated use of the extract, even at a lower dose (250-500 mg/kg), since it can cause significant toxic effects, e.g., alopecia, dry hair, tachypnea, low ALT, and mortality. These findings revealed that multiple oral doses of the extract for four weeks caused significant signs of toxicity. Caution should be taken if used repeatedly in ethno-medicine. Both acute and sub-acute toxicity of the

leaf extract hampers growth.

REFERENCES

Alara, O. R., and Abdurahman, N. H. (2019). GC–MS and FTIR analyses of oils from *Hibiscus sabdariffa, Stigma maydis and Chromolaena odorata* leaf obtained from Malaysia: Potential sources of fatty acids. *Chemical Data Collections* 20.

Asomugha, R. N., Ezejiofor, A. N., Okafor, P. N. & Ijeh, I. I. (2015). Acute and cytotoxicity studies of aqueous and ethanolic leaf extracts of *Chromolaena odorata. Pakistan Journal of Biological Sciences 18*: 46-49.

Anyanwu, S., Iinyang, I. J., Asemota, E. A., Obioma, O. O., Okpokam, D. C. & Aguc, V. O. (2017). Effect of ethanolic extract of C*hromolaena odorata* on the kidneys and intestines of healthy albino rats. *Integrative Medicine Research 6*: 292-299.

Atmani, D., Chaher, N., Atmani, D., Berboucha, M., Debbache, N., & Boudaoud, H. (2009). Flavonoids in human health: From structure to biological activity. *Current Nutrition & Food Science, 5(*4), 225-237.

Bunyapraphatsara, N. & Chokechaijaroenporn, O. (2000). Thai medicinal plants. Faculty of Pharmacy, Mahidol University and National Center for Genetic Engineering and Biotechnology 4: 622-626.

Cruttwell, M. (1989). Siam weed: a new threat to Australia's north. *Plant Protection Quarterly, 4*(1), 3-7.

Fu, P. P., Yang, Y. C., Xia, Q., Chou, M. C., Cui, Y. Y. & Lin, G. (2002). Pyrrolizidine alkaloids-tumorigenic components in Chinese herbal medicines and dietary supplements. *Journal of Food and Drug Analysis 10*(4): 198-211.

Gautier, L. (1992). Taxonomy and tropical distribution of a tropical weed: *Chromolaena odorata* (L.) R. King & H. Robinson. *Candollea*. 645-662.

Iwu, M. M. (1994). African medicinal plants in the search for new drugs based on ethnobotanical leads. In *Ciba Foundation Symposium 185-Ethnobotany and the Search for New Drugs* (pp. 116-129). John Wiley & Sons, Ltd.

Khoddami. A., Wilkes, M. A., & Roberts, T. H. (2013). Techniques for analysis of plant phenolic compounds. *Molecules, 18*(2), 2328-2375.

Kikiowo, B., Ogunleye, J. A., Iwaloye, O. & Ijatuyi, T. T. (2020). Therapeutic potential of *Chromolaena odorata* phyto-constituents against human pancreatic α-amylase, *Journal of Biomolecular Structure and Dynamics* 1-12.

OECD 423, (2001). Guidance document on acute oral toxicity. Environmental health safety monograph series on testing and assessment: 6-24.

Ogbonia, S. O., Mbaka, G. O., Anyika, E. N., Osegbo, O.M., & Igbokwe, N. H. (2010). Evaluation of acute toxicity in mice and subchronic toxicity of hydroethanolic extract of *Chromolaena odorata* (L.) King and Robinson (Fam. Asteraceae) in rats. *Agriculture and Biology Journal of North America, 1*(5), 859-865.

Panda, D., & Dash, G. K. (2010). Qualitative phytochemical analysis & investigation of anthelmintic and wound healing potentials of various extracts of *Chromolaena odorata* Linn. collected from the locality of Mohuda Village, Berhampur (South Orissa) *1*(2): 122-126.

Ping, K. Y., Darah, I., Chen, Y., Sreeramanan, S. & Sasidharan, S. (2013). Acute and Subchronic Toxicity Study of Euphorbia hirta L. Methanol Extracts in Rats. H*indawi Journal*. 1-14.

Putri, D. A. & Fatmawati, S. (2019). A New Flavanone as a Potent Antioxidant Isolated from *Chromolaena odorata* L. Leaves, *Evidence-Based Complementary and Alternative Medicine*, vol. 2019, Article ID 1453612, 12 pages.

Ramaty, E., Maor, E., Peltz-Sinvani, N., Brom, A., Grinfeld, A., Kivity, S., … & Segal (2014). Low ALT blood levels predict the long-term cause of mortality among adults. A historical prospective cohort study. *European Journal of Internal Medicine 25*: 919-921.

Saxena, M., Saxena, J., Nema, R., Singh, D., & Gupta, A. (2013). Phytochemistry of medicinal plants. *Journal of pharmacognosy and phytochemistry, 1*(6).

Tapas, A. R., Sakarkar, D. M., & Kakde, R. B. (2008). Flavonoids as nutraceuticals: a review. *Tropical Journal of Pharmaceutical Research, 7*(3), 1089-1099.

Vital, P. G. & Rivera, W. L. (2009). Antimicrobial activity and cytotoxicity of *Chromolaena odorata* (L.f) King and Robinson and *Uncaria* Perrotti Merr. Extracts. *Journal of Medicinal Plants Research 3*(7): 511-518.

Yakubu, M.T. (2012), Effect of a 60-Day Oral Gavage of a Crude Alkaloid Extract From *Chromolaena odorata* Leaves on Hormonal and Spermatogenic Indices of Male Rats. *Journal of Andrology, 33*: 1199-1207.