



Comparative Studies of Some Biochemical and Toxicological Investigations of *Chromolaena odorata* and *Ipomoea involucreta* from Lagos Suburb in Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The search for plants and plants products of medicinal potentials cannot be underestimated. Hence this study sought to investigate some parameters of medicinal importance. The phytochemical constituents, proximate and mineral composition, anti-nutrient contents, and acute toxicity of *Chromolaena odorata* and *Ipomoea involucreta* were analyzed using standard analytical methods. Phytochemical screening revealed the presence of reducing sugars, flavonoids, saponins, and

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alkaloids in both plants. Notably, *Ipomoea involucre* exhibited higher saponin levels, suggesting potential unique medicinal properties. Proximate and mineral composition analysis indicated that both plants have low moisture content, which helps in preserving the active compounds by minimizing chemical degradation. Additionally, the plants were found to be rich in crude fiber, protein, and nutritionally valuable elements. Anti-nutrient assessments showed that *Chromolaena odorata* had the lowest cyanide (5.45mg/100 g) and phytate (0.23mg/100 g) levels, while *Ipomoea involucre* had the lowest oxalate content (0.40 mg/100 g). Furthermore, no mortality was observed in tested animals following oral treatment with 2000 mg/kg body weight, indicating a high margin of safety. This study highlights that both *Chromolaena odorata* and *Ipomoea involucre* possess beneficial phytochemical and nutritional profiles. The low moisture content in these plants contributes to the stability of their active compounds, enhancing their potential medicinal value. *Ipomoea involucre* stands out for its higher saponin content, which may offer distinctive therapeutic benefits. The minimal presence of anti-nutrients and less lethal effects further support their safety and efficacy as medicinal plants. The findings in this study however underscore the therapeutic potential of these plants and their suitability for use in drug development and their application in therapeutic products.

Keywords: Medicinal plants; proximate composition; acute toxicity; phytochemical screening; nutritional profiles.

1. INTRODUCTION

In recent decades, there has been a growing interest in plant-derived drugs due to their high biocompatibility and reduced side effects compared to synthetic medications. Despite this interest, the productivity and quality of these natural products often fall short, primarily due to issues such as slow multiplication rates, overexploitation, and habitat degradation. These challenges hinder the ability to meet the ever-increasing market demand for natural remedies. Among the variety of plant species available worldwide, only a few plants have been studied for its phytochemical constituents and biological activity [1] that may serve as potential alternative sources of therapeutic agents. In the early 21st Century, more than half of all drugs used in clinical applications contained natural products in their formulations. Additionally, various medicines derived from plants, microorganisms, and fungi have been approved for medical use [2]. Natural products not only play a crucial role in existing treatments but also serve as a vital source for the development of new pharmaceutical products. *Chromolaena odorata*, a member of the Asteraceae family, which is the largest family of flowering plants with about 950 genera and 20,000 species worldwide [3], is commonly known by names such as Siam weed, devil weed, eupatorium, Jack-in-the-bush, kingweed, paraffinbush, and paraffinweed [4]. This plant is widespread in tropical Asia, West Africa, and parts of Australia [5]. It can grow in a variety of soil pH levels [6] but thrives best in acidic soils rich in potassium and phosphorus.

Traditionally, *Chromolaena odorata* has been used for its therapeutic effects. Its leaf extracts have demonstrated antioxidant, anti-inflammatory, analgesic, antimicrobial, and cytoprotective properties [1]. The plant contains phytochemicals including alkaloids, flavonoids, flavanones, essential oils, phenolics, saponins, tannins, and terpenoids. Notable constituents like chromomoric acid, quercetagenin, and quercetin enhance its medicinal benefits [7]. Studies have shown that these phytochemicals have various pharmacological activities, including antioxidant effects, hypoglycemic and hypocholesterolemic effects in animals [8], and modulation of wound healing [4].

Ipomoea involucre Beauv belongs to the Convolvulaceae family and is locally known as 'alukorese' in Southwest Nigeria and is commonly known as 'morning glory weed'. It is described by Akobundu and Agyakwa [9]. This plant is an annual or perennial twiner with hairy stems that root at the nodes. It is also known as 'lehowa dua' in Ghana and is widely distributed across Ghana, tropical West Africa, Tanzania, Zimbabwe, and northern South Africa [10]. *Ipomoea involucre* has a broad range of ethnobotanical uses in West Africa. Burkill [11] reported some traditional uses, including a decoction of fresh sap as a remedy for gonorrhoea in Sierra Leone. In Nigeria, the leaves are used to treat asthma. In Congo, leaf sap is applied to localized edema, used in eye infections, and an aqueous decoction is given to women at childbirth to facilitate afterbirth expulsion. Additionally, Okafor [12] noted that whole plant

parts are used for treating convulsions in Nigeria, while in Ghana, the stems and leaves are used to treat anemia by local herbalists [13].

Meira [14] reviewed the traditional, chemical, and biological activities of the *Ipomoea* genus, showing its use worldwide for treating various diseases such as diabetes, hypertension, dysentery, constipation, fatigue, arthritis, rheumatism, hydrocephaly, meningitis, kidney ailments, and inflammations. Wallace [15] assessed the nutritional quality and anti-nutritional composition of *Ipomoea involucreta* along with other non-conventional leafy vegetables. Ejimadu and Ogbeide [16] reported that petroleum ether and ethanol extracts of *I. involucreta* inhibited the growth of both Gram-positive and Gram-negative bacteria, including *Klebsiella* spp., *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus*. Additionally, antiviral activities of aqueous ethanolic extracts against herpes simplex virus have been documented [17]. The effectiveness of these plants as antimicrobial agents is attributed to the ability of their phytochemical constituents to inhibit pathogen growth [18]. This study aims to determine the phytochemical, proximate, mineral, and anti-nutrient constituent levels of *Chromolaena odorata* and *Ipomoea involucreta*. Understanding these components is essential for evaluating their potential therapeutic applications and effectiveness in treating various diseases.

2. METHODS AND MATERIALS

Collection and authentication of the plant's samples: Fresh samples of the *Chromolaena odorata* (Siam weed/bitter bush) and *Ipomoea involucreta* (Morning glory) plants were obtained from Anchor University, Ayobo, Lagos State, Nigeria. The plants were authenticated at the Plant Science Department of Ekiti State University, Ado-Ekiti, Nigeria by the Chief Technologist with voucher numbers UHAE 2020069 *Chromolaena odorata* Linn (L.) King & Robinson and UHAE 2020087 S *Ipomoea involucreta* Beauv. The leaves of the plants were cleansed and air-dried in the open laboratory, crushed, and subsequently ground to powder separately with a Marlex Excella laboratory blender.

2.1 Phytochemical Analysis of *Chromolaena odorata* and *Ipomoea involucreta*

2.1.1 Preparation of plant extract

20 g of each plant leaf was weighed and blended in 100 mL of distilled water and filtered to obtain

the aqueous solution which was used for the determination of the various parameters.

2.1.2 Test for flavonoids

Procedure: 2.5 ml of ammonia and 1ml of concentrated sulphuric acid was added to 5ml of the plant extract carefully. Indication of yellow color shows the presence of flavonoid in the plant sample.

2.1.3 Test for alkaloids

- Mayer's test:** A fraction of the extract was treated with Mayer's reagent (1.36g of mercuric chlorate and 5g of potassium iodide in 100ml distilled water) and noted for a cream-colored precipitate.
- Dragendorff's test:** A fraction of the extract was treated with Dragendorff's reagent and observed for the formation of reddish orange precipitate.
(Bismuth nitrate 1.7g, glacial acetic acid 20mL, water 80mL and 100ml of 50% solution of KI in water, mix together and keep as stock solution. 10ml of stock, 20mL of glacial acetic acid make up for 100ml in water for working solution)
- Wagner's test:** A fraction of the extract was treated with Wagner's reagent (1.27g of iodine and 2g of potassium iodide in 100ml of distilled water) and observed for the formation of reddish-brown precipitate.

2.1.4 Further test for alkaloids

For the purpose of phytochemical analysis of the selected plant, 0.2 g of the selected plant samples were added in each test tube and 3 ml of hexane were mixed in it, shaken well and filtered. Then 5 ml of 2% HCl was taken and poured in a test tube having the mixture of plant extract and hexane. The test tube with the mixture was heated, filtered few drops of picric acid was poured into the mixture. Formation of yellow color precipitate indicates the presence of alkaloids.

2.1.5 Test for saponin (Foam Test)

Procedure: 10 drops of distilled water was added to 20 drops of plant sample. After shaking vigorously persistence in the foam indicates the presence of Saponin.

2.1.6 Test for reducing sugar

Benedict's test: A fraction of the sample solution (2 mL) was mixed with an equal volume (2 mL) of Benedict's reagent in a test tube. The mixture was then heated in a boiling water bath

for 5 minutes. After cooling, the solution was observed for color changes. A reddish color indicated the presence of reducing sugars.

2.1.7 Proximate analysis, mineral composition, and anti-nutrient analysis of *Chromolaena odorata* and *Ipomoea involucre*

Proximate analysis was carried out on the dried powdered plant leaves according to the procedure of the Association of Official Analytical Chemists [19]. The percentages of moisture content, ash content, crude fibre, crude protein, crude fat, and carbohydrate were determined and calculated accordingly. The total carbohydrate in the sample was determined by difference. The sum of the percentage moisture, ash, crude fat, crude protein, and crude fiber was subtracted from 100 [20].

% Total carbohydrate = 100 – (% Moisture + % Ash + % Fat + % Protein + % Fibre).

Kjeldhal apparatus was used for the estimation of nitrogen content in the leaves and protein content was calculated as $N \times 6.25$.

The mineral element composition was determined from the obtained ash during proximate analysis which was digested and dissolved with dilute hydrochloric acid solution using Atomic Absorption Spectrophotometer (Buck Scientific, East Norwalk, CT, USA) and Flame Photometer (FP 202 PG).

The anti-nutrient analyses of phytate were done according to the Wheeler and Ferrel method [20], oxalate by the procedure of Day and Underwood [21] while the method of Association of Official Analytical Chemists [19] was used to determine the total cyanide content.

2.2 Acute Toxicity Investigation

Various concentrations of the extracts were prepared at 200, 400, 1000, and 2000 mg/kg body weight for each group of five mice weighing an average of 19.30 g respectively. A 0.2 mL each of extract of the plants was administered orally while the control group received 0.2 mL distilled water using an oral gavage feeding needle. The general, physical observations started after the mice were given the dosage, records were taken at intervals for 24 hours, physical observations like shivering, appetite, mortality were made. The mortality or otherwise of mice showed how toxic or nontoxic the leaves extract of the plants could be.

3. RESULTS AND DISCUSSION

Table 1 shows that phytochemical screening revealed the presence of reducing sugars, flavonoids, saponins, and alkaloids in both *Chromolaena odorata* and *Ipomoea involucre*. Notably, *Chromolaena odorata* demonstrated higher levels of saponins, which may suggest distinct medicinal properties. The medicinal properties of different plant extracts are attributed to their natural phytochemical compounds. Flavonoids, for example, are known as water-soluble antioxidants, which act as free radical scavengers by preventing oxidative cell damage and exhibiting anti-carcinogenic effects [22]. According to Okwu [23], flavonoids can reduce the risk of heart diseases and inflammation when present in the intestinal tract. Alkaloids, on the other hand, are recognized for their microbicidal properties and significant anti-diarrheal effects. Additionally, alkaloids have antihypertensive, antifungal, anti-inflammatory, and antifibrogenic effects [24].

Some alkaloids are also useful against HIV infection as well as intestinal infection associated with AIDS [25]. Adelani-Akande linked the antimicrobial activity observed in watermelon seeds to their saponin content [26]. Phytochemicals from plants play a crucial role in protecting against free radicals by neutralizing their harmful effects. This protective action helps prevent damage to nucleic acids, lipids, and proteins [27,28]. Our findings are consistent with those of Ezeabara [29] who reported that *Ipomoea involucre* leaves contained high levels of flavonoids, saponins, terpenoids, and tannins, while the roots had elevated concentrations of alkaloids and phenols. Similarly, Angitha Ajay [30] reported that *Chromolaena odorata* contains a variety of bioactive compounds, including alkaloids, flavonoids, saponins, cyanogenic glycosides, tannins, and phytic acid.

Table 2 presents the results of the proximate analysis (in %) for *Chromolaena odorata* and *Ipomoea involucre*. The moisture content of *Chromolaena odorata* and *Ipomoea involucre* leaves was found to be $36.44 \pm 0.45\%$ and $22.24 \pm 0.45\%$, respectively. These moisture levels are insufficient to promote microbial growth, mold formation, or degradation of the active compounds present in the plants. The observed moisture contents are lower than those reported by Abiodun Bukunmi [31] in the phytochemical and proximate analysis of some medicinal leaves.

Table 1. Phytochemical analysis of the samples aqueous extracts

Samples	Reducing sugar	Saponins	Flavonoids	Alkaloid
<i>Chromolaena odorata</i>	+	+++	+	+
<i>Ipomoea involucrate</i>	+	+	+	+

- = Not present, + = Present, ++ =High, +++ =Higher

Table 2. Proximate analysis of *Chromolaena odorata* and *Ipomoea involucrate*

Samples	Moisture Content %	Crude Fibre %	Crude Protein%	Ash%	Crude Fat %	CHO %	Total %
<i>Chromolaena odorata</i>	36.44±0.45	20.85±0.80	21.55±0.00	2.16±0.08	3.41±0.08	15.00±0.10	99.41±0.25
<i>Ipomoea involucrate</i>	22.24±0.45	17.75±0.26	12.18±0.03	3.02±0.07	18.63±0.05	25.37±0.08	99.19±0.15

Results show Mean±SD of three values

Table 3. Mineral elements composition of *Chromolaena odorata* and *Ipomoea involucrate* in part per million (ppm)

Samples	Fe	Na	K	Ca	Pb	Zn	Cr	Mn	Se	Co	Cu	Cd
<i>Chromolaena odorata</i>	0.37	20.10	31.70	16.56	0.001	0.35	ND	0.03	ND	0.001	0.19	0.005
<i>Ipomoea involucrate</i>	0.46	20.10	43.10	19.38	ND	0.26	ND	0.017	ND	ND	0.12	0.010

ND: Not detected, Fe: Iron, Na: Sodium, K: Potassium, Ca: Calcium, Pb: Lead, Zn: Zinc, Cr: Chromium, Mn: Manganese, Se: Selenium, Co: Cobalt, Cu: Copper, Cd: Cadmium

Table 4. Anti-nutrient Analysis of the Samples Aqueous Extracts

Samples	Cyanide content	Oxalate content	Phytate content
<i>Chromolaena odorata</i>	5.45±0.01	0.40±0.01	0.23 ± 0.02
<i>Ipomoea involucrate</i>	8.11 ±0.01	0.33 ±0.01	0.26± 0.02

Table 5. Acute toxicity study of the aqueous extract of *Chromolaena odorata* and *Ipomoea involucrate*

Responses	Control	200 mg/kg body weight dosage of each plant extract after 48 hrs.	400 mg/kg body weight dosage of each plant extract after 48 hrs.	1000 mg/kg body weight dosage of each plant extract after 48 hrs.	<i>Chromolaena odorata</i> 2,000 mg/kg body weight dosage after 48 hours	<i>Ipomoea involucrate</i> 2,000mg/kg body weight dosage after 48 hours
Consciousness	+	+	+	+	+	+
Shivering	+	+	+	+	-	-
Cuddling together	+	+	+	+	+	-
Scratching	+	+	+	+	-	+
Movement	+	+	+	+	+	+
Water consumption	+	+	+	+	+	+
Food intake	+	+	+	+	+	+
Lethargy	+	+	+	+	-	-
Salivation	+	+	+	+	+	+
Convulsion	+	+	+	+	+	+
Sound Response	+	+	+	+	+	+
Skin color	+	+	+	+	+	+
Diarrhea	+	+	+	+	+	+
Gripping Strength	+	+	+	+	+	+
Mortality	+	+	+	+	+	-
Morbidity	+	+	+	+	+	+

+ = Normal; - = Absent

The analysis revealed moderate crude fiber values for the leaves: $20.85 \pm 0.80\%$ for *Chromolaena odorata* and $17.75 \pm 0.26\%$ for *Ipomoea involucreta*. These fiber levels are not so high as to significantly hinder the absorption of active compounds or reduce the plants' therapeutic effectiveness, but are adequate to support bowel regularity and overall gut health. Both plants also exhibit moderate crude protein percentages, which are higher than the protein content found in *Telfairia occidentalis* (7.00%) and *Momordica balsamina* (11.29%) [32]. Thus, the leaves of these plants can be considered good sources of protein, as it gives us more than 12% of caloritic value from protein [33].

The ash content of *Chromolaena odorata* and *Ipomoea involucreta* was found to be low, at $2.16 \pm 0.08\%$ and $3.02 \pm 0.07\%$, respectively. Lower ash content typically indicates higher purity and a greater concentration of desired bioactive compounds, while higher ash content may signal impurities or excess minerals that could affect the plants' therapeutic efficacy or safety [34]. The crude fat content in both plants are moderate, aligning with recommendations that a diet providing 1.20% of its caloric energy from fat may be considered deficient, as excess fat consumption is associated with certain cardiovascular disorders [35]. The 15 to 25 percent carbohydrate content observed in both plants falls within the expected range for many medicinal plants and should provides a sufficient substrate for the synthesis of bioactive compounds [36].

Table 3 presents the mineral content of *Chromolaena odorata* and *Ipomoea involucreta* leaves. Potassium levels were the highest, measuring 31.70 and 43.10, respectively. These values are moderate and essential for maintaining fluid balance and proper cell function. However, excessive potassium can disrupt sodium balance and potentially affect heart and kidney function [37]. Sodium content was observed to be 20.10 for both plants, which is moderate and aligns with the recommendation that lower sodium levels are preferable to avoid hypertension and other health issues [38]. Elevated sodium levels can also interfere with the balance of other essential minerals.

A report by Shomar [39] highlighted that sodium and potassium in intracellular and extracellular fluids are crucial for maintaining electrolyte balance and membrane fluidity. Calcium levels in *Chromolaena odorata* and *Ipomoea involucreta*

were 16.56 and 19.38, respectively. These values are within appropriate ranges, as excessive calcium can interfere with the absorption of other essential minerals like magnesium and iron, potentially impacting the plants' bioavailability and therapeutic efficacy [40]. According to Hassan [41], adequate calcium intake is crucial and can be safer for cancer patients compared to some chemotherapeutic agents that may lead to osteopenia and osteoporosis.

Micro-elements such as zinc (Zn), copper (Cu), magnesium (Mg), and iron (Fe) were present in beneficial proportions. Zinc is vital for growth, development, and immune system function [42]. It plays a role in preventing and treating diarrhea, pneumonia, colds, respiratory infections, and malaria. Copper, an essential nutrient for various biological functions, including enzymatic and redox reactions, was also found [43]. Lead, cobalt, and cadmium levels were relatively low in both plants, posing no significant risk of harmful effects, while chromium and selenium were not detected. It is important to compare the concentrations of these metals with the safe limits established by WHO/FAO standards [44]. Overall, the mineral content of both plants appears to be within safe and beneficial ranges.

Table 4 presents the cyanide content in *Chromolaena odorata* and *Ipomoea involucreta* as 0.054 ± 0.01 mg/g and 0.081 ± 0.01 mg/g, respectively. Both values exceed the World Health Organization's recommended maximum level for cyanide in medicinal plants [45], which is set at 0.01 mg/g. Nevertheless, methods such as boiling, abrasion, and dehulling have been demonstrated to substantially lower cyanide levels [46,47,48]. As a result, the cyanide levels present are unlikely to cause adverse effects. Regarding oxalate content, *Chromolaena odorata* and *Ipomoea involucreta* have contents of 0.40 ± 0.01 mg/100g and 0.33 ± 0.01 mg/100g, respectively. These amounts are well below the 50 mg/100g threshold for high-oxalate plants, as identified by Judprasong [49]. Therefore, these plants are unlikely to form insoluble calcium salts that could lead to kidney stones, even if consumed in large quantities.

The phytate concentrations in *Chromolaena odorata* and *Ipomoea involucreta* are 0.23 ± 0.01 mg/100g and 0.26 ± 0.01 mg/100g, respectively. These levels are relatively low and should not significantly impede the absorption of crucial minerals such as iron, zinc, and calcium. Overall,

the concentrations of cyanide, oxalate, and phytate in these plants are insufficient to impact nutrient absorption or inhibit growth.

In Table 5, the result revealed that majority of mice across most groups exhibited no signs of illness or death, demonstrating survival throughout the 48-hour observation period. This outcome indicates that the extract at the maximum tested dose is likely non-toxic and safe when administered orally. Additionally, there were no significant variations in food and water consumption between the control and treated groups during this time. While acute toxicity assessments are crucial for understanding the toxicological profile of substances, evaluating food and water intake is also essential when assessing the safety of a product or extract intended for medicinal use. This is because the proper intake of supplements is vital for the animal's physiological health and the optimal response to the tested compounds. The study found that the administration of the extracts did not impact food and water intake, as there were no indications of appetite suppression or adverse effects. There were no notable differences in body weight gain between the control and treated groups, suggesting that the plant extracts tested at each dose were largely non-toxic.

The results of this study align with findings from previous research, including those by Ojo [50] on assessment of acute and sub-acute toxicity of *hibiscus sabdariffa* in rodents, Ali [51] on acute toxicity, brine shrimp cytotoxicity, and relaxant activity of *Callistemon citrinus*, Mishra [52] on acute and sub-acute toxicity evaluation of *azadirachta indica (neem)* leaf extracts and Roy [53] on the acute and sub-acute toxicity of *Senna alata* in Swiss Albino mice.

4. CONCLUSION

Conclusively, the analysis of *Chromolaena odorata* and *Ipomoea involucreta* demonstrates that both plants possess significant medicinal and nutritional value due to their diverse phytochemical profiles. *Chromolaena odorata*, with its higher saponin levels, may offer unique therapeutic benefits compared to *Ipomoea involucreta*. Both plants exhibit favorable proximate and mineral compositions, including moderate protein, fiber, and essential minerals such as potassium, calcium, and zinc. The low moisture content in these plants helps preserve their bioactive compounds, supporting their use in medicinal applications. Despite higher-than-

recommended cyanide levels, the plants' processing methods like boiling can mitigate this risk. The minimal presence of anti-nutrients and the lack of acute toxicity in animal testing further underscore their safety and efficacy. Overall, these findings validate the therapeutic potential of *Chromolaena odorata* and *Ipomoea involucreta*, highlighting their suitability for future research and medicinal use.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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