Standardization, Physicochemical, Elemental Analysis and Anti-diabetic activity of Powdered Leaves of Chromolaena odorata in Alloxan-induced diabetic Rats

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Abdulrasheed Ajao Abdullahi
University of Ilorin

abdullahiabdulrasheed26@yahoo.com Corresponding Author
ORCiD: https://orcid.org/0000-0002-1532-3158

B. A. Aremu
University of Ilorin

S.A. Atunwa
University of Ilorin

S.O. Usman
University of Ilorin

N.S. Njinga
University of Ilorin

F.A.U. Attah
University of Ilorin

B.A Lawal
University of Ilorin

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SUBJECT AREAS
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Abstract

Background

The prevalence of diabetes is increasing worldwide, but more evidently in developing countries where there is higher incidence of the risk factors. Plants have offered an effective medicine for the treatment of illnesses since the dawn of mankind.

The present study is aimed to standardize, determine the physicochemical parameters, element present and anti-diabetic activity of *Chromolaena odorata*. Elemental analysis was done using Atomic Absorption Spectroscopy, while Alloxan-induced model was used to determine anti-diabetic activity.

Methodology

The leaves were cleaned and air dried for some days. The following macroscopic characters of the fresh leaves were noted; shape, length, colour, apex, margin, base, leaf arrangement and odour. The microscopy of the surface preparation and cross section of the fresh leaves and powdered leaves were carried out using a light Microscope connected to a standard camera.

Alcohol soluble extractive was determined following the method used by Azwanida, (2015). Water soluble extractive was done on the powdered leaves.

The moisture content was determined following the method used by Pimentel (2006). An evaporating dish was heated to a constant weight and allowed to cool in a desiccator. Elemental Analysis (K, Na, Mn, Mg and Ca) was carried out on the powdered leaves of *Chromolaena odorata* using the method of Association of Official Analytical Chemist (AOAC, 1980) with the aid of Atomic Absorption Spectrometer (AAS) GBC Avanta Model. Standards and digested samples were aspirated and the mean signal responses were recorded at each of the element respective wavelengths.

The acute toxicity (LD$_{50}$) test was determined following the method used by Jonsson et al. (2013) with little modification.

Alloxan-induced model was used to determine the anti-diabetics activity following method by Rohilia and Ali, (2007) with slight modification.

Twenty-Five Albino rats of both sexes weighing 150–200g were used for the study.

The data were expressed as mean ± standard error of mean (SEM). One-way analysis of variance
ANOVA) with Student-Newman-keuls tests was used to analyze the data and results were considered statistically significant at P < 0.05 when compared to the control.

Results

The macroscopic evaluation reviewed a triangular shape, height of 6-10cm, pungent odour, acuminate apex, opposite leaf arrangement, dentate margin, hastate base and a green colour leaf. The microscopic study of both the fresh and powdered leaves of *C. odorata* showed the presence of anisocytic and anomocytic stomata, as well as multicellular uniseriate covering trichomes. The moisture content was 6.0 ±0.07%, the alcohol soluble extractive was 30±0.05%. while the water-soluble extractive was 40±0.05%. %. The elemental analysis of the powdered leaves of *C. odorata* showed that the leaves contain 29.00mg/L of K, 13.500mg/L of Na, 0.15mg/L of Mn, 4.78mg/L of Mg and 0.30mg/L of Ca. The powdered leaves showed a dose dependent anti-diabetic activity as 300 mg/kg significantly reduced the blood glucose level when compared to the negative control (p<0.05) on day 7, 14 and day 21. The 200 mg/kg dose showed significant reduction on day 14 and day 21 and the 100 mg/kg only on day 21.

Conclusion

The presence of phytochemicals such as alkaloids, tannins, terpenoids and flavonoids, as well as elements such as Na, K, Mn and Mg in *C. odorata* could be responsible for an increase stimulate the production of insulin from the pancreas thus leading to reduction in the blood glucose level. The study suggest that the powdered leaves of *C. odorata* possess anti-diabetic activity

Introduction

Herbs or plants have offered an effective medicine for the treatment of illnesses since the dawn of mankind (Falodun, 2010). Moreover, many orthodox drugs are derived from both nature and traditional remedies distributed around the world (Falodun, 2010). Plants have the ability to synthesize a wide variety of chemical compounds that possess important biological functions, and defense against the attack from predators such as insects, fungi and herbivores mammals (Tapsell et al., 2006; Abo et al., 2011). Many of these phytochemicals have beneficial effect on long-term health when consumed by humans, and can be used effectively to treat human diseases (Tapsell et al.,
Medicinal plants are used in herbal medicine because they are believed to ameliorate several health conditions and for a long time already, many novel chemotherapeutic agents have been derived from medicinal plants (Nweze et al., 2004). Fruits such as grapes and apples, vegetables such as onion, beverages such as red wine, spices such as turmeric, as well as many others has served as sources for phytochemicals (Doughari et al., 2009). Overtime, traditional medicines or alternative medicine have evolved from its use in the treatment of fever, headache, wounds; to its use in the treatment of more complicated diseases conditions such as diabetes mellitus (Falodun, 2010).

Diabetes mellitus is a chronic metabolic disorder characterized by high blood glucose associated with absent or inadequate pancreatic insulin secretion (due to the destruction of the beta cells of the pancreas), with or without concurrent impairment of insulin action (Zimmet et al., 2001), additionally increased risk of complications of various vascular diseases. Specialist suggested that diabetes is the third leading cause of death due to high percentage of morbidity and mortality after cancer and cardiovascular disorders (King et al., 2008).

The prevalence of diabetes is increasing worldwide, but more evidently in developing countries where there is higher incidence of the risk factors. The current estimate indicates a 69% increase in the number of adults that would be affected by the disease between 2010 and 2030, compared to 20% for developed countries (Shaw et al., 2010).

The early symptoms of diabetes include glycosuria (elevated blood sugar), polyphagia, weight loss, polyuria, polydipsia and blurred vision.

Complications of *Diabetes mellitus* includes Diabetic Ketoacidosis, Nonketotic hyperosmolar Coma, Severe Hyperglycemia, Retinopathy, Nephropathy, Neuropathy, Arthropathy etc. (Lyra et al., 2006).

Insulin therapy and life style modifications or long-term use of oral hypoglycemic agents, exercises with dietary control can be the medication of *Diabetes mellitus* (Lawalet et al., 2008).

In most developing countries, plants play an important role in the treatment of Diabetes. Report of ethnobotany revealed that about 800 medicinal plants have antidiabetic activity (Alarcon-Aguilara et al., 2004) and the bioactive compounds like alkaloids, glycosides, terpenoids, carotenoids and
flavonoids are very effective drugs both in preclinical and clinical studies (Marles et al., 2004; Loew et al., 2002). These medicinal plants are used either alone or in conjunction with conventional medicines (Marles et al., 2004). *C. odorata* (Asteraceae) is regarded as a highly invasive weed. It is found throughout the world especially in highly pacific region under different names like Siam weed, devil weed, French weed, Communist weed etc. It is an important weed that extends its territory from American to Asian countries like India, China, Bangladesh, Thailand etc. (Vaisakh and Pandey, 2012).

*C. odorata* is being used traditionally for its many medicinal properties, especially for external use as in wound, skin infections, inflammation etc. Studies have shown that the leaf extract have antioxidant, anti-inflammatory, analgesic, antimicrobial, cytoprotective and many other medicinally significant properties (Owoyele et al., 2005). Due to the increased interest in the plant, efforts have been made to formulate *C. odorata* to oral and topical preparations. In general, the compounds found in the leaves of *C. odorata* were alkaloids, carbohydrates, saponins, phenolics, tannins, flavonoids, terpenoids and steroids (Vaisakh and Pandey, 2012). The study aimed to standardize, the leaves of *C. odorata* and evaluate its anti-diabetic activity.

**Materials And Methods**

**Plant collection and identification:** Fresh leaves of *Chromolaena odorata* were collected around Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, kwara state Nigeria in the month of January, 2019. The plant specimen was identified by a taxonomist in Department of Plant Biology, University of Ilorin, Nigeria and a voucher specimen (UILH/001/1281) was deposited.

**Preparation of plant materials**

The leaves were cleaned and air dried for about five days. The following macroscopic characters of the fresh leaves were noted; shape, length, colour, apex, margin, base, leaf arrangement and odour. The dried leaves were milled into fine powder using Arthur milling machine and was stored in cellophane bags. The microscopy of the surface preparation and cross section of the fresh leaves and powdered leaves were carried out using a light Microscope connected to a camera.

**Physicochemical Screening of the Powdered Leaves.**

**Alcohol soluble extractive**
Alcohol soluble extractive was determined following the method used by Azwanida, (2015). The powdered leaves (5g) was weighed into a 250mL stoppered conical flask. Ethanol (100mL) was added and mixed on a mechanical shaker for 6 h. It was allowed to stand for 18 h and the extract was filtered. The weight of 20mL of the filtrate was evaporated to dryness on a hot plate. The residue was dried to constant weight at 105°C and the final weight was taken. The alcohol extractive value was calculated with reference to the initial weight of the powdered leaves using the following formula.

\[
\text{% Alcohol soluble} = \frac{\text{mass of dried residue}}{\text{Initial weight of the powdered leaves}} \times 100
\]

**Water soluble extractive**

The above procedure was repeated using Chloroform distilled water (0.25%v/v chloroform in distilled water) in place of ethanol as the extracting solvent.

**Moisture Content**

The moisture content was determined following the method used by Pimentel (2006). An evaporating dish was heated to a constant weight and allowed to cool in a desiccator. The powdered leaves (3g) was weighed into the dish and placed in an oven at 105°C to dry to a constant weight. This was achieved by checking the weight at 30 minutes interval after initial drying for 1h, two consecutive same weights confirmed a constant weight. The percentage of the moisture content was calculated with reference to the initial weight of the powdered drug using the following equation:

\[
\text{% Moisture Content} = \frac{\text{final weight of powdered leaves}}{\text{Initial weight of powered leaves}} \times 100
\]

**Elemental Analysis**

Elemental Analysis (K, Na, Mn, Mg and Ca) was carried out on the powdered leaves of *Chromolaena odorata* using the method of Association of Official Analytical Chemist (AOAC, 1980) with the aid of Atomic Absorption Spectrometer (AAS) GBC Avanta Model. Standards and digested samples were aspirated and the mean signal responses were recorded at each of the element respective wavelengths.
Experimental animals

Albino rats weighing 120–200g of either sex, purchased from Central Research Laboratory, University of Ilorin, bred at the animal house and were then moved to the Department of Pharmacology and Toxicology, University of Ilorin, Ilorin, for the study. The albino rats were used for the acute toxicity studies and the anti-diabetic studies. The animals were maintained in groups of five in animal cages at a temperature of 22±1°C and kept in the laboratory environment (12h dark/12h light cycle) for seven days for acclimatization. The animals were given standard feed and water *ad libitum*. The animals were treated in accordance with the guideline for the use of animals by University Ethical Review Committee, University of Ilorin, Ilorin, Nigeria. Ethics clearance with reference: UERC/ASN/2019/1876 was obtained.

**Acute toxicity (LD$_{50}$) studies**

The acute toxicity (LD$_{50}$) test was determined following the method used by Jonsson *et al.* (2013) with little modification. Three of the test animals were fasted overnight (approximately 12 hour) and weighed. Test doses of the powdered leaves were calculated in relation to the body weight of the rats. Each animal was administered 2000 mg/kg via oral gavage. The animals were carefully and individually observed for behavioural changes and the general toxicity signs after dosing for the first 24 hours. Special attention was given during the first 4 hours. The process was repeated with the remaining rats and regular observations were conducted daily on them over a period of 14 days.

**Evaluation of Anti-diabetic activity of the powdered leaves**

Alloxan-induced model was used to determine the anti-diabetics activity following method by Rohilia and Ali, (2007) with slight modification. Twenty-Five Albino rats of both sexes weighing 150– 200g were used for the study. The animals were fed standard feed and water *ad libitum*. The animals were fasted for 12h before the commencement of the experiment. After fasting, Diabetes was induced by intraperitoneal administration of Alloxan monohydrate dissolved in 0.5mL distilled water at a dose of 100mg/kg b.w. (body weight) The blood samples of the rats were taken five days later in order to check their blood glucose concentration
before commencement of the study. Animals with blood glucose of 200mg/dL and above were considered diabetic and were selected for the antidiabetic study.

**Experimental design**

The Alloxan-induced diabetic albino rats were randomly assigned into five groups (1-5) of five rats (n=5) each as follows;

Group 1: received 100mg/kg of *Chromolaena odorata* powdered leaves p.o
Group 2: received 200mg/kg of *Chromolaena odorata* powdered leaves p.o
Group 3: received 300mg/kg of *Chromolaena odorata* powdered leaves p.o
Group 4: received Glibenclamide 5mg/kg p.o (Positive control)
Group 5: Untreated diabetic rats (Negative control)
Group 6: Normoglycaemic rats

**Drug Administration**

Each rat in group 1-3 were given the appropriate dose of the powdered leaves orally (dissolved in 1ml distilled water) in relation to the dose of the drug (100mg/kg, 200mg/kg and 300mg/kg ) respectively per body weight of the rats once daily for 21days. Glibenclamide (5mg/kg) dissolved in 0.5ml of distilled water was administered to the rats in group 4 in relation to the body weight of the rats once daily for 21days

**Determination of Blood Glucose Level**

Blood samples were collected by cutting the tail-tip of the rats (Group 1-6), for blood glucose determination before administering the powdered leaves, on day 7, 14 and on day 21 using a glucometer kit and results were reported in mg/dL.

**Statistical Analysis**

The data were expressed as mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) with Student-Newman-keuls tests (primer) was used to analyze the data and results were considered statistically significant at P < 0.05 when compared to the control.

**Results**

Table 1: Macroscopy study of *C. odorata* leaves
| Parameters         | Result          |
|--------------------|-----------------|
| Shape              | Triangular      |
| Length             | 6-10cm          |
| Odour              | Pungent         |
| Apex               | Acuminate       |
| Leaf arrangement   | Opposite        |
| Margin             | Dentate         |
| Base               | Hastate         |
| Colour             | Green           |

**Microscopy Result**

**Physicochemical parameters**

Table 2: Physicochemical studies

| Physicochemical characteristics | Values (%) |
|---------------------------------|------------|
| Moisture content                | 6 ±0.07    |
| Alcohol soluble extractive      | 30±0.05    |
| Water soluble extractive        | 40±0.05    |

Values are expressed as Mean ±SEM (N=2)

**Elemental Analysis**

Table 3: Elemental analysis of C. odorata of powdered leaves
| Elements     | Values (mg/L) |
|--------------|---------------|
| Potassium    | 29.00±0.10    |
| Sodium       | 13.50±0.50    |
| Manganese    | 0.15±0.01     |
| Magnesium    | 4.78±0.44     |
| Calcium      | 0.30±0.01     |

Values are expressed as MEAN ±SEM (N=2)

**Acute Toxicity Results**

The fasted animals used in the first phase of the test were observed to be visibly calm after oral administration. No visible signs of pain/discomfort were observed. From the toxicity study, it was observed that the powdered leaves of *Chromolaena odorata* was non-toxic and caused no death up to 2000mg/kg orally.

**Effect of powdered leaves of Chromolaena odorata on blood glucose level in Alloxaninduced rats**

**TABLE 4: Percentage reduction in blood glucose level in diabetic rats**

| Dose        | Day 0 (Basal Value) | Day 7 (%) | Day 14 (%) | Day 21 (%) |
|-------------|---------------------|-----------|------------|------------|
| 100mg/kg    | 100.00              | 13.80     | 29.30      | 42.20      |
| 200mg/kg    | 100.00              | 9.40      | 37.40      | 55.00      |
| 300mg/kg    | 100.00              | 31.00     | 46.30      | 68.96      |
| Glibenclamide | 100.00            | 38.00     | 67.50      | 82.90      |
| Negative    | 100.00              | 100.00    | 100.00     | 100.00     |
Data show the mean ± SEM blood glucose level at the different time points expressed as percentages of levels at day 0 to 21.

Discussion
Some pharmacognostic parameters determined in this study help in standardization and identification of crude drugs. The macroscopic evaluation revealed that the leaf of Chromolaena odorata has triangular shape, height of 6-10cm, a pungent odour, an acuminate apex, opposite leaf arrangement, dentate margin, hastate base and has a green colour. The microscopic study of both the fresh and powdered leaves of Chromolaena odorata showed the presence of stomata and trichomes which is in agreement with literature (Adeboye et al., 2012).

Trichomes are outgrowths ranging from small hairs to larger outgrowths like thorns. The fresh and powdered leaves of C. odorata showed the presence of multicellular uniseriate covering trichomes which are not many at the base. This corresponds to the result that was obtained by Vaisakh and Pandey (2012).

Stomata are minute pores which occur in the epidermis of the plants. The fresh leaves and the powdered leaves of C. odorata showed an Anisocytic and Anomocytic type of stomata. The accessory or subsidiary cells were five in number thus confirming the study reported (Adeboye et al., 2012): (Vaiaksh and Pandey (2012) only observed the presence of anisocytic stomata during their study of the leaves of C odorata.

Moisture content is the amount of water in the sample given as a percentage of the sample’s original weight. Moisture content affects the process ability, shelf-life, usability and quality of a sample (Vaikash and Pandey, 2012). The low moisture content of the powdered leaves of C. odorata(6 ±0.07 %) makes the powder to have a long shelf-life as well as easy usability and good quality.

Extractive values are useful for evaluation of crude drugs and give an idea about the nature of the chemical constituents present in them (Usman et al., 2018).

The determination of the alcohol soluble extractive gave 30±0.05 % while that of soluble extractive gave 40±0.05 %.

This shows that C. odorata if extracted with water would contain high molecular weight substances
like saponins, flavonoids, alkaloids, tannins, and steroids. Phytochemical screening yielded alkaloids, cyanogenic glycosides, flavonoids (aurone, chalcone, flavone, and flavonol), phytates saponins and
tannins (Igboh, et al., 2009).

The elemental analysis of the powdered leaves of C. odorata showed that the leaves contains
29.00mg/L of potassium, 13.500mg/L of sodium, 0.15mg/L of Manganese, 4.78mg/L of Magnesium
and 0.30mg/L of Calcium. Low levels of any these elements have their parts in the progression of
Diabetes mellitus (Abou-Seif and Youssef, 2004).

The presence of these elements in C. odorata could also stimulates the production of insulin from the
pancreas which reduces the blood glucose level.

From the toxicity study, it was observed that the powdered leaves of Chromolaena odorata was non-
toxic and caused no death up to 2000mg/kg orally Antidiabetic effect of the powdered leaves of C.
odorata was evaluated in alloxan induced diabetic rats at the dosages of 100mg/kg, 200mg/kg and
300mg/kg and were compared with standard drug Glibenclamide (5mg/kg), the negative control
(untreated) and the normal control (normoglycaemic).

The 300mg/kg dose of powder leaves of C. odorata showed significant reduction in blood glucose level
when compared to the negative control (p<0.05) on day 7, 14 and day 21.

The 200mg/kg dose showed significant reduction in blood glucose when compared to the negative
control (p<0.05) on day 14 and day 21.

The 100mg/kg showed significant reduction in blood glucose when compared to the negative control
(p<0.05) only on day 21.

The 100mg/kg and the 300mg/kg were statistically significant when compared to the Glibenclamide
(p<0.05) on day 14 and day 21. The 200mg/kg was only statistically significant when compared to
Glibenclamide (p<0.05) on day 21.

The antidiabetic activity of C. odorata powdered leaves shows a dose – dependent activity. Bioactive
compounds like alkaloids, glycosides, terpenoids and flavonoids are very effective anti-diabetic drugs
both in preclinical and clinical studies (Marles and Farnsworth, 2004).

The presence of alkaloids, tannins, terpenoids and flavonoids in C. odorata leaves could be responsible
for the observed hypoglycaemic effect of the plant (Tapsell et al., 2006).

**Conclusion**

The study showed that the fresh and powdered leaves of *Chromolaena. odorata* has been standardized and the powdered leaves has dose-dependent anti-diabetic activity probably as a result of the elemental compounds present in the leaves. Further studies on the fractions and ashes of *C. odorata* however should be carried out to compare their anti-diabetic

**Declarations**

**Ethics approval**

The animals were treated in accordance with the international guideline for the use of animals. The proposal was reviewed by University Ethical Review Committee, University of Ilorin, Ilorin, Nigeria.

Ethics clearance with reference: UERC/ASN/2019/1876 was obtained

**Consent for publication**

Not applicable

The raw data analyzed in this manuscript were readily available and it will provided on request.

**Self funding Research**

**Competing interests**

No competing interest in this research.

**Authors' contribution**

A.A. Abdullahi: Corresponding Author

B. A. Aremu: Research assistance (project student),

S.A. Atunwa: Pharmacologist (acute toxicity study and administration of drugs to the animals) S.O. Usman; N.S. Njinga, F.A.U. Attah, and B.A. Lawal help to designed the research proposal and vetted the write up.

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Figures

Figure 1

Stomata of the stained Fresh Leaves of Chromolaenaodorata(Magnification×100)
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Stomata of the stained Fresh Leaves of Chromolaenaodorata(Magnification×100)
Figure 2
Unstain (A) and Stained (B) Trichomes of powdered Leaves of C. odorata (Magnification ×100)
Figure 3

Trichomes (A) and Stomata (B) of the fresh leaves of C. odorata (Magnification×100)
Figure 4

Effect of powdered leaves of C. odorata on blood glucose level in Alloxan-induced diabetic rats from day 0 to 21
Figure 4

Effect of powdered leaves of C. odorata on blood glucose level in Alloxan-induced diabetic rats from day 0 to 21
Figure 5

Statistical comparison of the blood glucose reduction produced by C. odorata at (100mg/kg, 200mg/kg and 300mg/kg) to the untreated group (Negative control). Values are statistically significant at *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.
Figure 5

Statistical comparison of the blood glucose reduction produced by C. odorata at (100mg/kg, 200mg/kg and 300mg/kg) to the untreated group (Negative control). Values are statistically significant at *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.
Figure 6

Statistical comparison of the blood glucose reduction produced by the Positive control Glibenclamide (5 mg/kg) and C. odorata (100 mg/kg, 200 mg/kg and 300 mg/kg). Values are statistically significant at **p<0.01, *p<0.05. One-way analysis of variance (ANOVA) followed by Student-Newman-keuls test for comparison.
Figure 6

Statistical comparison of the blood glucose reduction produced by the Positive control Glibenclamide (5 mg/kg) and C. odorata (100 mg/kg, 200 mg/kg and 300 mg/kg) Values are statistically significant at **p<0.01, *p<0.05. One-way analysis of variance (ANOVA) followed by Student-Newman-keuls test for comparison