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Pharmacognostic and Anti-diabetic Studies of C*hromolaena odorata* Linn. (Asteraceae) Powdered Leaves in Alloxan-induced Diabetic Rats

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: *Chromolaenaodorata*Linn. (Asteraceae)is being used traditionally for its many medicinal properties including lowering of blood glucose level. However, few and inconsistent information about its antidiabetic potential is available.

Objective: to standardize; determine physicochemical and elemental parameters; and evaluate anti-diabetic potential of *Chromolaena odorata* Linn. (Asteraceae) powdered leaves in alloxan-induced diabetic rats.

Materials and Methods: Physicochemical screening of fresh and powdered leaves of *C. odorata* leaves were respectively determined using a light microscope connected to a standard camera. Elemental analysis was done using Atomic Absorption Spectrometer (AAS) GBC Avanta Model. Thirty-three Wistar rats of either sex weighing 150 – 200 g were used in the procedures. Acute toxicity assessment (LD_{50}) was carried out using the guideline of Organization for Economic Cooperation and Development (OECD). *Chromolaena odorata* powdered leaves were evaluated using alloxan-induced model.

Results: Physicochemical screening of the fresh and powdered leaves confirmed the pharmacognostic parameters of *Chromolaena odorata.* 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 contains 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. *Chromolaena odorata* showed no toxicity when it was orally administered to rats ($LD_{50} \geq 2000$ mg/kg). The powdered leaves of *Chromolaena odorata* at 100, 200 and 300 mg/kg showed dose and time-dependent anti-diabetic activities.

Conclusion: The powdered leaves of *Chromolaena odorata* is non-toxic and preliminary data showed its anti-diabetic potential possibly due to the presence of some phytochemicals and mineral elements identified

Keywords: C*hromolaena odorata;* Asteraceae; Anti-diabetic potential; Alloxan-induced; Atomic absorption spectroscopy

INTRODUCTION

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. Globally, diabetes mellitus has been suggested as the third leading cause of death due to high percentage of morbidity and mortality after cancer and cardiovascular disorders (King *et al.,* 2008). Although, the prevalence of diabetes is increasing worldwide, it is more evident 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 among others. Similarly, complications from *diabetes mellitus* have been identified as diabetic ketoacidosis, non-ketotic hyperosmolar coma, severe hyperglycemia, retinopathy, nephropathy, neuropathy, arthropathy, to mention but a few (Lyra *et al.,* 2006). Meanwhile, insulin therapy or long-term use of oral hypoglycemic agents and life style modifications, such as moderate exercises, efficient dietary control are usually prescribed in the management of *diabetes mellitus* (Lawal *et al.,* 2008). Despite, an ideal therapeutic agent or conventional medication used in the management of *diabetes mellitus* remains elusive. Thus, efforts are being continuously made to explore natural remedies especially medicinal plants which are abound in developing worlds. This is with the view to discovering new, relatively safe and efficacious pharmacological moieties to be employed in the management of diabetes mellitus.

Herbs or medicinal plants have offered effective therapy for the management of pathological conditions *diabetes mellitus* inclusivesince the dawn of mankind (Falodun, 2010). Moreover, many orthodox drugs are derived from both natural and traditional remedies distributed globally (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, thus herbs are used to effectively treat human ailments (Tapsell *et al.,* 2006).

More so, since many novel chemotherapeutic agents have been derived from medicinal plants (Nweze *et al.,* 2004) which also served as sources for phytochemicals (Doughari *et al.,* 2009), the interest in herbal medicine has increased globally. Hence, herbal medicine has rapidly evolved from simple interventions in the treatment of common ailments to more complicated disease conditions such as diabetes mellitus (Falodun, 2010).In most developing countries, medicinal plants play important roles in the treatment of diabetes mellitus. These medicinal plants are used either alone or in conjunction with conventional medicines (Marles*et al.,* 2004). Ethnobotanical reports revealed that about 800 medicinal plants were used for diabetic (Alarcon-Aguilara *et al.,* 2004). Bioactive compounds such as alkaloids, glycosides, terpenoids, carotenoids and flavonoids are very effective phytochemicals both in preclinical and clinical studies (Marles *et al.,* 2004; Loew *et al.,* 2002).

Chromolaena odorata Linn.*(*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 exists in all continents of the world (Vaisakh and Pandey, 2012). *C. odorata* is being used traditionally for its many medicinal properties, such as its anti-inflammatory potential in the treatment of wound and skin infections. Studies have shown that the crude leaf extract has anti-oxidant, anti-inflammatory, analgesic, antimicrobial, cytoprotective activities among others (Owoyele *et al.,* 2005). However, due to the increased interest in *C. odorata,* efforts have been made to formulate it into oral and topical preparations. In general, the compounds found in the leaves of *C. odorata* are alkaloids, carbohydrates, saponins, phenolics, tannins, flavonoids, terpenoids and steroids (Vaisakh and Pandey, 2012). This study therefore aimed to standardize the leaves of *C. odorata* and evaluate its potential for anti-diabetic activity**.**

METHODOLOGY

Ethical approval

The study proposal was submitted and thereafter reviewed by the University Ethical Review Committee, (UERC) University of Ilorin, Ilorin, Nigeria. Ethical clearance with reference approval: UERC/ASN/2019/1876 was obtained.

Plant collection and identification

Fresh leaves of *Chromolaena odorata* were collected around Faculty of Pharmaceutical Sciences, University of Ilorin, Ilorin, Kwara State Nigeria in January 2019. The plant specimen was identified by a taxonomist in the 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 evaluation of the powdered leaves

Alcohol soluble extractive

Alcohol soluble extractive was determined following the method used by Azwanida, (2015). The powdered leaves (5g) were weighed into a 250 mL 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 20 mL of the filtrate was evaporated to dryness on a hot plate. The residue was dried to constant weight at 105 ^{o}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.

$$\%\ Alcoholsoluble = \frac{massofdriedresidue}{Initialweightofthepoweredleaves} x\ 100$$

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 analysis

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^{o}C.todry to a constant weight. This was achieved by checking the weight at 30 minutes interval after initial drying for1h, 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:

$$\%\ MoistureContent = \frac{finalweightofpowderedleaves}{Initialweightofpoweredleaves} x100$$

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

Thirty-three Wistar rats weighing 120 – 200g of either sex, were purchased from the animal house of Central Research Laboratory, University of Ilorin, Ilorin. They were then moved to the animal holding of the Department of Pharmacology and Toxicology, University of Ilorin, Ilorin, for the acute toxicity and anti-diabetic studies. The animals were maintained in groups of five in animal cages at a room temperature (12 h dark/12 h light cycle) for seven days for acclimatization. The animals were given a commercial standard feed and water *ad libitum.* The rats were treated in accordance with the guidelines for the use of animals by the University Ethical Review Committee, University of Ilorin, Ilorin, Nigeria. Ethical clearance with reference approval: UERC/ASN/2019/1876 was obtained.

Acute toxicity (LD_{50}) study

The acute toxicity (LD_{50}) test was determined withthe guideline of Organization for Economic Cooperation and Development (OECD, 2001).Three rats were fasted overnight (approximately 12 hours) 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. Further regular observations were conducted daily on therats for a period of 14 days.

Evaluation of anti-diabetic activity of the powdered leaves

Thirty Wistar rats of both sexes weighing 150 – 200 g were used for the study. They were fasted for 12 hours overnight before the commencement of the procedure. Diabetes was induced by intraperitoneal administration of alloxan monohydrate dissolved in 0.5 mL distilled water at a dose of 100 mg/kg b.w. (body weight) following the method of Rohilia and Ali, (2007) but with slight modification. The blood samples of the rats were taken five days after in order to check their blood glucose concentration before commencement of the study. Animals with blood glucose of 200 mg/dL and above were considered diabetic and thus were selected for the anti-diabetic study.

Experimental design

The Alloxan-induced diabetic Wistar rats were randomly assigned into five groups (1-5) of five rats (n=5) each (and treated for 21 days) as follows:

Group 1: received 100 mg/kg of *Chromolaena odorata* powdered leaves p.o

Group 2: received 200 mg/kg of *Chromolaena odorata* powdered leaves p.o

Group 3: received 300 mg/kg of *Chromolaena odorata* powdered leaves p.o

Group 4: received glibenclamide 5 mg/kg (in distilled water) p.o (positive control)

Group 5: untreated diabetic rats (negative control)

Group 6: normoglycaemic rats

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 \leave suspension on day 7, 14 and 21 using a glucometer kit and results were reported in mg/dL.

Data 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.

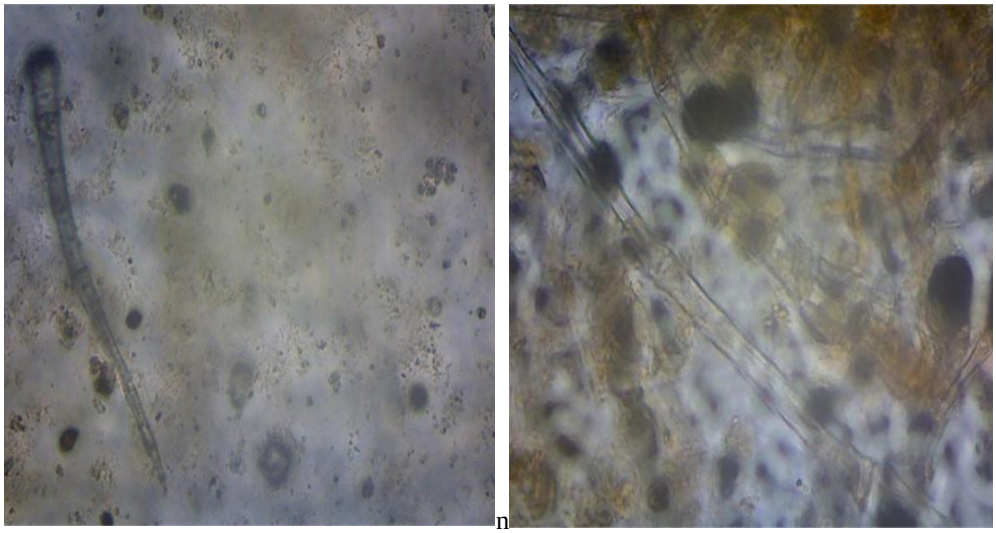
RESULTS

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

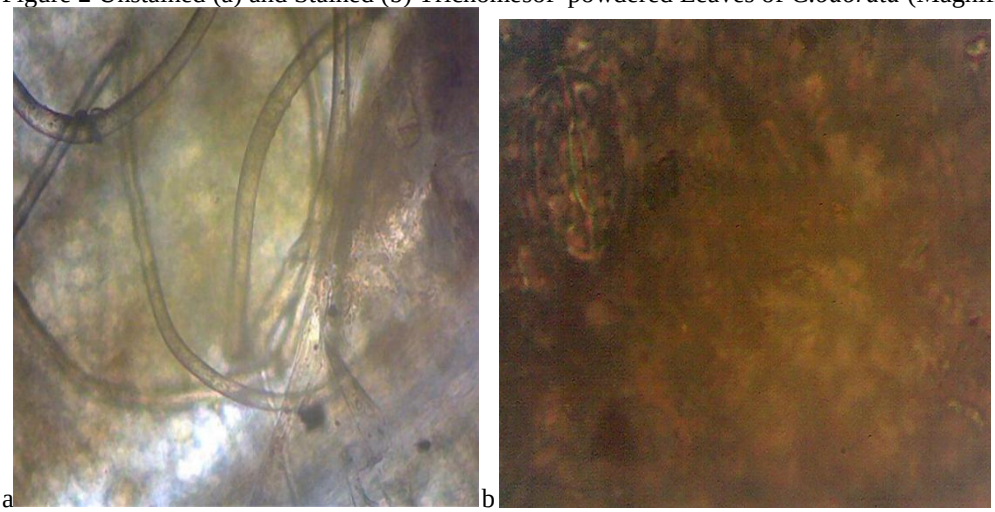
Microscopy result

Figure 1: Stomata of the stained Fresh Leaves of *Chromolaena odorata* (Magnification×100)



a n

Figure 2 Unstained (a) and Stained (b) Trichomesof powdered Leaves of *C.odorata* (Magnification×100)



a b

Figure 2: Trichomes (a) and Stomata (b) of the fresh leaves of *C. odorata* (Magnification×100)

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 fasting 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 2000 mg/kg orally.

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

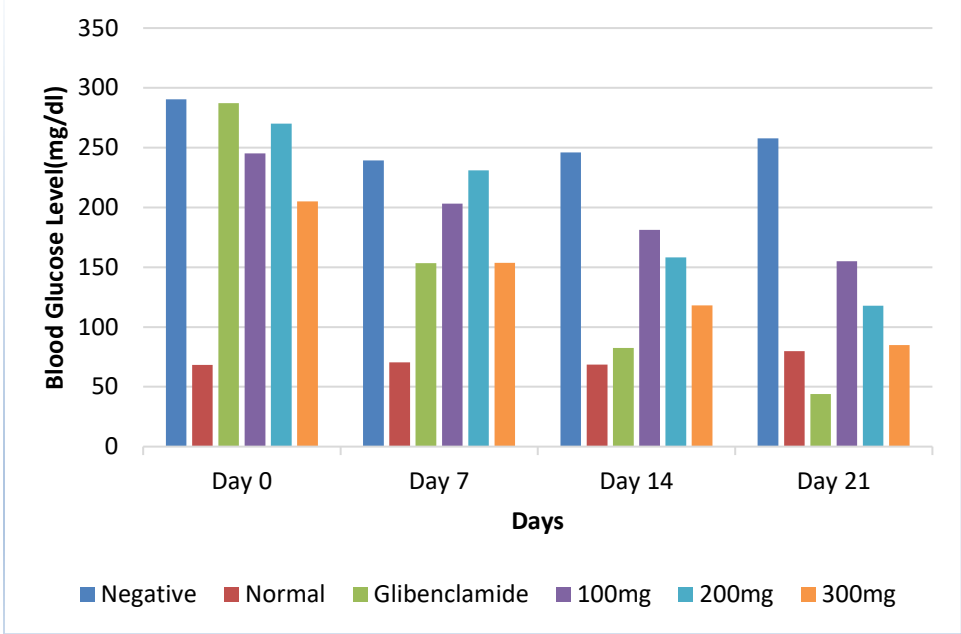


Figure 4: Effect of powdered leaves of *C. odorata*on blood glucose level in Alloxaninduced diabetic rats from day 0 to 21

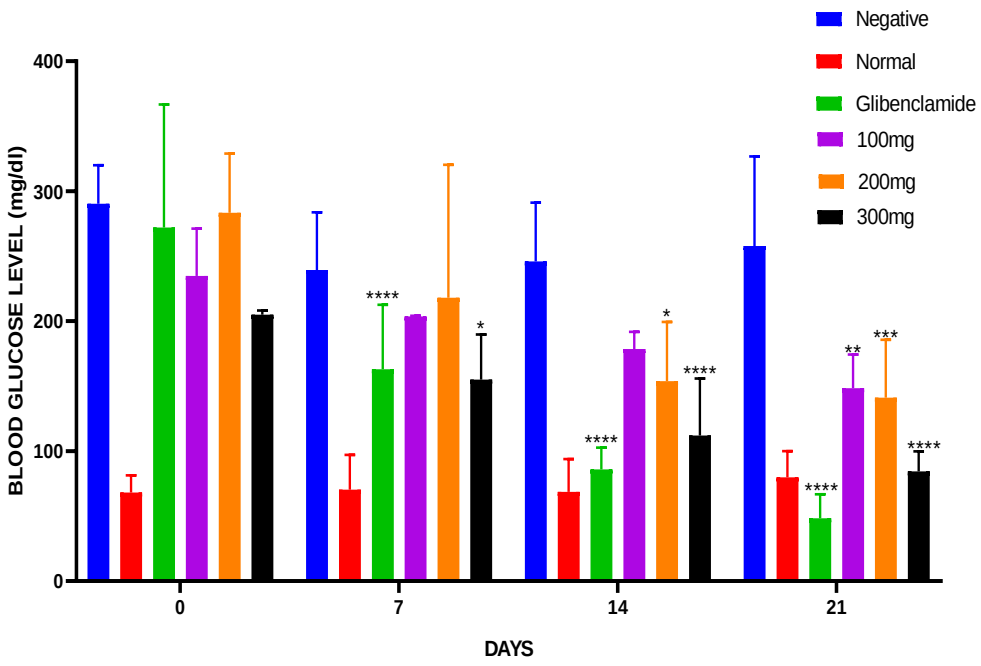


Figure 5: Statistical comparison of the blood glucose reduction produced by *C. odorata* at (100mg/kg, 200mg/kg and 300mg/kg) to the untreated group (Negative control). Values are statistically significant at *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001.

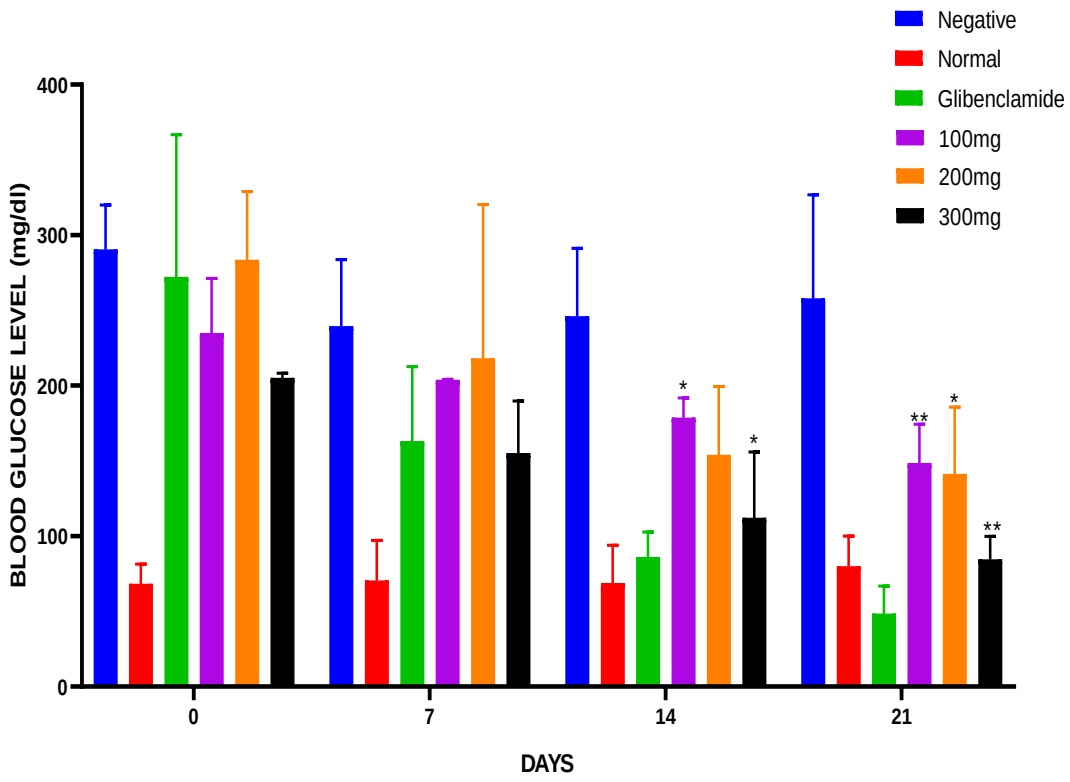


Figure 6: Statistical comparison of the blood glucose reduction produced by the Positive control Glibenclamide (5 mg/kg l). and *C. odorata* (100mg/kg, 200mg/kg and 300mg/kg) Values are statistically significant at **p<0.01, *p<0.05. One-way analysis of variance (ANOVA) followed by Student-Newman-keuls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

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

	Day 0 (Basal Value %)	Day 7 (%)	Day 14 (%)	Day 21 (%)
100mg/kg	100.00	15.08	26.3	39.88
200mg/kg	100.00	3.51	35.65	54.34
300mg/kg	100.00	35.75	52.03	67.03
Glibenclamide	100.00	35.69	66.40	82.93
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.

TABLE 5: Effect of powdered leaves of *Chromolaena odorata* on alloxan-induced diabetic albino rats

Group	Treatment	Dose (mg/kg)	Day 0 (basal value)	Day 7	Day 14	Day 21
1	Glibenclamide	5mg/kg	287.30±51.06	153.50±25.90	82.50±8.54***	44.00±8.93***
2	*Chromolaena odorata*	100mg/kg	245.30±26.84	203.30±0.33*	181.3±8.76*	155.00±18.25*
3	*Chromolaena odorata*	200mg/kg	270.00±33.78	231.00±65.52*	158.00±27.29*	117.70±21.40*
4	*Chromolaena odorata*	300mg/kg	205.00±1.90	153.80±20.00*	118.00±24.10*	85.00±8.90**
5	Normal Control		68.20±5.93	70.40±11.94**	68.60±11.32	79.80±9.10**
6	Negative control		290.40±13.20	239.40±19.85	246.00±20.22	257.80±30.86

Values (mg/2ml) are given as mean ± SEM (n=5), determined at different days before treatment. Experimental groups were compared with diabetic control (positive and negative). Values are statistically significant at *p<0.005, **p<0.01 and ***p<0.001.

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 – 10 cm, 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). Vaikash 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 29.00 mg/L

of potassium, 13.500 mg/L of sodium, 0.15 mg/L of Manganese, 4.78 mg/L of Magnesium and 0.30 mg/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 since it caused no death or observable signs of morbidity even at 2000 mg/kg when orally administered. Anti-diabetic effect of the powdered leaves of *C. odorata* was evaluated in alloxan-induced diabetic rats at the doses of 100 mg/kg, 200 mg/kg and 300 mg/kg and were compared with standard drug glibenclamide (5 mg/kg), the negative control (untreated) and the normal control (normoglycaemic).The 300 mg/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 200 mg/kg dose showed significant reduction in blood glucose when compared to the negative control ($p<0.05$) on day 14 and day 21. The 100 mg/kg showed significant reduction in blood glucose when compared to the negative control ($p < 0.05$) only on day 21..The 100 mg/kg and the 300 mg/kg were statistically significant when compared to the glibenclamide ($p < 0.05$) on day 14 and 21. The 200 mg/kg was only statistically significant when compared to Glibenclamide ($p<0.05$) on day 21. The anti-diabetic 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).

CONCLUSION

The study showed that the fresh and powdered leaves of *Chromolaena odorata* has been standardized. The powdered leaves of *Chromolaena. odorata* showed no toxicity; and time and dose-dependent anti-diabetic potentials possibly as a result of the phytochemicals and elemental compounds present in the leaves. However, further studies on the fractions, higher doses and ashes of *Chromolaen aodorata* are suggested.

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