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

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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 *Chromolaenaodorata*. 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 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.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 Mgin *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.,* 2006).

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* (Lawal*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 anti-oxidant, 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 *Chromolaenaodorata* 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 extractivewas determined following the method used by Azwanida, (2015). The powdered leaves (5g) was weighed into a 250mL stoppered conical flask. Ethanol (100mL) was addeand 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^{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.

$$\%\ Alcohol\ soluble\ = \frac{mass\ of\ dried\ residue}{Initial\ weight\ of\ the\ powered\ leaves} x\ 100$$

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

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:

$$\%\ \boldsymbol{MoistureContent}\ = \frac{\boldsymbol{final\ weight\ of\ powdered\ leaves}}{\boldsymbol{Initial\ weight\ of\ poweredleaves}} \boldsymbol{x\ 100}$$

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^{o}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 *Chromolaenaodorata* powdered leaves p.o

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

Group 3: received 300mg/kg of *Chromolaenaodorata* 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 *Chromolaenaodorata*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 muticellular 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 (Adeboyeet 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 29.00mg/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 ofChromolaena 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 inC.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 leanes 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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References

Abo, K.A., Ogunleye, V.O., &Ashidi, J.S. (2011). Antimicrobial potential of Spondiasmombin*, Croton zambesicus* and *Zygotritoniacrocea*. *Journal of Pharmacological Research,* 5, pp 494-497.

Abou-Seif, M.A. & Youssef, A.A. (2004). Evaluation of some biochemical changes in diabetic patients. *Clin Chim Acta*, 346, pp161–170.

Adeboye, O. C., Hunsche, M., Noga, G., &Lankes, C. (2012). Morphology and density of trichomes and stomata of Trichosanthescucumerina (Cucurbitaceae) as affected by leaf age and salinity. *Turkish Journal of Botany*, 36(4), pp 328-335.

Alarcon-Aguilara, F.J., Roman-Ramos, R., Perez-Gutierrez, S., Aguilar-Contreras, A., Contreras-Weber, C.C., & Flores-Saenz, J.L. (1998). Study of anti-hyperglycaemic effect of plants used as antidiabetics. *Journal of Ethnopharmacology*, 61(2),pp 101-110.

AOAC (Association of Official Analytical Chemists) (1980). Official method of analysis. 13th Ed., Washington D.C. 376-384

Azwanida, N. N. (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Medica Aromatic Plants*, *4*(196), pp 2167-0412.

Doughari, J.H., Human, I.S., Bennade, S., &Ndakidemi, P.A. (2009). Phytochemicals as chemotherapeutic agents and antioxidants: possible solution to the control of antibiotic resistant verocytotoxin producing bacteria. *Journal of Medicinal Plants Research*, 3, pp 839-848.

Falodun, A. (2010). An Herbal medicine in Africa-distribution, standardization and prospects. *Research. Journal of Phytochemistry*, 4(3), pp 154-161.

Jonsson, M., Jestoi, M., Nathanail, A. V., Kokkonen, U. M., Anttila, M., Koivisto, P., ...&Peltonen, K. (2013). Application of OECD Guideline 423 in assessing the acute oral toxicity of moniliformin. *Food and chemical toxicology*, *53*, *p*p 27-32.

Igboh, M. N.,, Ikewuchi, C. J., Catherine, C.(2009) Chemical Profile of *Chromolaena odorata L*. (King and Robinson) Leaves. *Pakistan Journal of Nutrition,* 8,pp 521-524.

King, H., Aubert, R.E., & Herman, W.H. (2008). Global burden of diabetes, 1995-2025: prevalence, Numerical estimates and Projections. *Diabetes care*, 22,e*p*p 1414-1431.

Lawal, H.A., Atiku, M.K., Khelpa, D.G., &Wannang, N.N. (2008). Hypoglycaemic and hypolipidaemic effect of aqueous leaf extract of MurrayaKoenigii in normal and alloxan-diabetic rats. *Nigerian Journal of Physiological Sciences*, 23(1-2), *p*p*p*p 37-40.

Lyra, R.M., Oliveira, D., Lins, N., &Cavalcanti, N. (2006). Prevention of type 2 diabetes mellitus. *ArquivoBrasileiros de Endocrinologia and Metabologia*, 50(2), *p*p239-249.

Loew, D., &Kaszkin, M. (2002). Approaching the problem of Bioequivalence of Herbal Medicinal products. *Phytotherapy Research*, 16(8),*p*p 705-711).

Marles, R.J., &Fransworth, N.R. (2004). Antidiabeticplants and their active constituents. *Phytomedicine*, 2, *p*p, 133-189.

Nweze, E.L., Okafor, J.L., &Njoku,O. (2004) Antimicrobial Activities of Methanol extract of *Trumeguineesis* and *Morinda Lucinda* used in Nigerian Herbal Medicinal Practice. *Journal of Biological Research and Biotechnology*, 2(1), pp 34-46.

Owoyele, V.B., Adediji, J.O., &Soladoye A.O. (2005). Anti-inflammatory activity of aqueous extract of *Chromolaenaodorata. Inflammopharmacology*, 13, *p*p 479-484.

Pimentel, F. A., Cardoso, M. D. G., Salgado, A. P. S., Aguiar, P. M., Silva, V. D. F., Morais, A. R. D., & Nelson, D. L. (2006). A convenient method for the determination of moisture in aromatic plants. *Química Nova*, *29*(2),*p*p 373-375.

Rohilla, A., & Ali, S. (2012). Alloxan induced diabetes: mechanisms and effects. *International journal of research in pharmaceutical and biomedical sciences*, ,*3*(2), *p*p 819-823.

Shaw, J.E., Sicree, R.A., &Zimmet, P.Z. (2010). Global estiate of prevalence of Diabetes for 2010 and 2030. *Diabetes Research and Clinical Practice*, 87, *p*p 4-14.

Tapsell, L.C., Hemphill. I., Cobiac, L., Patch, C.S., Sulivant, D.R. (2006). Health benefits of herbs and spices: the past, the present, the future. *Medical Journal Austr,* 185, pp s4-s24.

Usman, S. O.,Abdullah, **A.A.,** Ayanniyi, R., Musa, M., Lawal, B., Salawu, M., AttahA.A.(2018).Extractive Values and Diuretic Activity of Methanol Extract of Three Varieties of *Hibiscus sabdariffa* Linn (Malvaceae) in Wistar Albino rats.*Journal of pharmaceutical research development and practice,* 2(2) pp85-93

Vaisakh, M.N., & Pandey, A. (2012). The invasive weed with healing properties: a review on *Chromolaenaodorata*. *International journal of pharmaceutical Science Res*earch, 3(1), pp 80-83.

WHO (2006). Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia. World health Organization.www.who.int.2006.

Zimmet, P., Alberti, K.G.M., & Shaw, J. (2001). Global and Societal Implications of the Diabetes Epidemic Nature, 4141, *p*p 782-787.

Figures

Figure 2

Stomata of the stained Fresh Leaves of Chromolaenaodorata(Magnification×100)

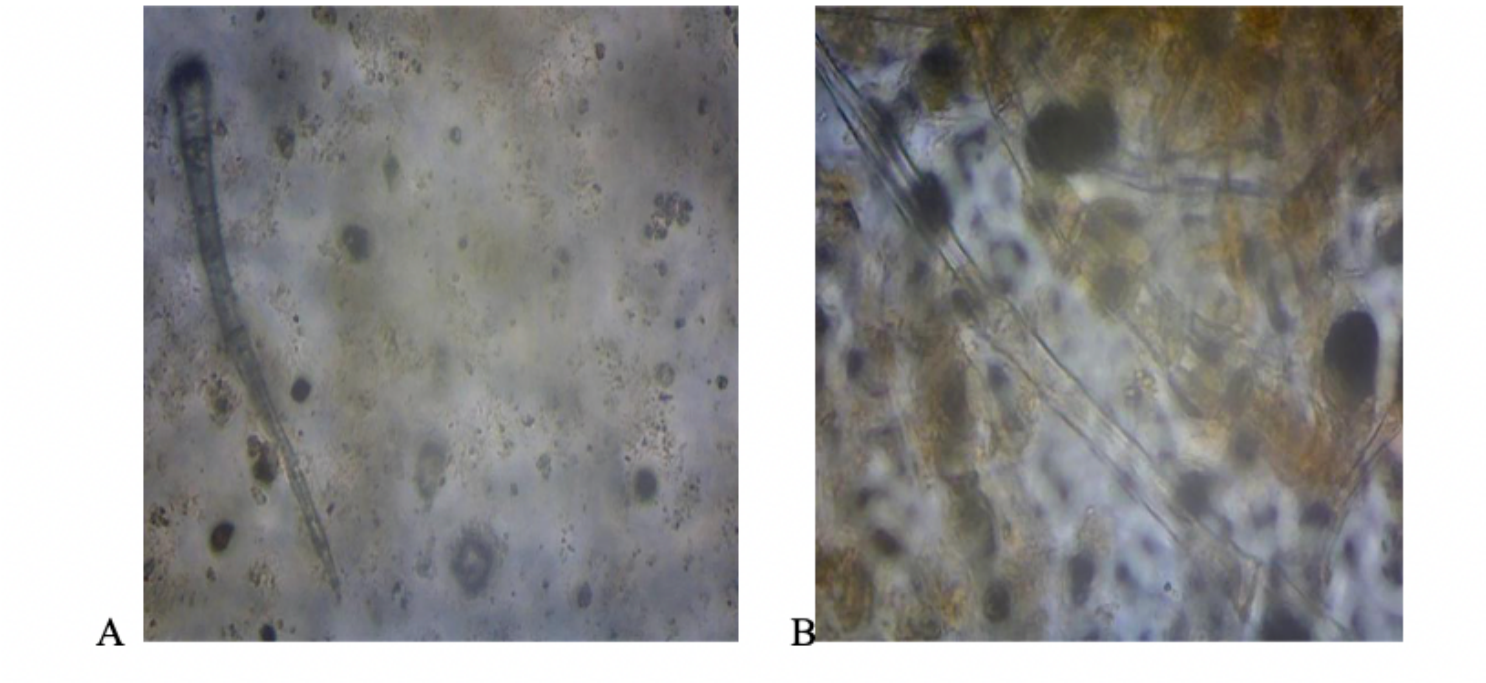

Figure 4

Unstain (A) and Stained (B) Trichomesof powdered Leaves of C.odorata (Magnification×100)

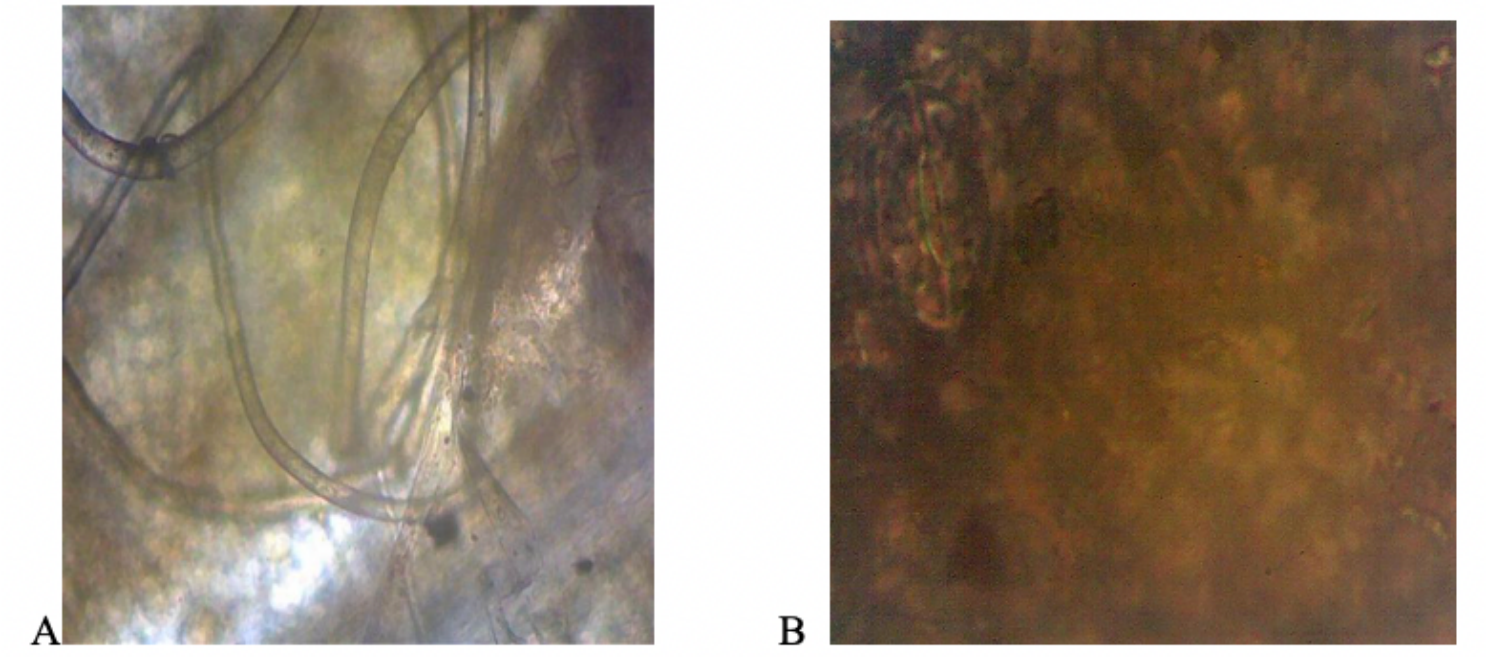

Figure 6

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

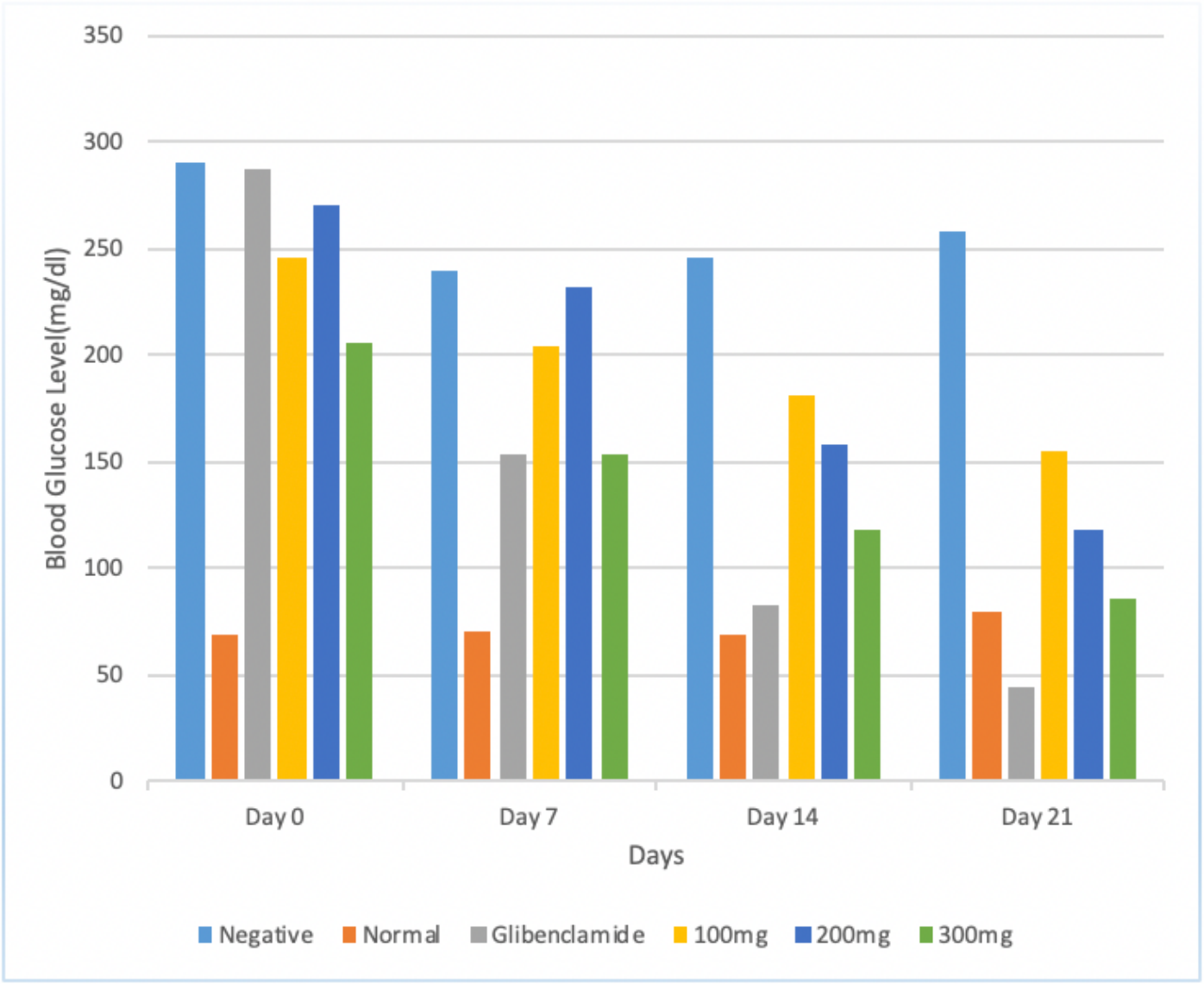

Figure 8

Effect of powdered leaves of C. odorataon blood glucose level in Alloxaninduced diabetic rats from day 0 to 21

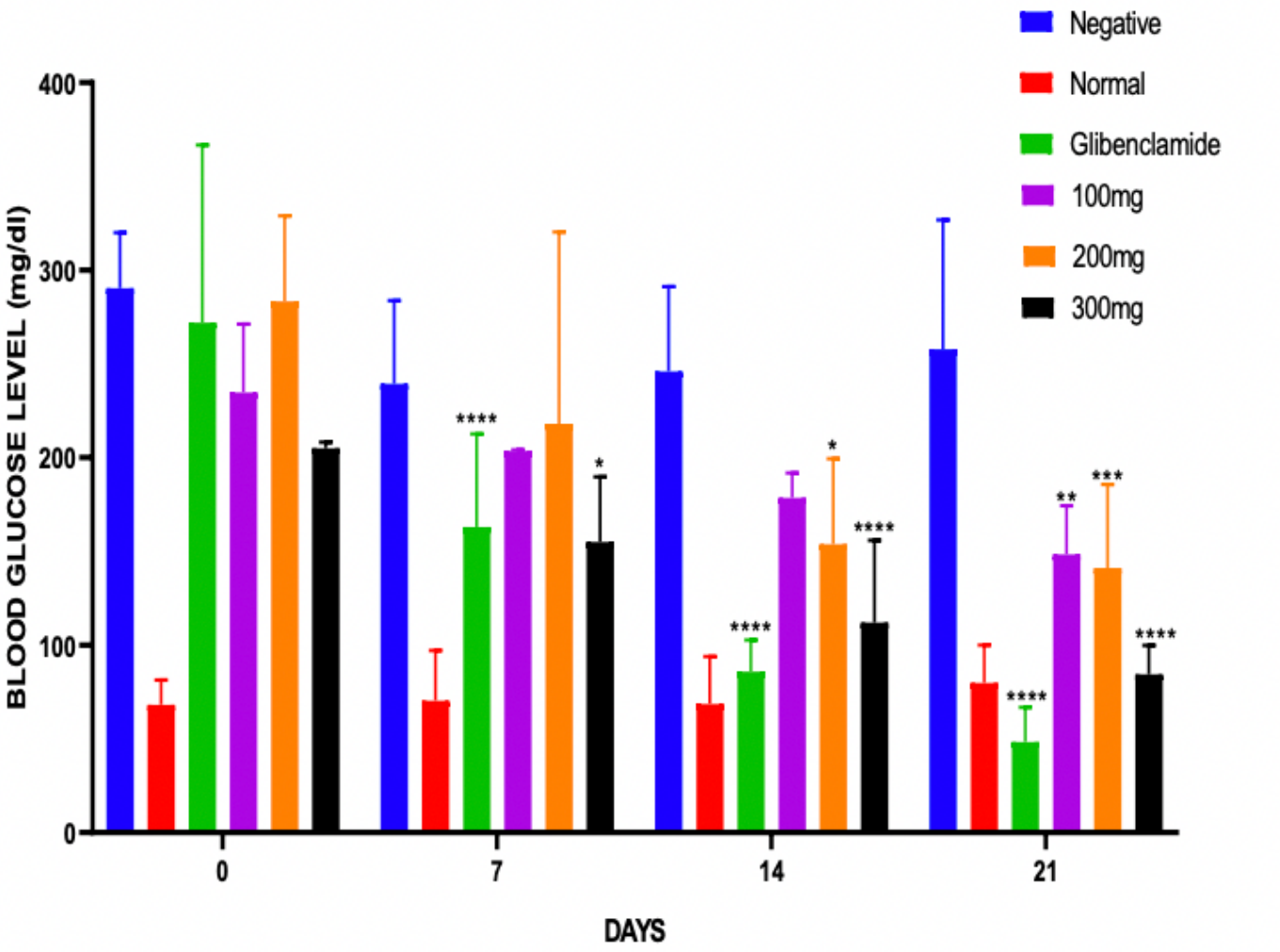

Figure 9

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.

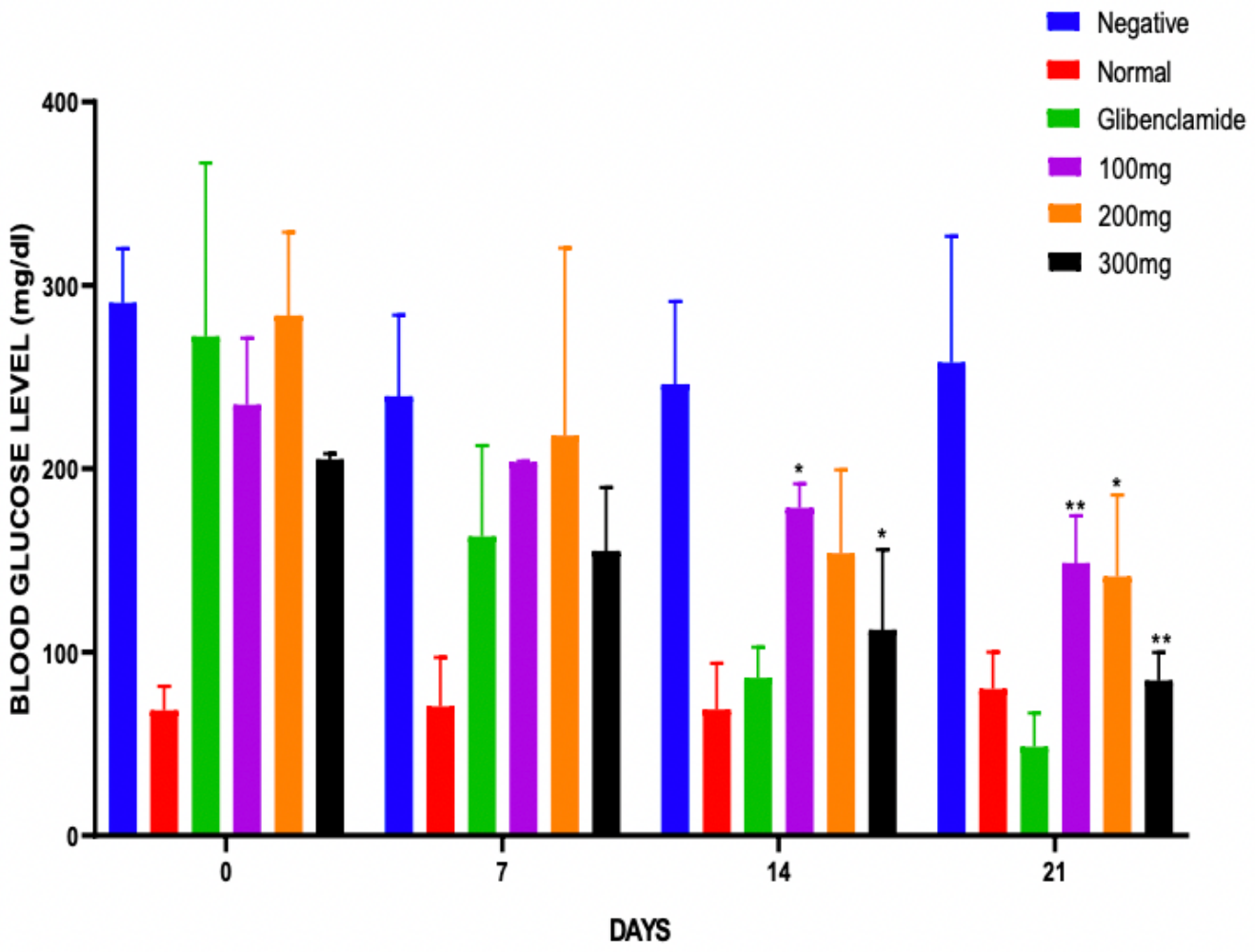

Figure 11

Statistical comparison of the blood glucose reduction produced by the Positive control Glibenclamide(5 mg/kg l). andC. 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