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(RESEARCH ARTICLE)

Pharmacognostic and antidiabetic evaluation of methanol extract of *Calyptrochilum christyanum* (RCHB.F.) Summerh. (Orchidaceae) and it's fractions

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Abstract

Plants are valuable sources of new compounds employed in the management of diabetes and other health conditions. This study aims to evaluate the pharmacognostic and antidiabetic activity of the methanol extract of Calyptrochilum christyanum and its fractions. The fresh leaf and crude extract were macroscopically and microscopically evaluated. The antidiabetic assay was carried out on the Alloxan-induced diabetic rats using glibenclamide as the standard antidiabetic drug. The microscopic evaluation showed paracytic stomata, unicellular trichomes, and collechymatous cells with lignified cell walls. The qualitative phytochemical screening of Calyptrochilum christyanum indicated the presence of secondary metabolites in this order: Flavonoids > Phenols > Tannins > Glycosides > Terpenoids > Saponins > Cyanides. The average percentage reduction in blood glucose showed that the methanol 200 mg fraction had the highest percentage reduction of 23.38%, followed by the crude extract 300mg at 22.60%. This, therefore, suggests that the plant indeed possesses antidiabetic activity.

Keywords: *Calyptrochilum christyanum*; Pharmacognostic; antidiabetic; Orchidaceae

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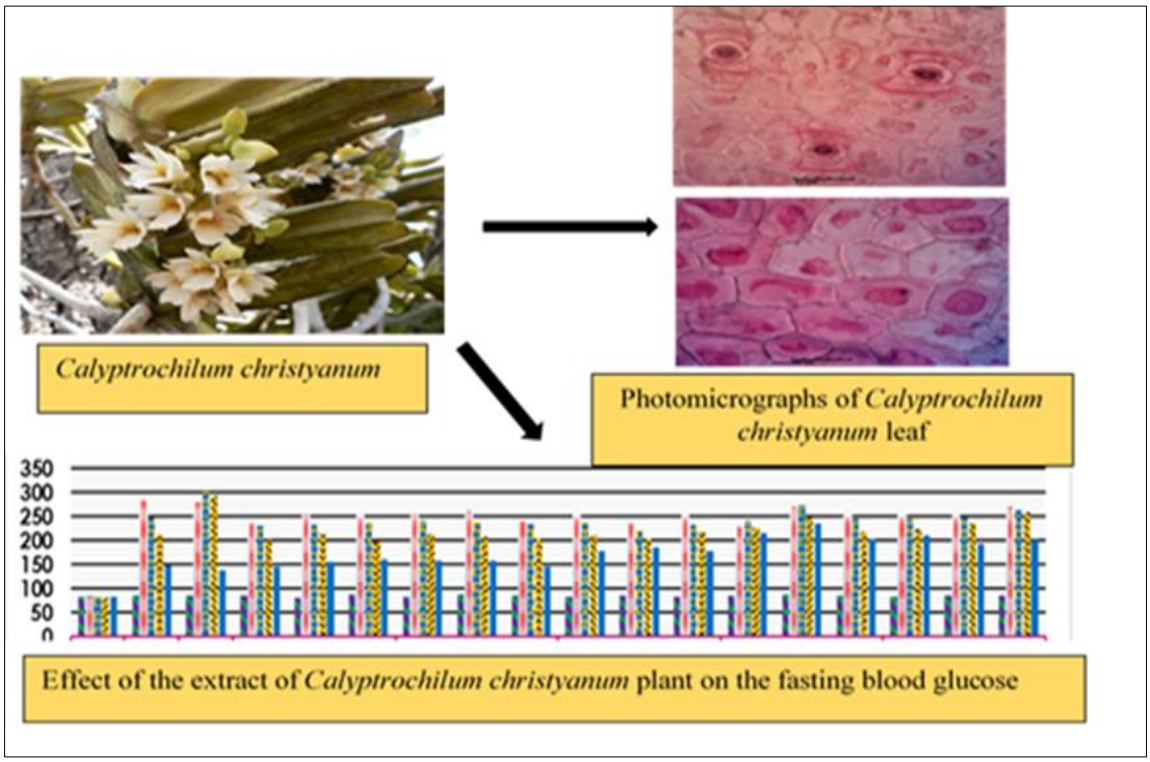
Graphics abstract



1. Introduction

Diabetes is a chronic condition characterized by elevated blood glucose levels arising from impaired insulin production or utilization (1). It is a significant global health issue, with its prevalence steadily increasing worldwide. According to the International Diabetes Federation, approximately 463 million adults (20-79 years) had diabetes in 2019 (2). This number will rise to 700 million by 2045 if current trends continue. According to the International Diabetes Federation, Nigeria has one of the highest rates of diabetes in Africa, with an estimated 5.3 million people living with the disease in 2019 (2). The landscape of diabetes management encompasses diverse therapeutic strategies, each aiming to achieve glycemic control and prevent complications (3). The measures include both pharmacological and non-pharmacological methods aimed at maintaining an optimum control of blood glucose to avoid complications. The associated complications, including cardiovascular diseases, neuropathy, and nephropathy, underscore the urgency to explore novel therapeutic avenues (4). Plant extracts are useful sources for new compounds to be employed directly or as targets that could be manipulated to improve glycemic control. Some hypoglycemic plants have been subjected to clinical tests for diabetic control (5). Calyptrochilum *christyanum* is a flowering plant from the orchid family Orchidaceae native to Africa (Senegal, Ethiopia, Nigeria, Zimbabwe and Mozambique) and the Bahia region of Brazil. The taxonomy of *Calyptrochilum christyanum* is presented in Table 1. The antioxidant and alpha-amylase inhibition activities of *Calyptrochilum christyanum* have been investigated(6,7). *Calyptrochilum christyanum*, commonly known as "Ela", is used ethnobotanically as an antidiabetic herbal remedy, and there is a need to evaluate this property scientifically using appropriate and adequate laboratory methods (8). Hence, our study aims to investigate the macroscopic and microscopic characteristics of the leaves of *Calyptrochilum christyanum* and its antidiabetic properties in Alloxan induced diabetic rat model.

Table 1 Taxonomy of *Calyptrochilum christyanum*

Kingdom	**Plantae**
Phylum	Streptophyta
Class	Equisetopsida
Subclass	Magnoliidae
Order	Asparagales
Family	Orchidaceae
Genus	*Calyptrochilum*
Species	*Calyptrochilum christyanum*

2. Methods

Whole plants of *Calyptrochilum christyanum*, were collected from Nsukka in the southeastern part of Nigeria and identified by Mr. Felix Nwafor, a taxonomist at the University of Nigeria, Nsukka. The collected plant was evaluated macroscopically (9). Microscopy of the fresh and powdered samples was done using standard methods. The plant was air-dried at room temperature for two weeks, pulverized to fine powder particles and stored in a moisture-free, airtight container for further evaluation and studies.

The extraction of the plant was carried out by the cold maceration method with a 1:1 ratio of methanol and dichloromethane followed by concentration in a rotary evaporator (10). Afterwards, the dried extract was weighed, and the weight value was used for the calculation of the percentage yield of the plant.

Quantitative phytochemical analysis was done according to standard methods (11,12). The fractionation process was carried out by Vacuum Liquid chromatography using dry-packing method and eluted with n-hexane, dichloromethane, ethyl acetate, and methanol (13).

Rats within the weight range of 150-200g were grouped into four animals per treatment group: non-induced, positive control, negative control, crude extract, and various fraction groups. The rats were fasted for 12 hours before inducing diabetes by injecting Alloxan at a dose of 130mg/kg intra-peritoneally. The plant extract fractions and standard drug (Glibenclamide) were dissolved using 12% Tween 80, and treatments were administered orally using a gavage. Blood samples were collected from the tail vein, and fasting blood glucose levels were measured using a glucometer at regular intervals.

3. Results

3.1. Macroscopic Evaluation Result

The results of the macroscopic evaluation of *Calyptrochilum christyanum* are presented in Table 2.

Table 2 Macroscopic features of the *Calyptrochilum christyanum* plant

Paramters	**Features**
Colour	Green
Odour	Slightly floral
Leaf appearance	The presence of midrib, absence of petiole, and leaves are narrow with unequal ends.
Leaf texture	Smooth and thick
Leaf arrangement	Alternate
Stem	Firm and woody
Venation	No venation

The results of the microscopic evaluation of *Calyptrochilum christyanum* is presented in Table 3.

Table 3 Microscopic features of C. christyanum

Parameters	**Features**
Epidermal cell	Epidermal cells are polygonally shaped with straight anticlinal cell walls on both the upper and lower surfaces.
Stomata type	The leaf is hypostomatic (stomata occur only on the lower surface) with paracytic (two subsidiary cells positioned parallel to the guard cells) type of stomata.
Trichome	Present - Unicellular covering trichomes
Stomata number (p.f.v.)	3.75 ± 0.48
Stomata density (mm^{-2})	22.06 ± 2.82
Stomata length (µm)	35.78 ± 0.59
Stomata width (µm)	32.40 ± 0.41
Stomata size ($µm^2$)	1158.82 ± 16.21
Epidermal cell number (p.f.v.)	45.00 ± 0.71
Stomata index (%)	8.30 ± 0.97

The result of the powder chemomicroscopy of *Calyptrochilum christyanum* leaves is presented in Table 4. The result shows the presence of starch grains, lignified tissues, tannin, cellulose, protein and oil. Calcium oxalates and gum were absent.

Table 4 Powder chemomicroscopy of the leaf of *C. christyanum*

Parameter	**Reagent(s)**	**Result**
Starch grains	Iodine solution	Present
Lignified tissues	Conc. HCl + Phloroglucinol	Present
Calcium oxalates	Iodine solution Conc. Sulphuric acid	Absent
Tannin	Ferric chloride	Present
Cellulose	Zinc chloride; Conc. Sulphuric acid	Present
Gum/Mucilage	Ruthenium red	Absent
Protein	Biuret reagent; Ninhydrin	Present
Oil	Sudan III reagent	Present

The photomicrographs of the leaf of *Calyptrochilum christyanum* show the adaxial and abaxial surfaces of the leaf with polygonal and straight-walled epidermal cells as presented in Fig. 1. The abaxial surface of the *Calyptrochilum christyanum* leaf has paracytic stomata while the adaxial surface is without stomata.

Figure 1 Photomicrographs of the leaf of *C. christyanum* A) Adaxial surface showing polygonal and straight-walled epidermal cells with no stomata B) Abaxial surface showing polygonal and straight-walled epidermal cells, and the presence of paracytic stomata Mg: X400

The transverse section of the *C. christyanum* leaf, stem and root is shown in Fig. 2. The photomicrograph of the whole powder of *C. christyanum* shows the presence of a pack of collenchymatous cells, lignified vascular tissues, leaf palisade cells, cork cells and some secretory glands/cells (Fig 3).

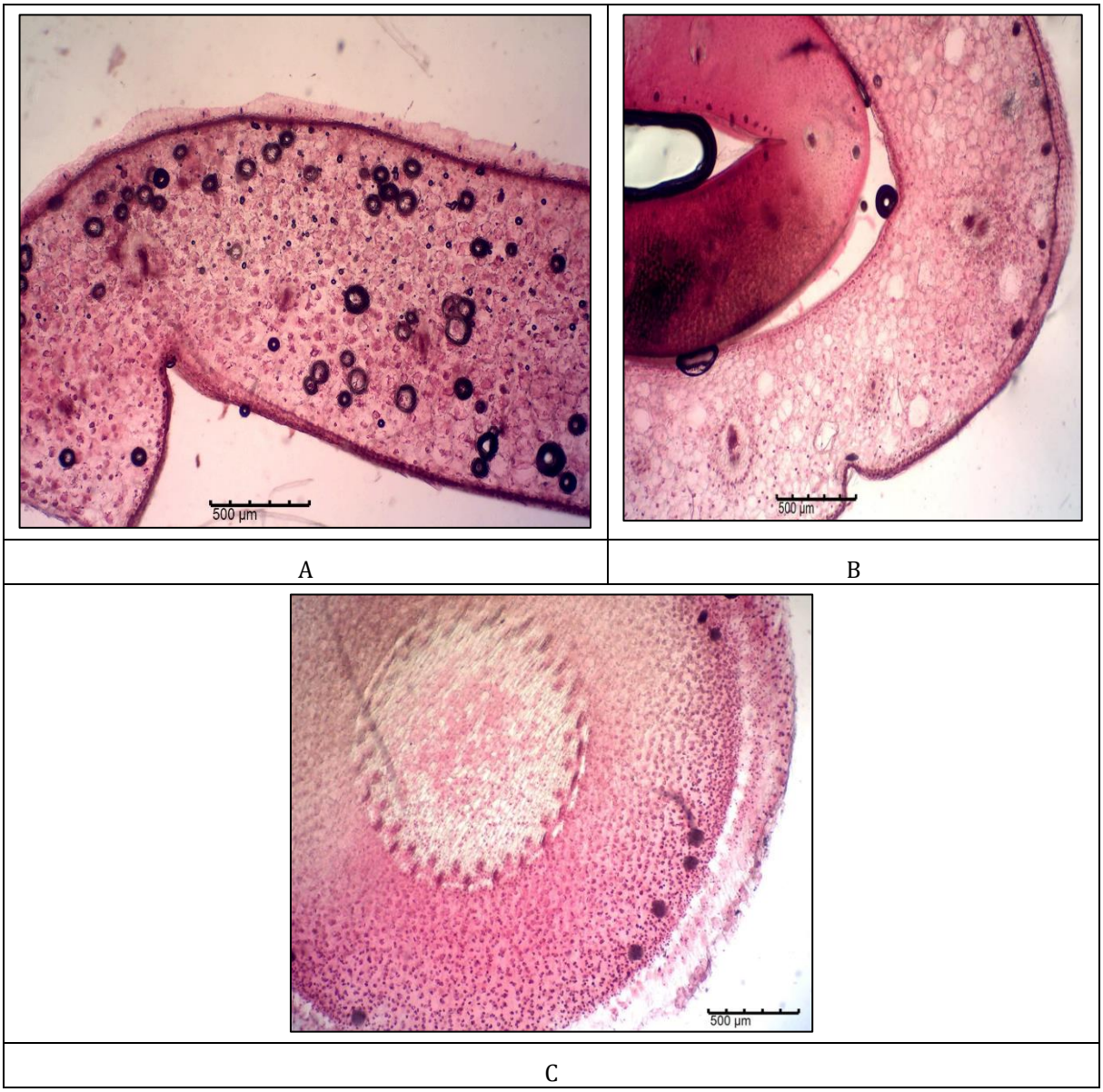


Figure 2 Transverse section of the different parts of *C. christyanum* A) the leaf showing unicellular trichomes on the lower surface B) the stem C) the root Mg X40

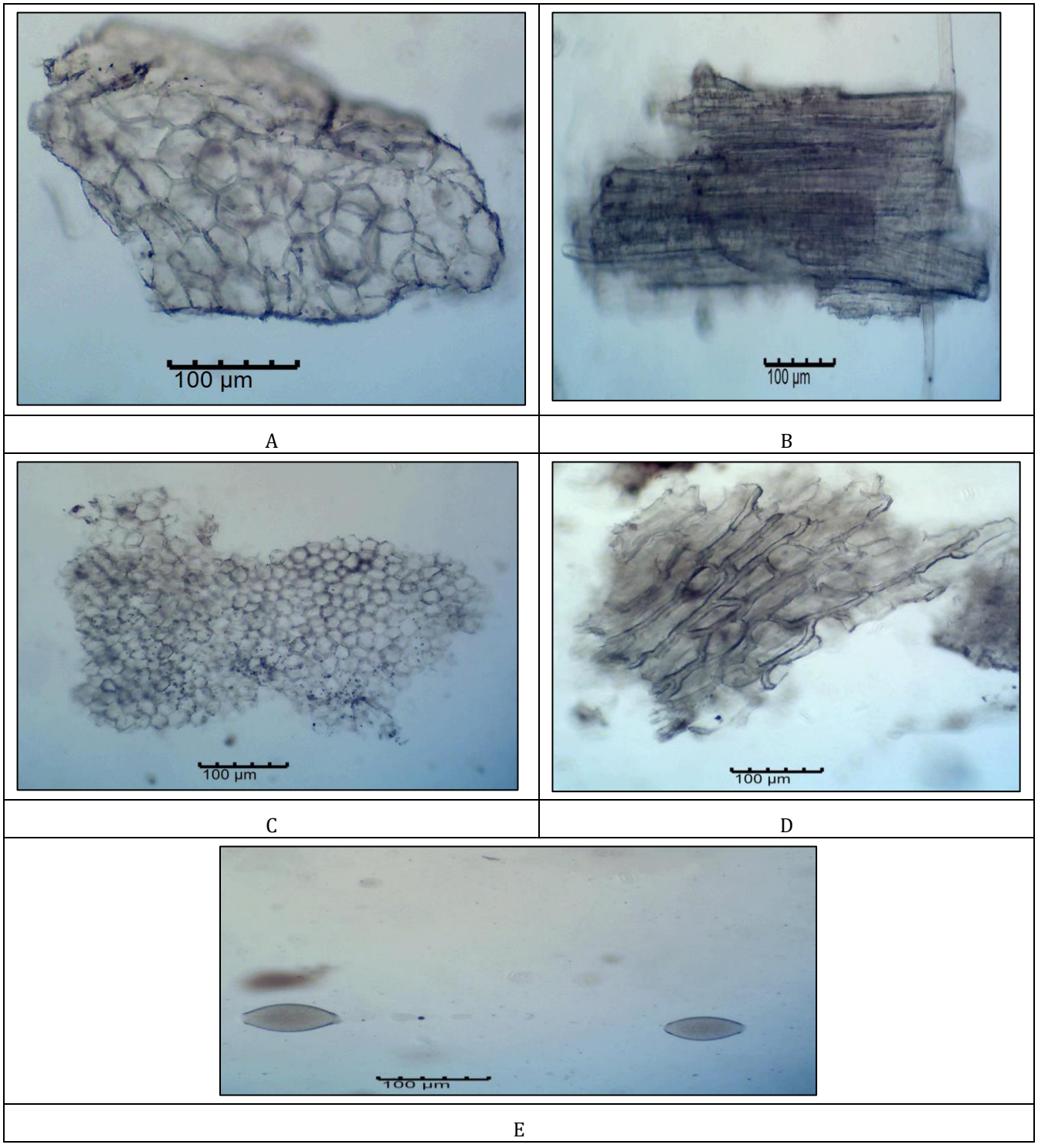


Figure 3 Photomicrograph of the whole powder of *C. christyanum* showing A) a pack of collenchymatous cells, B) a pack of lignified vascular tissues, C) leaf palisade cells, D) cork cells, E) Some secretory glands/cells

3.2. Result of Percentage Yield of Methanol Extract

A 45.8g of the extract was obtained from the extraction of a 1 kg sample using methanol as the solvent. This means that the percent yield is 4.5%. The yield of the different fractions of *Calyptrochilum christyanum* was highest with ethyl acetate and lowest with methanol, as presented in Table 5. The findings from the quantitative phytochemical analysis of *Calyptrochilum christyanum* show the presence of many phytochemicals in this order: flavonoid> phenol > tannin > glycoside > alkaloid > terpenoid > saponin > steroid > hydrogen cyanide (See Fig. 4).

Table 5 Percentage yield of the different fractions of *Calyptrochilum christyanum*

Solvents	Yield (%)
N-Hexane	22.3
Ethyl acetate	37.1
Dichloromethane	30.0
Methanol	9.1

Table 6 Quantitative Phytochemical Results of constituent of Calyptrochilum christyanum

Phytochemical	Quantity (Mg/100g)
Tannin	1021.80
Saponin	0.77
Flavonoid	1650.62
Alkaloid	643.02
Steroid	0.65
Phenol	1195.80
Hydrogen Cyanide	0.37
Glycoside	721.05
Terpenoid	450.26

3.3. Results of In Vivo Antidiabetic Study

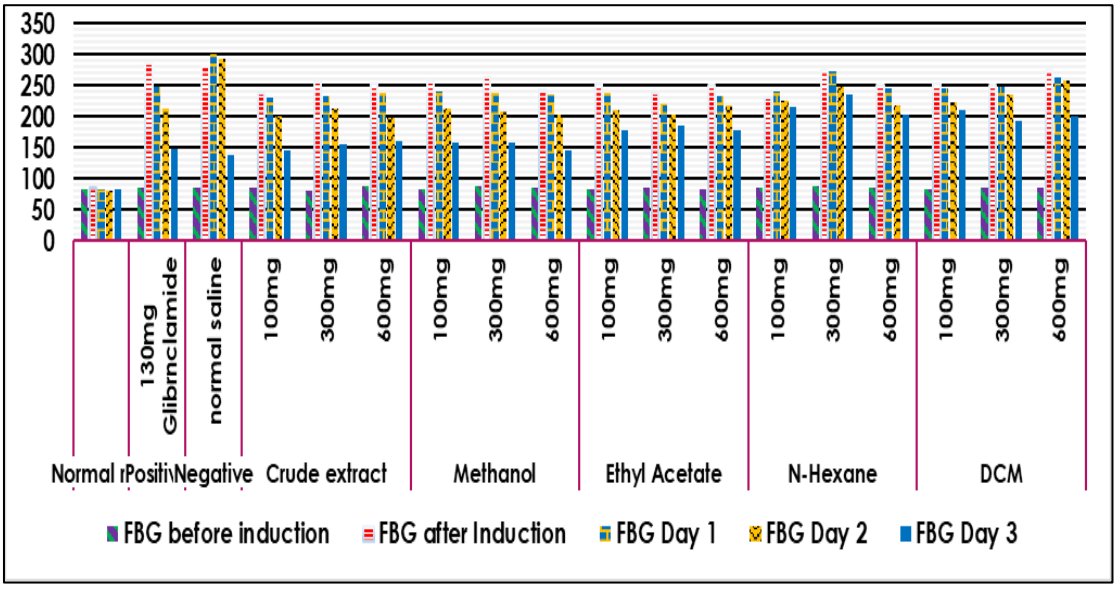


Figure 5 Effect of the crude extract and methanol fractions of the *Calyptrochilum christyanum* plant on the fasting blood glucose of alloxan-induced rat model

The glucose-lowering effect of the different concentrations of the methanol fractions of the *Calyptrochilum christyanum* plant on the alloxan-induced rat model is presented in Fig. 5. Methanol and ethyl acetate fractions gave higher glucose reduction, which were comparable to the standard drug. In contrast, DCM and n-hexane fractions gave lower blood glucose reduction (see Fig. 6).

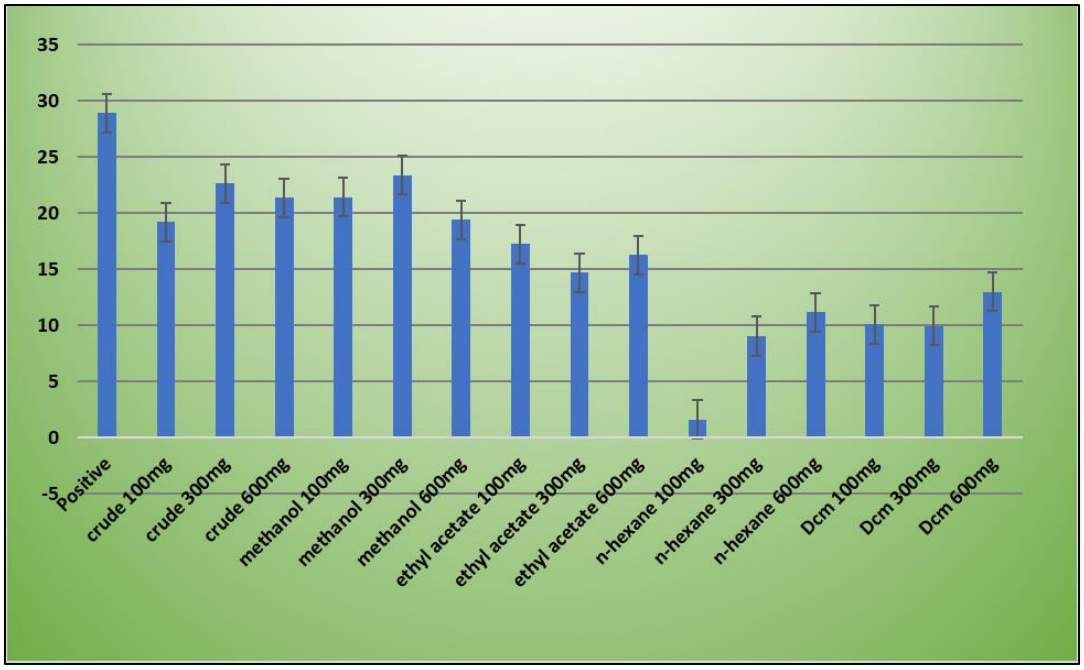


Figure 6 Average Percentage Reduction in Blood Glucose from the methanol fractions of the *Calyptrochilum christyanum* on alloxan-induced diabetic rats

4. Discussion

The macroscopic evaluation of the plant showed that *Calyptrochilum christyanum* is a green-coloured plant with narrow leaves with unequal ends, no venation and petiole, and a midrib alternately arranged. It has a smooth, thick texture and a firm, woody stem with a slightly floral odour. The microscopic analysis (Table 3) shows that epidermal cells of the leave of *C.christyanum* is polygonally shaped, forming an interlocking pattern that helps create a strong barrier protecting it from physical damage, water loss and pathogens. The photomicrographs of Calyptrochilum christyanum (Fig. 1) reveal various cellular structures indicative of its anatomical characteristics. For instance, collenchymatous cells found in clusters provide structural support and flexibility, especially in young plant tissues. Their thickened cell walls are evident in the photomicrograph, suggesting their role in supporting growth. Fig. 3C shows leaf palisade cells depicting elongated, tightly packed cells beneath the upper epidermis. These cells are rich in chloroplasts and are essential for photosynthesis, maximizing light capture. The cork cells observed in the photomicrograph (Fig. 3D) have a protective function. Their suberized walls form a barrier against water loss and pathogens, indicating their role in the plant's defence and water retention. Secretory glands or cells highlight the plant's ability to produce and secrete substances, potentially for defence or attracting pollinators. These structures can be identified by their distinctive morphology and position within the tissue. The lignified vascular tissues observed indicate areas where support and transport occur. These tissues, comprising xylems, are essential for water and nutrient transport, and their lignification strengthens the plant structure. Overall, the photomicrographs collectively illustrate the complex anatomy of *Calyptrochilum christyanum*, reflecting its adaptations for growth, protection, metabolic functions and medicinal use. Unicellular trichomes (as shown in Fig. 2A) were also seen on the surface of the leaves, providing extra protection to the plant. The stomata occur only on the lower surface and are paracytic (two subsidiary cells parallel to the guard cells.

The pulverized plant also showed a 4.58% percentage yield on extraction. For efficient extraction, methanol and dichloromethane were used to extract solvents in a 1:1 ratio instead of water to curtail microbial infestation and obtain higher extractive values. According to (14), the efficiency of the extraction process is directly determined by the properties of the extraction solvent(s). Thus, a solvent that can dissolve most, if not all, of the secondary metabolites, is usually chosen for the extractive function. The crude extract obtained after extraction was fractionated using N-hexane, Dichloromethane, Methanol, and Ethyl acetate as suitable solvents. Thus, various fractions were obtained with weights recorded as 7.8g for N-hexane, 13g for Ethyl acetate, 10.5g for dichloromethane, and 3.2g for methanol, respectively. The percentage yield from the fractionation of the extracts of *Calyptrochilum christyanum* gave 22.3%, 37.1%, 30% and 9.1% for the n-hexane, ethyl acetate, DMC and methanol fractions, respectively. The ethyl acetate fraction gave the highest yield (37.1%) while the methanol fraction gave the lowest yield (9.1%) for plants.

The quantitative phytochemical screening results indicate the presence of various metabolites in the following order of abundance Flavonoids > Phenols > Tannins > Glycosides > Terpenoids > Saponins > Cyanides. It is worth noting that the secondary metabolites are responsible for the antidiabetic properties of this plant, as they act either independently or synergistically to enhance the antidiabetic activity of the plant. Also, phytochemicals such as flavonoids, saponins, steroids, tannins, and glycosides are accountable for the numerous biological properties exhibited by plant extracts (15).

The findings from the evaluation of the antidiabetic activity of *Calyptrochilum christyanum* demonstrated that it has significant hypoglycemic activity. The graphical representation of Fig. 5 shows that the crude extract, alongside its various fractions, reduced the fasting blood glucose from the first to the third day of treatment. In contrast, the fasting blood glucose level of rats that received no treatment (negative control) after diabetes was induced remained high throughout the study, while the fasting blood glucose of the normal rats that were neither induced nor treated remained within the normal fasting blood glucose range.

Also, Fig. 6 shows the average percentage reduction in blood glucose levels of each treatment group. The methanol fraction at 200mg concentration showed the highest blood glucose reduction of 23.38%, followed by 300mg crude extract of 300mg with a decrease of 22.60%. The N-hexane fraction of 100mg concentration showed the lowest average percentage reduction in blood glucose at 1.59%.

5. Conclusion

The findings from our study suggest that the *Calyptrochilum christyanum* plant possesses glucose lowering activity and could be a potential antidiabetic agent.

Compliance with ethical standards

Disclosure of conflict of interest

The authors report there are no competing interests to declare.

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