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In vitro Antioxidant and Antidiabetic Potentials of Extracts of the Stem Bark of *Cylicodiscus gabunensis* (Harms) Mimosaceae

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ABSTRACT

The study evaluated the antioxidant and antidiabetic activity of extracts of *Cylicodiscus gabunensis* (CG) by *in vitro* assay methods. The powdered plant material was successively extracted to yield n-hexane extract (CG-HX), ethyl acetate extract (CG-EA), and methanol extract (CG-ME), respectively. The crude extract (CG-TE) was separately obtained from methanol. Phytochemical screening revealed the presence of alkaloids, flavonoids, phenolic compounds, steroids, and reducing sugars. The total phenolic content (TPC) was highest in the CG-ME (122.559 mg GAE/g extract), followed by CG-TE (121.913 mg GAE/g extract), while the total flavonoids content (TFC) was highest in the CG-EA extract (159.351 mg QE/g). The percentage DPPH free radical scavenging assay shows that the potency of the CG-EA extract (95.4±0.40 %) was comparable to ascorbic acid (96.22±0.692 %), $p<0.05$. In the ferric power-reducing antioxidant assay, CG-TE (2.246±0.1796) and CG-ME (2.140±0.1227) exhibited the highest antioxidant activity compared to others. The extracts significantly inhibited α-Amylase and glucose uptake in yeast cells in the *in vitro* study. This inhibitory potential was concentration-dependent with crude methanol extract (CG-TE) showing comparable inhibitory potency with Acarbose (45.7%) at 0.05 mg/mL but 40.10% at 4.00 mg/mL as against 51.27 % for Acarbose. Similarly, CG-TE was more potent than metronidazole, with percentage glucose uptake inhibitory potency of 50.13% and 76.465% at concentrations of 0.05 mg/mL and 4.00 mg/mL, respectively. We conclude that the plant extracts possess significant antidiabetic activity, which correlated with the antioxidant effects and could be further investigated for use in managing diabetes and related diseases.

***Keywords*:** *Cylicodiscus gabunensis*, Antidiabetic activity, Antioxidant activity, α-amylase, Acarbose.

Introduction

Diabetes mellitus (DM) is a metabolic disease complex characterised by chronic and sustained hyperglycemia caused by a deficiency in either insulin production or action and in some cases, both.[1] This defect causes metabolic irregularities in carbohydrates, lipids, and protein metabolism.[2] Uncontrolled diabetes has been linked with various physiological conditions, ketoacidosis, stupor, coma, and death.[3] The IDF report of 2021 showed the global prevalence of diabetes amongst different age groups and sex. It reported that in the adult (20-79 years old) population of 382 million people, 8.3% were diabetic.[4] The disease was most prevalent among adult males (198 million) compared to females (184 million) of ages 40-59 years. The report projected a rise in the number of cases to over 592 million by 2035, with a global prevalence of > 10%. There are about 175 million undiagnosed cases of diabetes, with 21 million pregnant women affected by the disease.[4] A global IDF survey showed that populations in the Middle East and North Africa have the highest prevalence of diabetes, with about 138.2 million cases, while 8% of patients are in low and middle-income countries, where studies also show more people being diagnosed with the disease.[4]

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Insulin is critical to many patients' Type 1 and sometimes Type 2 diabetes treatment plans.[5] Insulin comes in various formulations, each with its peak onset and duration of action. In Type 2 diabetes, oral hypoglycemic medications are utilised, while insulin is needed in around a third of patients. Oral antidiabetic medicines are classified based on their mechanisms of action. These include insulin secretagogues (sulfonylureas and meglitinide analogues); insulin sensitisers (biguanides and thiazolidinediones); α-glucosidase inhibitors, and Dipeptidyl peptidases-IV inhibitors.[6] Oral hypoglycemic medications and insulin therapy (injectables) are the mainstay of diabetes treatment. They, however, have some unwanted side effects and sometimes fail to alter or amend diabetic complications. As a result, there is an urgent need to search for alternatives. Plants have been known to be the largest reservoir of medicinal compounds that man has harnessed for healthcare purposes for centuries.[7] Herbal drugs have various advantages, including a lower chance of side effects, effectiveness against chronic illnesses, extensive availability, and affordability.[8] There is a general acceptance of herbal medicines worldwide. Some Nigerian medicinal plants (*Persea Americana*, *Parkia biglobosa*, *Sida acuta*, *Tithonia diversifolia*, *Aframonium melegieta*, *Azadirachta indica*, *Carica papaya*, *Ocimum gratissimum*, *Moringa oleifera*, etc.) have been evaluated for activities against various diseases including cancers, inflammations, hypertension, malaria, and diabetes.[9] For instance, the fruit juice of *Citrus paradisi* was reported to improve different haematological and histopathological parameters in streptozocin-induced diabetic rats.[10]

Cylicodiscus gabunensis (Harms), Mimosaceae, is distributed in the deciduous and evergreen forests of equatorial Africa; Cameroon, Central African Republic, Congo, Equatorial Guinea, Gabon, Ghana, Ivory Coast, and Nigeria. It has long been recognised among the different tribes in West and Central Africa for its ethnomedicinal uses.

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The Bokis (in Cross River State, Nigeria) and other tribes use the decoction of the stem bark to treat fevers, infections, stomach issues, diabetes, stroke, and other ailments such as headaches, filariasis, rheumatism, and migraine headaches.[11,12] The stem bark extract is also reported to possess antiplasmodial activity.[13] It is also used to treat erectile dysfunction in traditional medicine, possibly due to its antioxidant property[14] and inhibition of the PDE5 (phosphodiesterase-5) enzyme implicated in erectile dysfunction.[15] Phytochemical separation of the stem bark extract of *C. gabunensis* resulted in the isolation and characterisation of some triterpenes, such as cylicodiscic acid, cyclodione, and coumestan glycosides.[16,17,18] Using other chromatographic techniques, GC-MS and LC-MS, gallic acid, ethyl gallate, and other phenolic compounds were identified and may have contributed to its antimicrobial activity.[16] Also, further chemical analysis of the stem bark of *C. gabunensis* led to the isolation of three previously undefined glycosides 'coumestoside A, coumestoside B, and erythrodiside A', erythrodiol, and sojagol (IV) were also reported.[19]This study aims to evaluate the *in vitro* antioxidant and antidiabetic activities of various extracts of the stem bark of *C. gabunensis* to validate its ethnomedicinal use in treating diabetes.

Materials and Methods

Sample Collection
The stem bark of *C. gabunensis* was purchased from a local market in Benin City, Edo State, in March 2020. The sample was identified by Professor Macdonald Idu, a taxonomist, at the herbarium unit of the Plant Biology and Biotechnology Department of the University of Benin, Edo State. A voucher number UBF-V261 was assigned.

Solvents and Reagents
All reagents and solvents used for this study were of analar grade. Acarbose was purchased from (Intra Labs India Pvt. Ltd), Methanol (JDH, China), Potassium dihydrogen phosphate (Qualikems, India), Sodium chloride, Sodium hydroxide (Merck), soluble starch, dinitrosalicylic acid (DNSA), α-amylase, Metronidazole (Sanofi), sodium phosphate buffer, baker's yeast, glucose powder, were all purchase from Pyrex Chemicals, Nigeria.

Preparation of extracts
The dried stem bark was ground to powder using a mechanical blender (PCG-890, Germany) and weighed. An amount (200 g) of the ground material was macerated with 1L of methanol for 48 hours. The crude extract solvent was removed with a rotary evaporator at 40 ºC to give 9.92 g of a dark solid (total extract, TE). Another 500 g of the powdered stem bark was successively extracted by macerating in 2L of hexane for 48 hours, followed by ethyl acetate and then methanol, respectively. The extracts were concentrated under reduced pressure in a rotary evaporator at 40 ºC to yield 1.98 g, 1.27 g, and 10.18 g, respectively.

Phytochemical screening
The aqueous extract of the powdered plant material was screened for alkaloids, tannins, saponins, terpenoids, steroids, anthraquinones, flavonoids, proteins, and other phenolic compounds using established methods.[20,21]

Statistical analysis
All the experiments were carried out in triplicates, and data were analysed with Microsoft Excel 2010 and expressed as mean ± SEM. One-way ANOVA was carried out using SPSS statistical package. $P<0.05$ was considered significant.

Results and Discussion

The dried stem bark of *C. gabunensis* was extracted successively with hexane, ethyl acetate, and methanol to obtain corresponding extracts coded as CG-HX (2.00 g), CG-EA (1.30 g), and CG-ME (10.20 g), respectively. Also, a portion of the powdered plant material was extracted with methanol to obtain a crude extract (CG-TE, 15.95 g). The qualitative phytochemical test of the extracts of *C. gabunensis* revealed that alkaloids, tannins, phenols, flavonoids, saponins, steroids, carbohydrates, reducing sugars, and deoxysugars are present, while proteins were not detected (Table 1).

Total Phenolic Contents (TPC)
The phenolic contents of the crude extract (TE), hexane extract (CG-HX), ethyl acetate extract (CG-EA), and methanol extract (CG-ME) were obtained from the equation of the calibration curve of Gallic acid, $Y = 0.0031X + 0.0314$ ($R^2 = 0.991$), as shown in Figure 1.

Total Flavonoids content (TFC)
Similarly, the flavonoids content of the extracts was obtained from the equation of the calibration curve of Quercetin, $Y = 0.0073X - 0.0296$ ($R2 = 0.9902$), as shown in Figure 2.
Diabetes is associated with the inability of the pancreas to produce insulin or to utilise insulin effectively.[29] Millions of people worldwide are affected by the disease. It is reported by the International Diabetic Federation (IDF) that over 637 million people worldwide were living with diabetes in 2021.[4] It is known to cause micro and macrovascular changes that often results to other complications (heart attack, kidney failure, leg amputation, vision loss), and peripheral neuropathies in the body's extremities.[30] Reports show that excess free radical generation induces oxidative stress, exacerbating diabetes.[31,32] Antioxidants are known to quench free radicals in the body, and reduce oxidative stress.[33] Several plant secondary metabolites have been implicated in managing various diseases (diabetes[34] and cancer[35]) in humans and animals.

Table 1: Chemical constituents of *Cylicodiscus gabunensis* stem bark

Extracts	Results
Alkaloids	+
Saponins	+
Proteins	-
Phenolics	+
Tannins	+
Flavonoids	+
Steroids	+
Deoxysugars	+
Carbohydrates	+
Reducing sugars	+

Present = +; Absence –

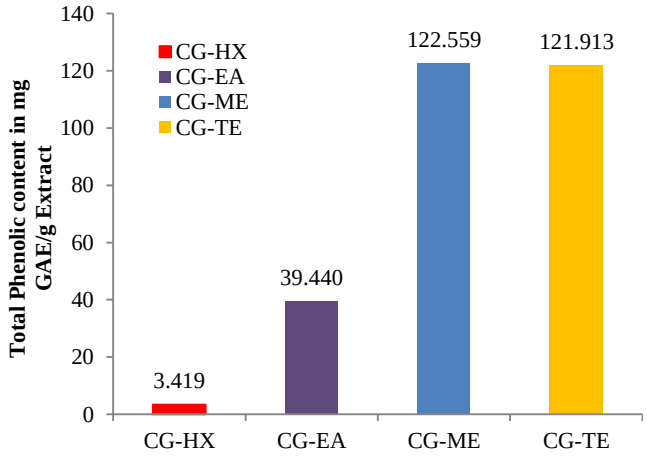

Figure 1: Showing the TPC of the extracts of *Cylicodiscus gabunensis*

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It has been reported that over 40 % of drugs in clinical use today were either natural products or their derivatives.[36] Plant secondary metabolites, like alkaloids, flavonoids, and Phenolics, are known to prevent inflammatory disorders, cardiovascular diseases, cancers, oxidative stress, and diabetes.[37,38,39,40] Flavonoids (quercetin, rutin, apigenin, and luteolin) can exhibit various biological activities, and are known for their antioxidant and free radical quenching potentials,[41,42,43,44] antitumour,[45] and their ability to prevent cardiovascular diseases.[46] The results of the quantitative phytochemical screening (total phenolic and flavonoid contents) of the plant extracts are shown in figures 1 and 2. This study shows that the ethyl acetate extract has the highest flavonoid content (159.351 mg QE/g extract), Figure 2, and antioxidant activity (95.42+0.400%) Table 2. A similar study by Ganuyi *et al*. on the aqueous extract of CG, the total phenolic and flavonoid contents were 41.82 mgGAE/g and 36.06 mgQE/g, respectively. The extract also exhibited significant DPPH free radical scavenging effects with an IC_{50} value of 0.60±0.03 mg/ml.[15]The total phenolic content of the extracts in this study is shown in Figure 1. The methanol extract had the highest total phenol content (122.45 mgGAE/g), while the n-hexane extract had the least (3.42 mgGAE/g). These values also translate to their antioxidant activities, as seen in Table 2, where CG-HX has a percentage antioxidant activity of 17.68+3.955%, while CG-ME had 70.17+1.631%.[47,48] Similarly, in the reducing power assay using butylated hydroxytoluene (BHT), all the extracts at the different concentrations tested showed concentration-dependent antioxidant activities. The CG-TE and CG-ME exhibited the highest antioxidant activity, 2.246±0.1796 and 2.140±0.1727, respectively, compared to the other extracts (Table 3).

The study also evaluates the antidiabetic activity of the crude, methanol, ethyl acetate, and hexane extracts of stem bark of *C. gabunensis* using *α*-amylase inhibitory activity and glucose uptake inhibition in yeast cells. In the *α*-amylase inhibition assay, *α*-amylase was subjected to increasing concentrations (0.050-4.00 mg/mL) of the test samples (CG-TE, CG-HX, CG-EA, and CG-ME) using Acarbose as a standard. All the extracts showed a concentration-dependent *α*-amylase inhibition activity. The CG-ME at 0.05 mg/mL exhibited the highest *α*-amylase inhibitory activity with a significant ($p<0.05$) percentage inhibition of 45.70% (figure 3 and table 5), with an IC_{50} value of 0.3476 mg/mL (Table 4). This inhibitory activity of the extract compares with Acarbose at the same concentration (45.70%). There was a decrease in the inhibitory activities at 0.10 mg/mL, followed by a steady increase in the inhibitory activity (figure 3). Acarbose, a complex polysaccharide, delays glucose absorption by competitive but reversible inhibition of pancreatic *α*-amylase and membrane-bound intestinal *α*-glucoside hydrolases. This inhibition leads to lower blood glucose concentrations.[49]Inhibitors of the *α*-amylase enzyme block the cleavage of 1,4-glycosidic linkages of starch and other oligosaccharides into simple sugars, thus preventing starch's absorption into the body.[50] Ganiyu et al. reported a similar concentration-dependent inhibitory effect of the extract of CG on the activity of *α*-amylase, which was claimed to be related to the copious amounts of phenolic compounds in the plant samples.[15] The *α*-amylase inhibitory effects observed with CG-ME may be due to the presence of polar phytoconstituents. It should be noted that all the extracts cause the inhibition of *α*-amylase activity (figure 3). The results of this assay show that the extracts of *C. gabunensis* inhibited the *α*-amylase enzyme significantly ($p<0.05$). It is evident from this study that the inhibition of *α*-amylase by the extracts of the study plant impedes the breakdown of starch in the small intestine and has antidiabetic potentials and can therefore be used to regulate post-prandial hyperglycemia.[51] Similarly, the glucose uptake in the yeast cell model shows that increasing concentrations (0.05-4.00 µg/mL) of the different extracts of *C. gabunensis* exhibited a significant and dose-dependent inhibition of glucose uptake in yeast cells (figure 4 and table 5). The CG-TE exhibited the highest glucose uptake inhibitory activity (76.46%), figure 4. The extracts of *C. gabunensis* showed better inhibition of glucose uptake in all the concentrations (0.05 – 4.00 mg/mL) compared to metronidazole at the same concentration, the positive control drug. The IC_{50} values of the extracts CG-TE, CG-HX, CG-EA, and CG-ME, are shown in Table 4.

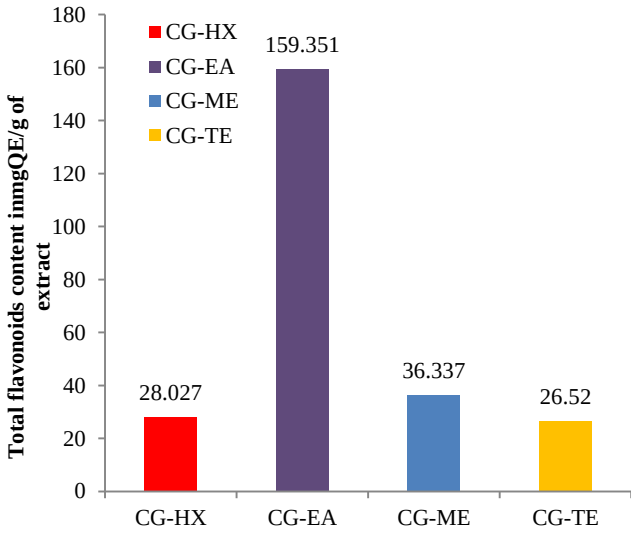

Figure 2: Showing the TFC of the extracts of *Cylicodiscus gabunensis*.

Table 2: Comparison of the DPPH Free radical scavenging activity of Extracts and Ascorbic Acid

Extracts	% Free Radical Scavenging Activity (mean ±SD)
CG-TE	75.94±0.200
CG-HX	17.68±3.955
CG-EA	95.42±0.400
CG-ME	70.17±1.631
Ascorbic acid	96.22±0.692

Where CG-TE = crude; CG-HX = Hexane extract; CG-EA = Ethyl acetate extract, CG-ME = extract.

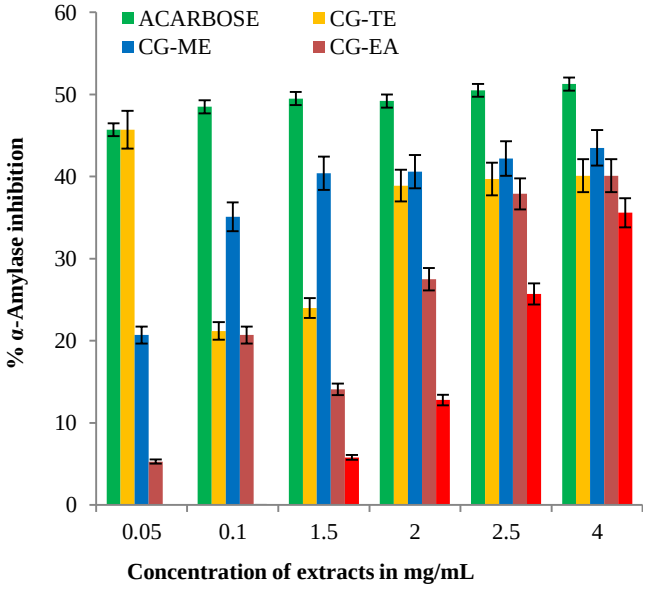

Figure 3: Inhibitory potency of extracts of *Cylicodiscus gabunensis* against *α*-amylase activity. The values are expressed as means ± SEM of triplicate tests

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Table 3: Antioxidant activity of CG extracts by reducing power assay, with BHT as a positive control agent (Absorbance is expressed as mean ± SD; 700 nm)

Conc (µg/mL)	BHT	CG-TE	CG-ME	CG-EA	CG-HEX
200	1.168 ± 0.0175[a]	0.918 ± 0.0991[b]	0.848 ± 0.0306[b]	0.359 ± 0.0654[c]	0.281 ± 0.0078[d]
400	1.580 ± 0.2136[a]	1.074 ± 0.1390[b]	1.359 ± 0.0085[a]	0.413 ± 0.0467[c]	0.429 ± 0.0240[c]
600	1.925 ± 0.1534[a]	1.214 ± 0.1027[b]	1.447 ± 0.0921[c]	0.507 ± 0.1004[d]	0.453 ± 0.1107[e]
800	2.683 ± 0.2716[a]	2.096 ± 0.1863[b]	1.607 ± 0.0793[c]	0.544 ± 0.0860[d]	0.496 ± 0.0446[e]
1000	3.705 ± 0.2540[a]	2.246 ± 0.1796[b]	2.140 ± 0.1727[b]	0.825 ± 0.0201[c]	0.506 ± 0.0151[e]

Values are expressed as mean ± SEM of replicate readings. Values with the same superscript along the same row are not statistically different (p<0.05).

Table 4: IC_{50} values for α-amylase and Glucose uptake inhibitory potentials of *Cylicodiscus Gabunensis* stem bark extracts.

Extracts	IC_{50} (mg/mL) α-Amylase	Glucose uptake
CG-TE	1.946[a]	2.248[b]
CG-HX	2.232[a]	2.230[a]
CG-EA	2.182[a]	2.354[a]
CG-ME	0.3476[a]	2.227[b]

Some studies involving the transport of non-metabolisable sugars suggested that stereo-specific membrane carriers mediate sugar transport across the yeast cell membrane through facilitated diffusion.[52,53] Glucose uptake by yeast cells may be affected by cellular glucose concentrations and metabolism in the cells. The conversion of internal sugars into other metabolites reduces internal glucose concentration, favouring high glucose uptake into the cells. Therefore, there is a possibility that the plant extracts induce glucose uptake into yeast cells by facilitated diffusion and elevated glucose metabolism. It is necessary to explore further the activity of the extract *in vivo*, to establish the mechanism by which it enhances glucose uptake by muscle cells and fatty tissues. The inhibitory activity of the extracts on α-amylase, an enzyme considered important in carbohydrate metabolism, and inhibition of glucose uptake in yeast may be due to the presence of bioactive phytoconstituents in the plant's extracts and could be of great pharmacological importance in the management of diabetes.

Table 5: Comparative results for total phenolic content (mg GAE/g extract), total flavonoids content (mg QE/g extract), Reducing Power Antioxidant assay (1 mg/mL), % DPPH free radical scavenging assay, *in vitro* percentage α-Amylase inhibition (0.05 and 4.00 mg/mL) and percentage glucose uptake inhibition potential in yeast cells at 0.05 & 4.00 mg/mL)

EXTRACT	TPC (mg GAE/g extract)	TFC (mg QE/g extract)	RPA (Extract conc. 1 mg/mL)	% DPPH free radical scavenging activity	% amylase inhibition		% Glucose uptake in yeast cells	
					(0.05 mg/mL)	(4.0 mg/mL)	(0.05 mg/mL)	(4.0 mg/mL)
CG-TE	121.913[a]	26.52[a]	2.246[a]	75.94[a]	45.70[a]	40.10[a]	50.13[a]	76.46[a]
CG-HX	3.419[b]	28.027[b]	0.506[b]	17.68[b]	nd	35.60[c]	24.73[b]	50.76[b]
CG-EA	39.440[c]	159.351[c]	0.825[c]	95.42[c]	5.30[b]	40.10[a]	37.00[c]	52.00[c]
CG-ME	122.559[a]	36.337[d]	2.140[a]	70.17[d]	20.70[c]	43.50[b]	47.40[d]	52.00[c]

Where: TPC (total phenolic content); TFC (total flavonoids content); RPA (reducing power assay); nd (not detected); values in vertical columns not sharing common superscripts are significantly different (p<0.05).

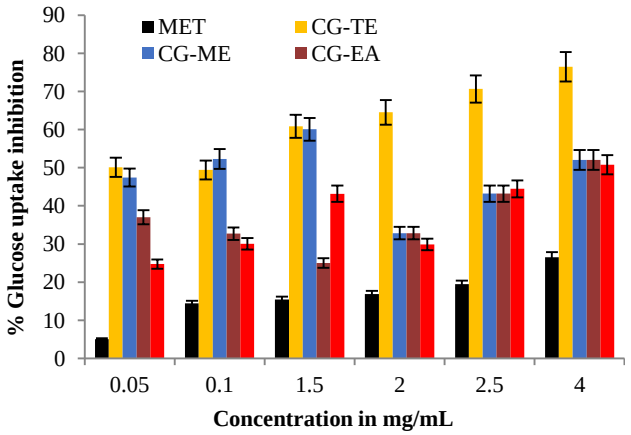

Figure 4: Glucose uptake inhibitory potential of extracts of *Cylicodiscus gabunensis* in Yeast cells. The values are expressed as means ± SEM of triplicate tests.

Conclusion

The study has revealed that the extracts of *Cylicodiscus gabunensis* contain phytochemicals with significant antioxidant activities, which may be responsible for the observed biological activities. The stem bark extracts exhibited modest inhibition of α-amylase (45.7% at 0.04 mg/mL) comparable to that of Acarbose and promoted glucose uptake in yeast cells. Therefore, this study conclude that the inhibitory effects of *Cylicodiscus gabunensis* on α-amylase and glucose uptake in yeast cells and their antioxidant activities suggest that these plant extracts could be harnessed to manage diabetes and other oxidative stress-related diseases.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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