

In vitro Antidiabetic and Antioxidant Activities of *Alchornea cordifolia* stem bark

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ABSTRACT

Alchornea cordifolia, commonly known as the Christmas bush, is a popular medicinal plant utilised in the management of several diseases including diabetes mellitus. The study aimed to comparatively assess the phytochemical components, *in vitro* antioxidant, and antidiabetic properties of the aqueous and methanol extracts of *Alchornea cordifolia* stem bark. The extracts of *A. cordifolia* were screened for their phytochemicals by standard procedures, and examined for antioxidant activity using various *in vitro* models: 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and Nitric oxide (NO). The antidiabetic capability of extracts was measured by assessing their inhibitory effects against α -amylase and α -glucosidase activities. The phytochemical inquiry revealed the presence of alkaloids, phenols, flavonoids, saponins, tannins, anthocyanins, glycosides, and terpenoids in the methanol extract. While the phytochemicals found in the methanol extract were also present in the aqueous extract except for terpenoids, the extracts exhibited dose-reliant free radical scavenging effects in the tried models, with IC_{50} values of 0.24 ± 0.01 and 0.30 ± 0.01 for the methanol and aqueous extracts, in the DPPH assay, IC_{50} values of 0.23 ± 0.05 (methanol extract) and 0.31 ± 0.01 (aqueous extract), in the ABTS method, and IC_{50} values of 0.31 ± 0.03 and 0.24 ± 0.06 respectively for the methanol and aqueous extract in the NO method. Hence, the best radical scavenging result was gotten from the methanol extract. Also, the methanol extracts of *A. cordifolia* exhibited considerably higher inhibition against α -amylase and α -glucosidase activities than the aqueous extract, as reflected in their IC_{50} values. Thus, the methanol was a better solvent in extracting the active components of the plant. This study validates the antidiabetic and antioxidant activities of the *A. cordifolia* stem bark, which explains its usage in folkloric medicine.

Keywords: *Alchornea cordifolia*, Phytochemical, α -glucosidase, α -amylase, Antidiabetic, Antioxidant.

Introduction

Alchornea cordifolia is a small plant (5 – 8 m high), with numerous stems, armed with blunted spines. The leaves are long-petiolate; cordate at base, apex curvly acuminate, sketchily ovate, entire or marginally dentate margin. It is slightly

glabrescent beneath or stelate-puberulous; with greenish-white to lax pendulous spikes flowers; the styles are lengthy and permanent on its fruit; the fruit is two-celled, stellate pubescent and small (Ishola *et al.*, 2014; Boniface *et al.*, 2016). *A.*

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cordifolia is the most studied species in the Alchornea genus. It occurs mostly in the west to central Africa. It is found in countries like Congo, Nigeria, Ivory Coast, and Ghana. The common names for *A. cordifolia* comprise buissondenoel (French) and Christmas bush (English). In Nigeria, it is known as “Bambami” in Hausa, “Ububo” in Igbo, and “Ewe Ipa”, “Esinyin” in Yoruba, “mbom” in Efik (Noundou *et al.*, 2016; Boniface *et al.*, 2016).

The different parts of *A. cordifolia* have diverse medical uses and applications, which have been validated scientifically by several researchers (Mohammed *et al.*, 2013; Ishola *et al.*, 2014; Noundou *et al.*, 2016; Boniface *et al.*, 2016). Although the leaves are commonly used, the stem bark, leafy stems, stem pith, root bark, fruits, and root also have diverse medical applications locally (Boniface *et al.*, 2016). Traditionally, *A. cordifolia* is used for boosting fertility and in treating genital-urinary complications including venereal diseases. The stem bark, roots, seeds, and leaves are utilized to induce labor, useful for abortifacation, and diabetes management. The leaf’s powder is applied locally for ulcers, and wounds healing. Decoction of the stems, and leaves is used for treating diarrhea, malaria, toothache, dermatitis, and other diseases (Ishola *et al.*, 2014; Ajibade and Olayemi, 2015).

Diabetes mellitus (DM), characterized by chronic hyperglycemia, is an endocrine disease caused by a deficiency or reduced efficiency of insulin. Chronic hyperglycemia results in reactive oxygen species (ROS) production, leading to the incidence of organ damage in diabetes. The antioxidant defense structure of organisms performs an essential role to improve the damaging effects of ROS. Thus, intake of antioxidant-rich foods is critical to stoppage of ROS-induced tissue and organ impairments in diabetes (Robertson and Harmon, 2007).

Humans have employed different plants for several centuries in their milieu for healing purposes. Plants are considered medicinal, and they serve as vital sources of raw materials for obtaining active substances necessary for drug manufacture. Oxidative stress has been fingered as the cause of numerous pathological disorders. Progress in the subject of biological antioxidants is emphasized to finally utilize them in healthcare, for chronic ailments, and in the field of nutrition (Pizzino *et al.*, 2017; Lekouagheta *et al.*, 2020).

Orthodox antidiabetic drugs normally act by either inhibiting the break of alimentary carbohydrates to glucose or precluding the diffusion of glucose across the membrane of the intestine into circulation. Inhibiting alpha-glucosidase and alpha-amylase enzymes is a significant approach to managing the post-prandial level of blood glucose in patients with type 2 diabetes. All the currently existing antidiabetic agents have been linked with considerable undesirable effects (Anbu *et al.*, 2012). Thus, herbal medicines have been the focus in recent times, as a safer and cheaper means for hypoglycemic agents (Sharifi-Rad *et al.*, 2020). Herbal preparations from medicinal herbs are more active, cheaper, safer, and compatible with the human body than synthetic modern drugs. Besides, rural communities rely on herbal drugs largely for primary healthcare in order to trounce the little coverage of the orthodox system (Conde *et al.*, 2014).

A. cordifolia is traditionally utilized for diabetes treatment and this practice has been authenticated scientifically by several authors (Mohammed *et al.*, 2013; Martínez *et al.*, 2017). Although the stem bark is also utilized by some traditional healers in Nigeria for the management of diabetes, most reports on the antidiabetic property of the plant are centered on the leaf extract. Hence, this research was targeted at investigating the phytochemicals, *in vitro* antidiabetic, and antioxidant activities of methanol and aqueous extracts of *A. cordifolia* stem bark.

Materials and Methods

Chemicals

Alpha-amylase, α -glucosidase, 2, 2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), methanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ascorbic acid and 4-Nitrophenyl- α -D-glucopyranoside (pNPG) were gotten from Sigma-Aldrich Chemicals (St. Louis, MO, U.S.A.). All other used reagents/chemicals were of analytical rating.

Collection, provision, and extraction of Sample

Alchornea cordifolia stem bark was collected from farmland in Abraka, Delta State, and validated at the Department of Plant Biology/Biotechnology, the University of Benin, Nigeria (Voucher Number: UBH-A502). The samples were shade dried and powdered by mortar and pestle, then the powder was macerated in distilled water for 72 h with occasional shaking and filtered by filter paper (Whatman No. 1). The sample: solvent mixture for extraction was in the ratio of 10 g of sample (stem bark) to 100 mL of water. The methanol extraction was performed following the aqueous extract procedure. The filtrate of each sample was concentrated at 40°C under decreased pressure in a Rotary Evaporator, and stored at 4 °C until use.

Phytochemical screening

The qualitative phytochemical analysis for the occurrence of alkaloids, phenols, flavonoids, saponins, tannins, anthocyanins, glycosides, and terpenoids in the crude extracts of *Alchornea cordifolia* stem bark was done by standard protocols (Harbone, 1984; Trease and Evans, 1998).

Antioxidant activity

DPPH radical test

The effect of *A. cordifolia* extracts on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical was done as described by Rahman *et al.* (2015), while the 2, 2'-azino-bis (3-ethylbenzthiazoline-6-

sulphonic acid) (ABTS) radical-scavenging activity of *A. cordifolia* extracts was evaluated by the method of Re *et al.* (1999). The capability of *A. cordifolia* extracts to scavenge nitric oxide radical was measured by the method reported by Shankar and Salini (2014). All three methods were done using ascorbic acid as standard.

Alpha-amylase and alpha-glucosidase inhibitory assays

Alpha-amylase inhibitory assay

The assay for the effects of *A. cordifolia* extracts on α -amylase, and α -glucosidase activities were done by the methods of McCue *et al.* (2004) and Kim *et al.* (2005) respectively, using acarbose as a standard.

Statistical analysis

All the tests were conducted in triplicates. The results were stated as mean \pm SD of triplicates. Statistical assessment was done using Graph Pad Prism, Version 6.0. Software (Graph Pad, San Diego, CA, USA). Values at $p < 0.05$ were considered significant.

Results

Qualitative Evaluation of Phytochemicals

The qualitative phytochemical assessment of the stem bark extracts of *A. cordifolia* showed mild presence of alkaloids, saponins, anthocyanins, glycosides, and terpenoids in the methanol extract, and moderate to high presence of phenols, flavonoids and tannins, in the methanol extract. While the phytochemicals noticed in the methanol extract were also mildly present in the aqueous extract, except terpenoids (Table 1).

Inhibitory activities of α -Amylase and α -Glucosidase

α -Amylase inhibitory activity of *A. cordifolia*

The inhibitory activity of variable concentrations of *A. cordifolia* extracts and acarbose on α -amylase is shown in Figure 1. There was a dose-dependent

inhibitory power of α -amylase by the extracts and acarbose. Considerably ($p < 0.05$) higher inhibitory activity was exhibited by the methanol extract (IC_{50} value of 0.41 ± 0.01 mg/mL), relative to the aqueous extract (IC_{50} value of 0.47 ± 0.03 mg/mL). Thus, comparing extracts, the methanol extract exhibited higher inhibition than the aqueous extract (Table 2).

Table 1: Phytochemical components of the stem bark extract of *A. cordifolia*

Phytochemicals	Aqueous Extract	Methanol Extract
Alkaloids	+	+
Phenols	+	++
Flavonoids	++	+++
Saponins	+	+
Tannins	+	++
Anthocyanins	+	+
Glycosides	+	+
Terpenoids	-	+

Where +: Presence (+ mild, ++ moderate, +++ high), -: Absence

Table 2: The IC_{50} values for *A. Cordifolia* extracts (α -amylase, α -glucosidase)

Parameters	IC_{50} values (mg/mL)		
	Acarbose (mg/ml)	Methanol Extract of <i>A. Cordifolia</i> Stembark (mg/ml)	Aqueous Extract of <i>A. Cordifolia</i> Stembark (mg/ml)
% of α -amylase inhibition	0.26 ± 0.04^g	0.41 ± 0.01^h	0.47 ± 0.03^j
% of α -glucosidase inhibition	0.27 ± 0.01^k	0.29 ± 0.03^{kn}	0.32 ± 0.00^n

Values are stated as mean \pm SD ($n = 3$). Values with unlike superscripts across rows contrast significantly ($p < 0.05$)

α -Glucosidase inhibitory activity of *A. cordifolia*

Shown in Figure 2 is the inhibitory activity of variable concentrations of *A. cordifolia* extracts and acarbose on α -glucosidase. Though higher inhibitory activity was noticed with the methanol extract, the difference was not significant ($p < 0.05$), as indicated by the IC_{50} values (Table 2).

Antioxidant activity

DPPH Radical Scavenging Action

The DPPH radical scavenging action of *A. cordifolia* extracts is presented in Figure 3. There was a dose-dependent increase in the percentage inhibition of DPPH for all extracts and the standard ascorbic acid. Lower radical scavenging activity was seen in the aqueous extract in comparison with the methanol extract, but as shown in the IC_{50} values (Table 3), the difference between the extracts was not significant ($p < 0.05$).

ABTS Radical Scavenging Action

The antioxidant actions of the *A. cordifolia* extract evaluated by ABTS radical scavenging activities (Figure 4), showed significantly ($p < 0.05$) higher scavenging actions by the methanol extract (IC_{50} value of 0.23 ± 0.05 mg/mL) relative to aqueous extract (IC_{50} values of 0.31 ± 0.01 mg/mL) (Table 3).

Nitric Oxide Radical Scavenging Action

Shown in Figure 5 is the Nitric oxide radical scavenging action of *A. cordifolia* extracts and ascorbic acid. A dose-dependent increase in the radical scavenging activities was noticed among the extracts and ascorbic acid. Although higher scavenging activity was noticed in the aqueous extract when equaled with the methanol extract, the difference between both extracts was not significant ($p < 0.05$) (Table 3).

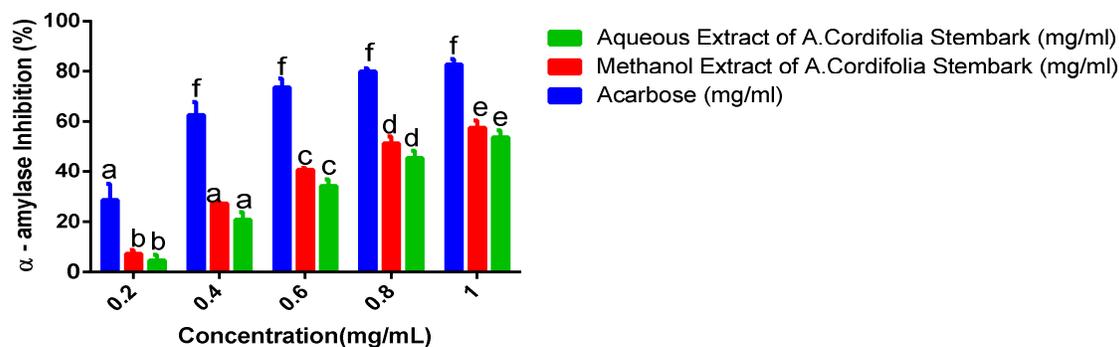


Figure 1: Inhibitory activity of varying concentrations of *A. cordifolia* extracts and Acarbose on α -amylase. Data are stated as mean \pm SD (n = 3). Bars with unalike letters contrast significantly (p < 0.05).

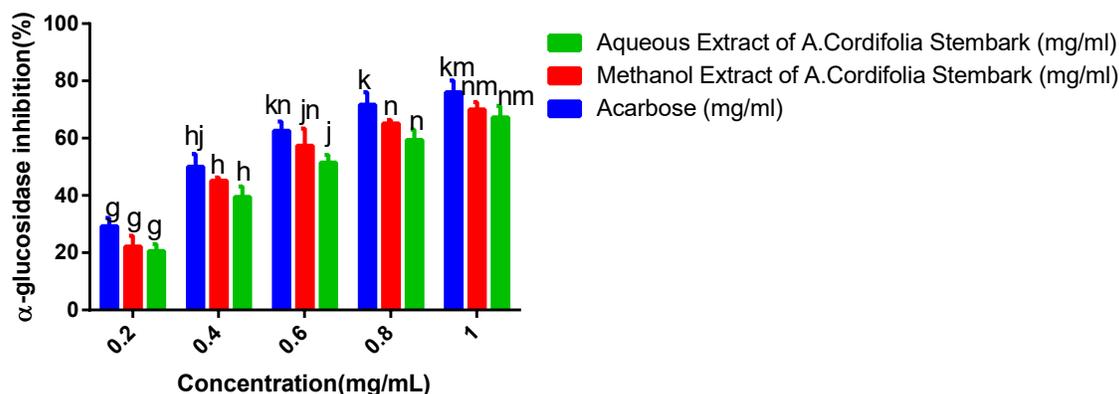


Figure 2: Inhibitory activity of varying concentrations of *A. cordifolia* extracts and Acarbose on α -glucosidase. Data are stated as mean \pm SD (n = 3). Bars with unalike letters contrast significantly (p < 0.05).

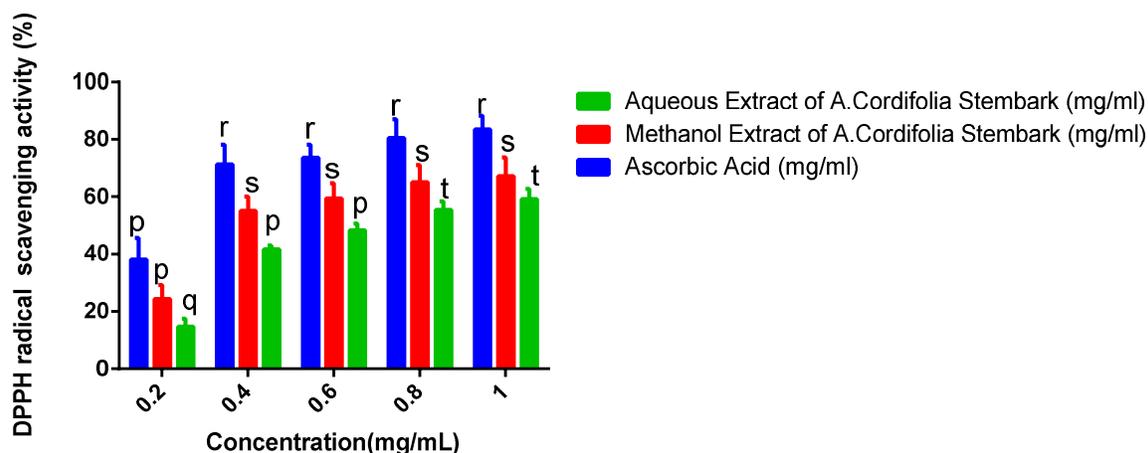


Figure 3: Radical scavenging action of *A. cordifolia* extracts (DPPH). Data are stated as mean \pm SD (n = 3). Bars with unalike letters contrast significantly (p < 0.05).

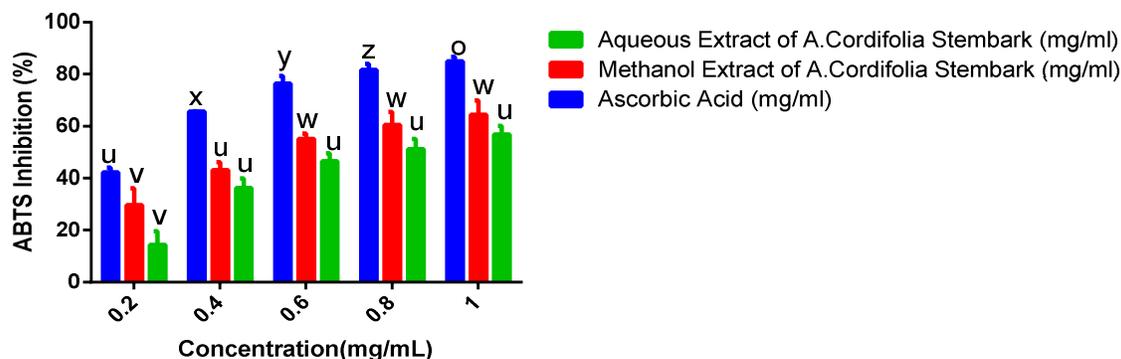


Figure 4: Radical scavenging action of *A. cordifolia* extracts (ABTS). Data are stated as mean \pm SD (n = 3). Bars with unalike letters contrast significantly (p < 0.05).

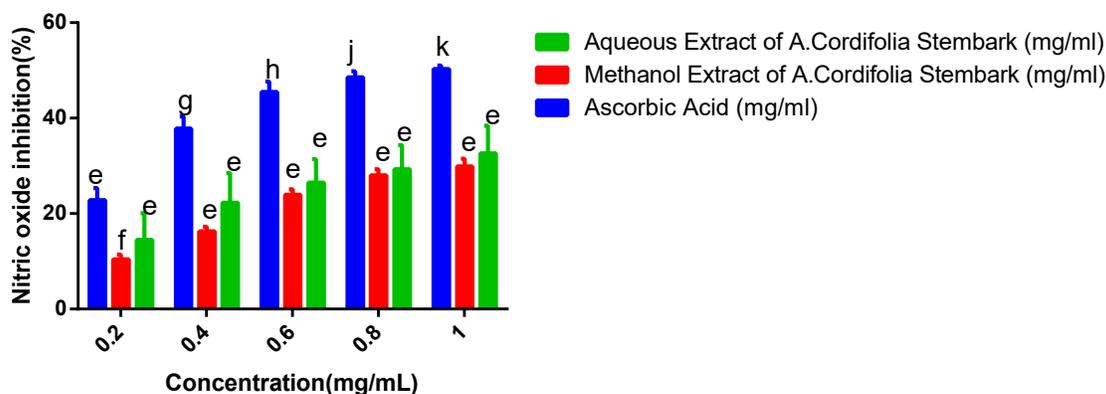


Figure 5: Radical scavenging action of *A. cordifolia* extracts (Nitric oxide). Data are stated as mean \pm SD (n = 3). Bars with unalike letters contrast significantly (p < 0.05).

Table 3: The IC₅₀ values for *A. Cordifolia* extracts (DPPH, ABTS, and NO)

Methods	IC ₅₀ values (mg/mL)		
	Ascorbic Acid (mg/ml)	Methanol Extract of <i>A. Cordifolia</i> Stembark (mg/ml)	Aqueous Extract of <i>A. Cordifolia</i> Stembark (mg/ml)
% of DPPH inhibition	0.21 \pm 0.02 ^a	0.24 \pm 0.01 ^{ab}	0.30 \pm 0.01 ^b
% of ABTS inhibition	0.21 \pm 0.01 ^c	0.23 \pm 0.05 ^c	0.31 \pm 0.01 ^d
% of Nitric oxide inhibition	0.22 \pm 0.02 ^e	0.31 \pm 0.03 ^f	0.24 \pm 0.06 ^{ef}

Values are stated as mean \pm SD (n = 3). Values with unalike superscripts across rows contrast significantly (p < 0.05)

Discussion

The quest for bioactive compounds of natural source, having low toxicity with significant antioxidant potential and inhibitory activities against carbohydrate hydrolyzing enzymes has taken the central stage in recent times (Sharifi-Rad *et al.*, 2020). Currently, all existing antidiabetic agents have been linked with considerable undesirable effects and are expensive (Anbu and Velpandian, 2012). Thus, herbal medicines have been the focus in recent years as a safer and cheaper means for hypoglycemic agents. Free radicals are linked with numerous lifestyle-related diseases like cancer and diabetes (Antonioni *et al.*, 2019). Medicinal plants have therapeutically effective

compounds that act as scavengers of free radicals, thereby alleviating oxidative stress-associated pathologies (Sharifi-Rad *et al.*, 2020). *Alchornea cordifolia* is a popular medicinal plant utilized in the management of several diseases including diabetes mellitus (Ajibade and Olayemi, 2015).

Qualitative assessment of plants crude extract offers preliminary evidence about the chemical constitution of plant products (Okoro *et al.*, 2017). Plant secondary metabolites comprising phenolics/ flavonoids are known bioactive compounds described to have anti-inflammatory, antioxidant, antimicrobial and other pharmacological actions (Okoro, 2020b). The phytochemicals of crude extracts are stated to have a varied range of pharmacological actions due to the existence of phenols, tannins, alkaloids, saponins, terpenoids, flavonoids and carotenoids. The phytochemicals noticed in the extracts of *A. cordifolia* have been reported to enhance the antioxidant potential of plants (Shankar and Salini, 2014; Ruslan *et al.*, 2018).

α -Amylase is a critical enzyme in saliva and pancreatic juice, secreted by the salivary and pancreas glands. It plays a key role in the digestion of starch by hydrolyzing the inner alpha 1, 4-glucosidic links in starch. The hydrolysis process of dietary complex carbohydrates to disaccharides starts at the mouth before they are further broken down in the small intestine by other digestive enzymes. Hence, the inhibition of α -amylase will pause starch and oligosaccharide digestion with a subsequent reduction in glucose absorption and postprandial blood glucose (Okoro *et al.*, 2015; Cherbal *et al.*, 2017). In this study, α -amylase inhibition evaluation was done to measure the antidiabetic capability of the stem bark extracts of *A. cordifolia*. Comparatively, the methanol extract of the stem bark gave the highest inhibitory action against α -amylase (IC₅₀, 0.41±0.01). This observation is in concord with earlier reports on the antidiabetic property of the

leaves extracts (Mohammed *et al.*, 2013; Martínez *et al.*, 2017).

It is recognized that carbohydrates are finally digested into monosaccharides by the intestinal enzymes. α -Glucosidase is one of the most important enzymes concerned in the course of carbohydrate digestion (Okoro, 2020a). α -Glucosidase inhibition by methanol and aqueous extracts of *A. cordifolia* stem bark was assessed and equated with acarbose, the reference drug. The results displayed dose-dependent α -glucosidase inhibitory activity by the extracts, with the methanol extract demonstrating the most promising inhibition potential of α -glucosidase, with an IC₅₀ value of 0.29±0.03 mg/mL. This observation concurs with reports of previous *in vivo* studies on the antidiabetic property of the leaves of *A. cordifolia* (Mohammed *et al.*, 2013; Martínez *et al.*, 2017). Also, it has been stated that the ideal antidiabetic agent would have a robust α -glucosidase inhibitory activity (Kwon *et al.*, 2007). Plant's phenolic phytochemicals have been depicted to be active α -glucosidase inhibitors. Thus, the inhibitory activity shown by the extracts of *A. cordifolia* against the enzyme may be a result of the existence of its polyphenolic constituents (Silva *et al.*, 2017).

Thus, the extracts were generally observed to show stronger inhibitory actions against α -glucosidase than the α -amylase. This observation concurs with the report of Costamagna *et al.* (2016), that a number of traditionally used plants for the managing of diabetes, exert a strong inhibitory influence on α -glucosidase and modest or slight effect against the α -amylase activity. Polyphenols have been stated to display inhibitory activities against α -amylase and α -glucosidase. Also, the inhibitory activity of flavonoids against α -glucosidase and α -amylase has been described. Flavonoids are the main class of polyphenolic compounds. It has also been reported that medicinal plants with alkaloids, glycosides, terpenoids, flavonoids, and phenolics display antidiabetic potential (Spínola *et al.*, 2020). Thus,

the antidiabetic potential displayed by the extracts of *A. cordifolia* could be ascribed to the presence of these phytochemicals.

Since diverse antioxidant assays are known to give broadly differing results, no single technique can be employed for assessing the antioxidant effects of foods (Frankel and Meyer, 2000). The present study estimated the antioxidant potential of *A. cordifolia* extracts (their capacity to scavenge the free radicals) by three models (DPPH, ABTS, and NO). The proton-donating ability of the extract was assessed by DPPH assay, one of the methods for antioxidant activities' evaluation, which assesses the hydrogen atom donating capability of plant extract through free radical scavenging (Okoro, 2017). Thus, their inhibition is a critical method for managing oxidative stress engendered ailment. In the present experiment, there was a dose-dependent radical scavenging action of DPPH displayed by the extracts, with the methanol extract giving the highest inhibition as shown by its IC_{50} value of 0.24 ± 0.01 mg/mL, which was comparable with that of the standard ascorbic acid (IC_{50} , 0.21 ± 0.02 mg/mL).

ABTS assay is intensely fascinating in plant extracts because at 734 nm wavelength absorption, color interference is eliminated (Ruslan *et al.*, 2018; Okoro, 2020b). In this study, the extracts displayed their inhibitory actions dose-dependently. Thus, the plant extracts showed antioxidant property by the scavenging action for the ABTS protonated radical dose-dependently, whereby the least inhibition was seen in the aqueous extract (IC_{50} , 0.31 ± 0.01 mg/mL), while the highest inhibition among the extracts was given by the methanol extract with IC_{50} value of 0.23 ± 0.05 mg/mL.

Nitric oxide (NO) is an effective pleiotropic inhibitor of known physiological processes like neuronal signaling, platelet aggregation, smooth muscle relaxation, and control of cellular mediated toxicity. Prolonged manifestation of NO

radical is linked with inflammatory disorders involving juvenile diabetes, ulcerative colitis, multiple sclerosis, and arthritis (Shi *et al.*, 2022). Thus, in this experiment, the extracts inhibited the production of stable nitrite compounds from unsteady nitric oxide which is connected with ailments such as diabetes through a direct antagonism with oxygen (Król, 2021). The antioxidative action of extracts may be ascribed to their phenolic compounds. Hence, the *in vitro* antioxidant and antidiabetic actions displayed by the extracts of *A. cordifolia* may be attributed to their phytochemicals like alkaloids, glycosides, tannins, and saponins (Tran *et al.*, 2020).

Conclusion

The study indicated that extracts of *A. cordifolia* stem bark have considerable antioxidant and inhibitory activities against α -amylase and α -glucosidase. The activity displayed by the methanol extract was superior to that of the aqueous stem bark extract. The preliminary phytochemical evaluation corroborated the antidiabetic and antioxidant results of the plant's extract examined. Thus, the *in vitro* antidiabetic and antioxidant activities of *A. cordifolia* stem bark may be as a result of the presence of its different phytochemicals and the methods of extraction. But, further comprehensive studies are required towards isolating the active compound(s) responsible for its medicinal properties.

Conflict of Interest

The authors hereby state that there is no existing conflict of interest.

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