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Antioxidant and α-Glucosidase Inhibitory Activities of Phenolic Compounds Isolated from the Aerial Parts of *Ampelocissus africanus*

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ABSTRACT

Ampelocissus africanus is an important medicinal plant with diverse ethnomedicinal uses. The purpose of this study was to investigate the antioxidant and α-glucosidase inhibitory activities of phenolic compounds isolated from the aerial parts of *Ampelocissus africanus*.Two known compounds were isolated using a combination ofcolumn and thin layer chromatography. The structures were elucidated usingUV and NMR spectroscopy. The antioxidant activity of the compounds was evaluated using 2,2^1-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays, while the antidiabetic potential was investigated using the α-glucosidase inhibition assay. Ascorbic acid and acarbose were used as standard and data obtained were expressed as mean ± SEM.The IC_{50} of the two compounds were calculated using a suitable regression analysis and graph pad prism software 6.0 using t-test.Compounds **1** and **2** were isolated as a yellow solids and identified as methyl gallate and quercetin, respectively. Result of the antioxidant assay using ABTS revealed that compound **1** gave a percentage inhibition of 98.22±0.79, while compound **2** gave 99.04±0.39% inhibition. The DPPH assay revealed inhibition at 59.38±3.23% and 63.45±2.56% for compounds I and 2, respectively. The values for DPPH assay were significantly different at p value <0.05 when compared to ascorbic acid. Compound 2 exhibited α-glucosidase inhibitory activity (IC_{50} 4.02±0.37 μg/mL) though lower than acarbose (IC_{50} 0.98± 0.57 μg/mL). Compound **1** however, showed weak α-glucosidase inhibitory activity with IC_{50}>100μg/mL. These results might justify the ethnomedicinal uses of this plant in ameliorating metabolic disorder such as diabetes.

Keywords: *Ampelocissus africanus*, α-glucosidase, Antioxidant assays, Quercetin, Methyl gallate

Introduction

Nitric oxide and superoxide are reactive nitrogen and oxygen species that have been implicated in DNA damage which in turn affects oxidation of lipids and proteins in cells.[1] Endogenous antioxidants present in human body are radical scavengers which in turn maintain the balance between oxidization and antioxidation. High intake of exogenous antioxidants has served as source of reduction in induced tissue damage via the inhibition of oxidative chain reaction.[2] Medicinal plants have been the source exogenous antioxidants from plants parts such as fruits, flowers, vegetables among others.[3] Polyphenols such as phenolic acids, Flavonoids, anthocyanins among others are the source of natural antioxidants derived from plants.[4]

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Diabetes mellitus is a disorder manifested by high blood glucose levels. Postprandial hyperclycemia plays an important role in development of type 2 diabetes. Carbohydrates-hydrolysing enzyme inhibitors such as α-glucosidase and α-amylase offer an effective strategy to regulate and prevent hyperglycemia by controlling the breakdown of carbohydrates. Natural α-amylase and α-glucosidase inhibitors, as well as antioxidants derived from plants offer a source of dietary ingredients that affect human physiological function in order to treat diabetes. Several research studies have investigated the effectiveness of plant based inhibitors of α-glucosidase and α-amylase as well as their antioxidant activity.[5]
Medicinal plants are still crucial sources of medicines particularly in less developed countries of the continent, where about 80% of the population primarily depend on these plants for their health needs.[6,7] Natural purified extracts from plants and microorganisms have been a source of bioactive metabolites which are known to possess multiple bioactivities and are considered inexpensive.[8] This has necessitated intensive studies for the unfolding of novel chemical compound suitable for drug development through purification and chemical characterization of active constituents.[9]
Ampelocissus africanus(Lour) Merr is of the family Vitaceae, a genus consisting of over 100 species widespread in the tropical and sub-tropical parts of the continent.[10] *Ampelocissus africanus* is a type of vine that is woody, or liana of the grape family, bearing edible fruit. It is a scrambling shrub, climber or extensive liana with stems that can

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vary from 0.5 – 9 meters long. It is known for its thick root that can grow up to 1 metre long and edible fruits that is used for local consumption. This fruit can be described as round berries that are about 1 cm in diameter. It has a sweet flavor when fully ripen and can be red to purplish-black in colour. The seeds are brown and shiny. The leaves are ovate to sub orbicular in shape with an acute apex, cordate base and serrate or crenate margins.[11]
Previous ethnobotanical reports have shown that *Ampelocissus africanus*is used alone or in combination with other medicinal plants to treat inflammation and cancer.[12] It is also used to treat wounds, cancer, diarrhea, rheumatism and muscular pain.[13-15] In Tanzania the leaf sap is drunk as a remedy for malaria fever, while in Nigeria, the decoction of the root is used by women to increase lactation.[10] Belem Kabre,[11] have reported the anti-inflammatory and antioxidant activities of the crude extracts of *Ampelocissus africanus*. In this present study, we report herein the antioxidant, α-amylase and α-glucosidase inhibitory activities of methyl gallate and quercetin isolated from ethyl acetate fraction of the hydroalcoholic extract of the aerial parts of *Ampelocissus africanus.*

Materials and Methods

General experimental procedure
Column chromatography was carried out on a column of dimension (2.5x35cm), with silica gel G (200-400 mesh, Silicycle) as the stationary phase. Thin layer chromatography (TLC) was performed using silica gel TLC plates aluminum backed (0.2 mm, TLC (F_{254}, Silicycle), and final purification was carried out usingSephadexLH-20 (Sigma). Proton and carbon-13 Nuclear magnetic resonance spectroscopy (NMR) was performed using a 400MHz spectrophotometer (BrukerAvance) $400MH_Z$ for (^{1}H) and 100MHZ for (^{13}C) respectively, while CD_3OD was used as dissolution solvent and TMS as internal standard.
Potassium persulfate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol, glucose, (Bovine serum albumin (BSA), sodium phosphate buffer, ascorbic acid, ethanol, Acarbose, $2,2^1$-azino-bis(3-ethylbenzothiazoline-6-sulphonicacid), para-nitrophenyl-alpha-glucopyranoside, were all purchased from Sigma-Aldrich chemicals, USA, Merck Limited, and S.D. Fine Chemicals Limited India.

Plant collection and extraction
The fresh aerial parts of *Ampellocissus africanus* was collected in Basawa, Zaria Kaduna State in July, 2019 by U.S. Gallah and was authenticated at the herbarium of Biology Department, Kaduna State University, Kaduna state. A voucher sample number KASU/BSH/ 991 was deposited in the herbarium of the university.
The fresh aerial plant parts were shade dried under room temperature for eight weeks and the air dried aerial part was powdered using wooden mortar to a weight of 389 g.The weighed coarsed powdered sample (389 g) was extracted to exhaustion by maceration using2x 2.5L of 70% ethanol at room temperature for 7 days, and the removal of the solvent from the combined filtrate using a rotary evaporator at reduced pressure afforded a brownish black colored extract which weighed 24.7 g. The crude ethanol extract (10 g) was dissolved in 100 ml of distilled water and partitioned with 500 mL of the ethyl acetate and the process repeated to exhaustion. The ethyl acetate soluble fraction was dried at room temperature and a dark green extract, 1.7 g was obtained.

Isolation
A portion of the ethyl acetate soluble fraction (1.2g) was packed in a column of dimension (50x5 cm) and elution commenced in a stepwise gradient manner using dichloromethane (100%) then dichloromethane: methanol (99:1, 98:2, 97:3, 96:4, 95:5, 90:10, 80:20, 70:30, 60:40, 50:50), followed by methanol (100%). The chromatography separation of the constituents were monitored on TLC using the solvent system dichoromethane: methanol (9:1), dichloromethane:ethyl acetate (2:3) and ethylacetate: chloroform:methanol:water (15:8:4:1) respectively. Fractions (15-27) eluted with 5% methanol in dichloromethane revealed the presence of 3 spots, which were combined together and subjected to gel filtration using sephadex LH-20 eluting with puree methanol to give compound**1** (3 mg) and compound **2** (2 mg).

Alpha-glucosidase inhibition assay
The two compounds were tested for their ability to inhibit α-glucosidase from *Saccharomyces cerevisiae* using *in vitro* assay adopting the method of Fajirah et al.[17] The samples were prepared with DMSO in 6 different concentrations (from 3.125 to 100 μg/mL) in test-tubes. To 200 μL volume of each sample concentration was added to 1000 μL of phosphate buffer (pH 6.8) and 1 U/mL of α-glucosidase enzyme. The mixture was incubated at 37°C for15 min. After the incubation, 0.5mL of 3 mM substrate, *p*-nitrophenyl-*α*-D-glucopyranoside (PNP-G), was added to the mixture. After 5 min, the inhibition was measured spectrophotometrically at wavelength of 405 nm after quenching of the reaction with 2M NaOH. The blank and control were prepared by replacing the tested samples with diluents (DMSO) and standard drug (Acarbose) respectively. All readings were taken in triplicate.
Percentage of enzyme inhibition was obtained using equation 1:

$$\% \, inhibition = \frac{Ao-A1}{Ao} X \ 100 \ \ldots\ldots\ldots\ldots \text{Equation 1}$$

Where Ao = absorbance of control; A_1 = absorbance of compound
All readings were taken in triplicates for each concentration and IC_{50} of the compounds were using a suitable regression analysis.

Antioxidant investigation using 2, 2′-diphenyl-1- picrylhydrazyl) free radical scavenging assay (DPPH)
The free radical scavenging activity ofcompound**1** and **2** was determined using the (DPPH) scavenging assay. The decolorisation of DPPH radical was determined by reported standard method of Proenca *et al.*[18]The reaction mixture containing 0.025 mL of various dilutions of compound 1, 0.1 mL of Tris HCl buffer (0.1 M, pH 7.4) and 0.125 mL DPPH solution (0.5 mM in methanol) were added in a 96-well micro plate and incubated in the dark for 15 min. The colour change from deep violet to yellow colour was measured. The intensity of the yellow colour depends on the level of the antioxidant in the test sample or the standard sample. The spectrophotometer was set at 517 nm and the absorbances were recorded. The percentage of DPPH scavenging by the compound was calculated from Equation 2:

$$\% \, inhibition = \frac{Ao-A1}{Ao} X \ 100 \ \ldots\ldots\ldots\ldots \text{Equation 2}$$

Where Ao = absorbance of DPPH control with solvent; A_1 = absorbance of decolorized DPPH in the presence of compound
Ascorbic acid was used as standard and data obtained were expressed as mean ± SEM.

2,2'-Azino-bis(3-Ethylbenzothiazoline-6-sulfonic acid Antioxidant assay (ABTS)
The ABTS radical scavenging activity was determined according to a method described by Tiwari et al.[18] The working solution of ABTS was obtained by the addition of 1mL potassium persulfate (6.89 mM in phosphate buffer saline (PBS) pH 8.0. The resulting mixture was stored in dark for 16h to produce the ABTS cation. In a 96-well plate, 10 μL of compound **1** (5μg/mL in PBS was added to 190μL of $ABTS^+$ and the absorbance was recorded at 734 nm with Trolox as the standard. The same procedure was repeated for compound **2**. The percentage scavenging radical of $ABTS^+$ by the two compounds were calculated from the equation:

$$\% \, inhibition = \frac{Ao-A1}{Ao} X \ 100 \ \ldots\ldots\ldots\ldots \text{Equation 3}$$

Where Ao = absorbance of control; A_1 = absorbance of reaction mixture
The tests were repeated in triplicates and the data obtained were expressed as mean ± SEM.

Statistical analysis
All values are expressed as means ± SEM. The data obtained were subjected to one-way analysis of variance (ANOVA) and the level of

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significant was evaluated using Duncan's multiple test ($P < 0.05$), with Graph pad prism, version 6.0.

Results and Discussion

Gel filtration over sephadex afforded compound **1**, which was obtained as a yellow solid, UV spectrum in methanol revealed a λmax of 274 nm which depict a substituted Benzene ring. The ^{1}H spectra of compound 1 (Supplementary material) revealed a signal at δ= 3.81 (s,OCH_3), and 7.04ppm (s, H-2,H-6) assigned to a methoxy and an aromatic protons typical o a trihydroxysubstituted benezene ring and consistent with that of methyl gallate.[19] The ^{13}C-NMR spectrum of compound **1(Supplementary material)** revealed signals at δ=50.9 ppm assigned to the methoxy carbon, and the low field carbon signal at δ=167.6 ppm typical of the carbonyl signal. All other signals due to the aromatic carbon signals at δ= 120.0 (C-1), 108.0 (C2, C-6), 145.1 (C-3, C-5), 138.3, (C-4) are consistent with that of methyl gallate. Comparison of the spectral data with literature depict compound 1 to be methyl gallate.[20,21]

Compound 2 was obtained as a yellow solid. The UV spectrum (Supplementary material) gave λmax at 254 and 370 nm ascribable to the Band II and Band I of a flavonoid nucleus.[22] The mass spectral data revealed an M+1 peak at *m/z* 303 which translates to a molecular weight of *m/z* 302 that points to a molecular formula of $C_{15}H_{10}O_7$. The proton NMR Spectrum revealed five aromatic proton signals δ=6.18 (H-6) and 6.38 ppm (H-8) assigned to the ring A protons of a 5,7-dihydroxy flavonoid nucleus, and the ABX protons of the ring B flavonoid nucleus appeared at δ = 6.87 (H-5^1) 7.62 (H-2^1) and 7.73 (H-6^1) ppm respectively. Compound 2 was found to be quercetin by comparing the NMR and mass spectral data with literature.[23]

Alpha glucosidase inhibitory and antioxidant activities

The result of the α-glucosidase inhibitory activity of the isolated compounds revealed that the compound 1 (methyl gallate) gave an IC_{50} greater than 100 µg/mL, thus compound 1 was found to exhibit weak activity against the enzyme, in contrast however, compound 2 (quercetin) gave an IC_{50} of 4.02 ± 0.37 µg/ml, while the standard drug acarbose gave an $IC_{50}$of 0.98 ± 0.57 µg/ml (Table 1 and Figure 2).The anti-oxidant activity revealed that compound**1**has a percentage inhibition of59.38±3.23 and 98.22±0.79% against DPPH and ABTS respectively, while compound **2** gave percentage inhibition of 63.45 ± 2.56 and 99.04 ± 1.39% indicating free radical scavenging activity of the two compounds. Ascorbic acid at 2mg/mL gave 81.00 ± 0.89% and 99.43 ± 0.12% respectively (Figure 3).

Alpha glucosidase inhibition is a metrical analysis for the determination of anti-diabetic drugs. The result of α-glucosidase inhibitory activity revealed that quercetin, compound 2was the most effective α-glucosidase inhibitor with IC_{50} value of 4.02±0.37µg/mL, in contrast however, methyl gallate (compound **1**) showed a weak activity against α-glucosidase with an IC_{50} >100 µg/mL, while acarbose the standard drug gave an IC_{50} of 0.98±0.57 µg/mL (Table 1 and Figure 2). Quercetin and methyl gallate (compound 1 and 2)both exhibited antioxidant activity against DDPH and ABTS free radical assays (Table 3 and Fig3).Free radicals have been implicated in the pathogenesis of diseases mediated via cell membrane damage and gene mutation which leads to diseases such as liver damage, cardiovascular disease, diabetis among others,[24,25] they react with different enzymes through chelation mechanisms.[26] Quercetin has been reported to be the most effective free radical scavenger among the flavonoid family.[27] The antioxidant activity of quercetin has been attributed to the hydroxyl group or benzo-dipyrone ring of the nucleus hence its ability to remove free radicals produced in the body.[28] The antioxidant property of quercetin has also been reported to be due to the reversal of oxidative stress mediated by streptozocin induced diabetis in rats.[29] Methyl gallate and quercetin the two compounds isolated from this plant both belong to the class of phenolic compounds which are very important plant constituents that are known for their antioxidant activity.[30] In this study, both methyl gallate and quercetin showed good scavenging properties, with quercetin showing a higher percentage inhibition at 99.04±1.39% and 63.45±2.56% against ABTS and DPPH radicals respectively. Methyl gallate possesses antioxidant activity and is known to inhibit lipid peroxidation.[31]We have previously reported the anti-inflammatory and analgesic activity of methyl gallate.[32] Methyl gallate has also been reported to inhibit cell proliferation and apoptosis in hepatocellular carcinoma cells[33]. Plants are well known for their medicinal values which are related to their phytochemical constituents such as phenolics and flavonoids, thus the presence of methyl gallate and quercetin might justify some of the ethnomedicinal uses of this plant.

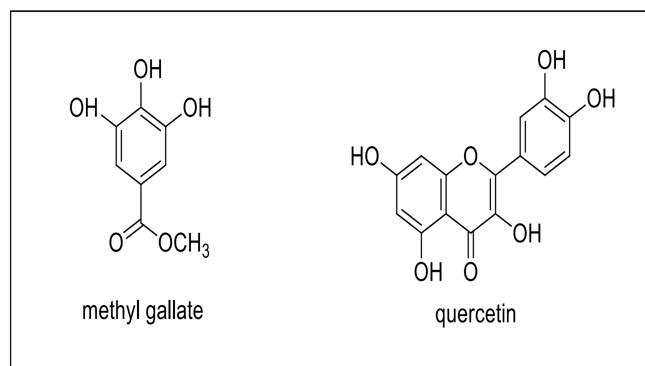
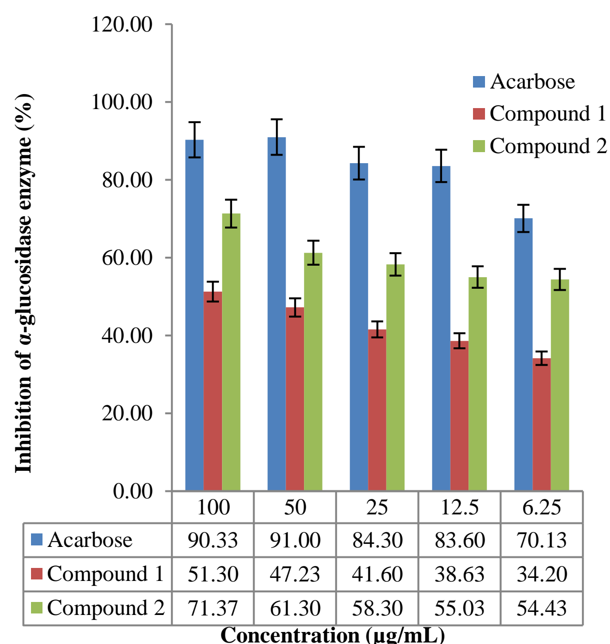


Figure 1: Chemical structures of isolated compounds

Table 1: α-glucosidase inhibitory activity of the isolated compounds

Compounds	IC_{50} (µg/mL)
Compound **1**	>100
Compound **2**	4.02 ± 0.37**
Acarbose (645 g/mol)	0.98 ± 0.57

Data expressed as mean ± SEM (n = 3)
SEM = Standard error of mean; **p<0.05; is considered significantly different when compared with the values for the positive control, acarbose (students' unpaired t-test)



	100	50	25	12.5	6.25
Acarbose	90.33	91.00	84.30	83.60	70.13
Compound 1	51.30	47.23	41.60	38.63	34.20
Compound 2	71.37	61.30	58.30	55.03	54.43

Figure 2: α-glucosidase inhibition activity of isolated compounds from *A. africanus*
Each point represents mean ± SD; n = 3

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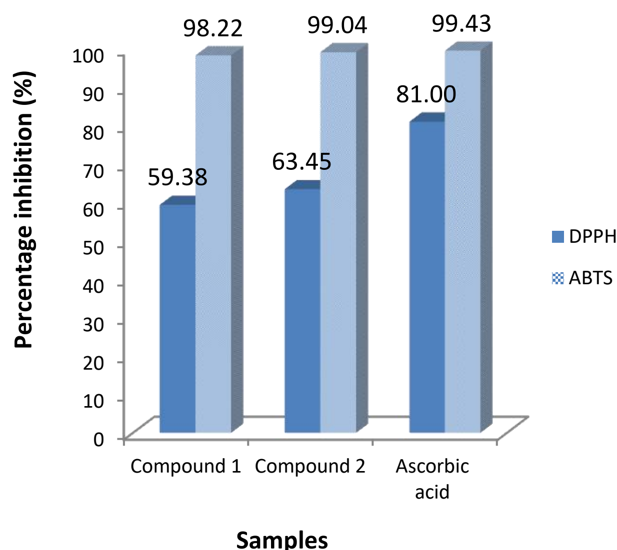

Figure 3: Percentage inhibition of free radical scavenging activities of compound 1 and 2

Conclusion

Quercetin and methyl gallate isolated from the ethyl acetate soluble fraction of the hydroalcoholic extract of A.africanus were found to exhibit antioxidant activity, while quercetin showed significant α-glucosidase inhibitory activity. The presence of these two phytoconstituents could be responsible for the antioxidant and antidiabetic potential of this plant.

Conflict of Interest

The authors declared 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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