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Experimental Article

Antioxidant properties and in vitro α-amylase and α-glucosidase inhibitory properties of phenolics constituents from different varieties of *Corchorus spp.*

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الملخص

هدف البحث: بحثت هذه الدراسة خصائص المكونات الفينولية المضادة للأكسدة وتأثيرها من المستخلصات المائية لبعض أنواع الملوخية على أنزيم ألفا أميليز وألفا جلوكوسيداز (على بنكرياس الفئران في المختبر).

طرق البحث: تم استخدام الاستشراب الغازي مع كاشف اللهب الأيوني لتمييز المكونات الفينولية. تم تحضير المستخلصات المائية وتحديد خصائص الكسح الجذرية، وتأثيراتها على أنشطة ألفا أميليز، وألفا جلوكوسيداز, والمواد المضادة لأكسدة الحديد الناتجة من بيروكسيد الدهون, كما درست قدرات الحديد المخلبية وقدرات الحديد المثبطة لمضادات الأكسدة.

النتائج: أغلب المكونات الفينولية التي تم كشفها بواسطة الاستشراب الغازي مع كاشف اللهب الأيوني هي كيمبفرول، وروتين، وابيجنين، وليتونلين، وحمض الكافيك، وكوارستين. أظهرت النتائج أن جميع أنواع الملوخية ثبطت بشكل كبير أنشطة ألفا أميليز وألفا جلوكوسيداز خاضعة للجرعة. لكن جميع أنواع الملوخية ثبطت نشاط ألفا جلوكزسيداز أكثر من ألفا أميليز وأيضا أظهرت خصائص مضادة للأكسدة.

الاستنتاجات: يمكن أن يعزى تثبيط الإنزيمات والخصائص المضادة للأكسدة التي ظهرت بأنواع الملوخية للمركبات الفينولية ومحتوى فيتامين سي، وهي الآلية المحتملة التي تجعل للملوخية تأثيرات علاجية على النوع الثاني من مرض السكري كما تم الحصول عليها في الطب الشعبي.

الكلمات المفتاحية: ألفا أميليز؛ الفا جلوكوسيداز؛ مضادات الأكسدة؛ أنواع الملوخية؛ المركبات الفينولية

Abstract

Objectives: This study investigated the phenolic constituents, antioxidant properties and effect of aqueous extracts from some *Corchorus species* (C. aestuans, C. bougoudo, C. capsularis, C. olitorius and C. urtifolicus) on α-amylase and α-glucosidase activities in vitro.

Methods: Gas Chromatography with Flame Ionization Detector (GC-FID) was used to characterize the phenolic constituents. Aqueous extracts were prepared weight/volume (w/v) and their effects on α-amylase and α-glucosidase activities, Fe^{2+} induced lipid peroxidation, and 1,1-diphenyl-2 picrylhydrazyl (DPPH) radical scavenging properties were determined. Fe^{2+} chelating abilities and Ferric Reducing Antioxidant Power (FRAP) properties were also studied.

Results: The predominant phenolics detected by GC-FID were kaempferol, rutin, apigenin, luteolin, caffeic acid and quercetin. The results showed that all the *Corchorus species* significantly ($P < 0.05$) inhibited α-amylase and α-glucosidase activities dose-dependently (0–8 μg/ml). C. olitorius had the highest α-amylase inhibitory ability while C. bougoudo demonstrated the maximum α-glucosidase inhibition. However, all the *Corchorus species* inhibited α-glucosidase better than α-amylase and also exhibited antioxidant properties.

Conclusions: The enzyme inhibitory and antioxidant properties exhibited by the *Corchorus species* could be attributed to their phenolic constituents and vitamin C content; a possible mechanism by which *Corchorus species* could elicit therapeutic effects on type 2 diabetes mellitus as obtained in folklore medicine.

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Keywords: α-amylase; α-glucosidase; Antioxidants; *Corchorus spp.*; Phenolics

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Introduction

Type 2 diabetes mellitus (T2DM) accounts for about 90–95% of all cases of DM[1]; a metabolic disorder characterized by chronic hyperglycemia due to defects or alterations in either the secretion or action of insulin.[2] Controlling postprandial hyperglycemia, via inhibition of α-amylase and α-glucosidase (carbohydrate hydrolyzing enzymes) present in the gastro-intestinal tract is one of the major management therapy.[3] Unfortunately, therapeutic drugs designed as inhibitor of these enzymes come with several side effects,[4] as well as associated financial constraint. Hence, the need to search for potent and cheap alternatives, with little or no side effect.

Pancreatic cell damage caused by reactive oxygen species (ROS) such as the superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH–) is a known etiology of T2DM.[5] Recent report by Jaganjac et al.[6] on elevated malondialdehyde (MDA) content in pancreatic tissue of diabetic animal models caused by lipid peroxidation also confirmed the role of free radicals in pathogenesis and progression of T2DM. However, the use of phytochemicals such as phenolics to augment endogenous antioxidants, help to effectively protect biological cells against deleterious effect caused by oxidative stress, has been reported in both in vitro and *in vivo* studies.[7,8]

Jute (*Corchorus spp.*) is a native plant of tropical Africa and Asia, and has since spread to Australia, South America and some parts of Europe. The leaves are consumed as vegetables and used in preparing different types of delicacies in different parts of the world.[7] Leaves from different Jute (*Corchorus spp.)* species have been used in folklore medicine for managing diabetes mellitus and hypertension. As reported for *Corchorus olitorius*, the mechanism could be due to α-amylase and α-glucosidase inhibitory effect of some constituent phenolic compounds such as caffeic acid.[7] This study therefore, evaluated other species of C*orchorus* on their ability to inhibit α-amylase and α-glucosidase in vitro, characterize their phenolic constituents and assessed their antioxidant properties.

Materials and Methods

Sample collection

Five jute (*Corchorus spp.)* plants (*Corchorus aestuans, C. Bougoudo, C. Capsularis, C. olitorius* and, *Corchorus urtifolicus)* were collected from the Botanical garden of Federal University of Technology, Akure, Nigeria. The samples were identified by Mr. Shorungbe of Biology Department, Federal University of Technology, Akure, Nigeria. The leaves were separated from the stem, washed under running tap water, air dried under shade and pulverized.

Aqueous extract preparation

Ten (10 g) grams of each pulverized samples was homogenized with 100 ml of distilled water. The homogenate was filtered through Whatman (No. 2) filter paper and later centrifuged at 2000 rpm for 10 min to obtain clear supernatant. The supernatant was then freeze dried into powdered form with the aid of freeze drier. 1 g of each freeze dried extracts was then dissolved into 100 ml of distilled water, stored at 4 °C and used for subsequent analysis. The freeze dried samples were used for GC-FID analysis.

Chemicals and reagent

Except otherwise stated, all chemicals used were of analytical grade. Glass distilled water was used.

Handling of animal

The handling and use of animals for this study was approved by the institution's ethical committee for the use of animals in laboratory experiments (reference number FUTA/SOS/1403). In this study, Wister strain albino rats weighing 200–210 g were purchased from the breeding colony of Department of Veterinary Medicine, University of Ibadan, Nigeria. Rats were maintained at 25 °C, on a 12 h light/12 h dark cycle, with free access to food and water. They were acclimatized under these conditions for one week before the experiment.

α-amylase inhibition assay

Appropriate dilutions of extracts (50 µl) and 500 µl of 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) containing Hog pancreatic α-amylase (EC 3.2.1.1) were incubated at 25 °C for 10 min. Then, 500 µl of 1% starch solution in 0.02M sodium phosphate buffer (pH 6.9 with 0.006M NaCl) was added to each tube. The reaction mixture was incubated at 25 °C for 10 min and stopped with 1.0 ml of dinitrosalicylic acid color reagent. Thereafter, the mixture was incubated in a boiling water bath for 5 min and cooled to room temperature. The reaction mixture was then diluted by adding 10 ml of distilled water and absorbance measured at 540 nm in a spectrophotometer.[9] The results were expressed as percentage enzyme inhibition using the formula:

$$\text{Inhibition}(\%) = \left(\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}}\right)/\text{Abs}_{\text{ref}}{*}100$$

where, Abs_{ref} is the absorbance without sample and Abs_{sam} is the absorbance of the extract

α-glucosidase inhibition assay

Appropriate dilution of the extracts (50 µl) and 100 µl of the α-glucosidase solution (EC 3.2.1.20) (0.5 mg/ml) in 0.1M phosphate buffer (pH6.9) was incubated at 25 °C for 10 min. The 50 µl of 5 mM p-nitrophenyl-α-D-glucopyranoside solution in 0.1 M phosphate buffer (pH6.9) was added. The

mixtures were incubated at 25 °C for 5 min, before reading absorbance at 405 nm in the spectrophotometer.[10] The results were expressed as percentage inhibition of α-glucosidase activity, calculated using the formula:

$$\text{Inhibition}(\%) = \left(\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}}\right)/\text{Abs}_{\text{ref}}*100$$

where, Abs_{ref} is the absorbance without sample and Abs_{sam} is the absorbance of the extract

Determination of total phenol content

The total phenol content of the extracts was determined according to the method of Singleton et al.[11] Briefly, appropriate dilutions of the extracts were oxidized with 2.5 ml 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45 °C and the absorbance was measured at 765 nm in the spectrophotometer. The total phenol content was subsequently calculated as gallic acid equivalent.

Determination of total flavonoid content

The total flavonoid content of the extracts was determined using a slightly modified method reported by Meda et al.[12] Briefly, 0.5 ml of appropriately diluted sample was mixed with 0.5 ml methanol, 50 µl 10% $AlCl_3$, 50 µl 1M Potassium acetate and 1.4 ml distilled water, followed by incubation at room temperature for 30 min. The absorbance of the reaction mixture was subsequently measured at 415 nm in the spectrophotometer. The total flavonoid content was subsequently calculated as quercetin equivalent.

Determination of vitamin C content

The vitamin C content of the various *Corchorus spp.* leaves were determined by AOAC[13] method. Briefly, 5 g of the sample was extracted by 100 ml H_2O and 10 ml of the extract was mixed with 100 ml 5% glacial acetic acid and titrated against standardized 2, 6- dichloroindophenol (0.005 g/100 ml) solution.

GC-FID characterization of constituent phenolics in the various Corchorus species

The qualitative-quantitative analysis of the phenolic compounds of the samples was carried out using the method reported by Kelley et al.[14] The phenolic compounds were extracted as described by Kelley et al.[14] and Provan et al.[15] and the purified phenolic extracts (1 µl: 10:1 split) were analyzed for composition by comparison with phenolic standards (Aldrich Chemical Co., Milwaukee, W1) on a Hewlett-Packard 6890 gas chromatography (Hewlett-Packard Corp., Palo Alto, CA) equipped with a derivatized, non-packed injection liner, a Rtx-5MS (5% Diphenyl-95% Dimethyl polysiloxane) capillary column (30 m length, 0.25 µm film thickness), and detected with a flame ionization detector (FID). The following conditions were employed PA separation; injector temperature, 23 °C; temperature ramp, 80 °C for 5 min then ramped to 250 °C at 30 °C/min; and a detector temperature of 320 °C.

Determination of Fe^{2+} chelating ability

The Fe^{2+} chelating ability of the extracts was determined using a modified method of Minotti and Aust,[16] with a slight modification by Puntel et al.,[17]. Freshly prepared 500 µM $FeSO_4$ (150 µl) was added to a reaction mixture containing 168 µl 0.1M Tris − HCl (pH 7.4), 218 µl saline and the extracts (0−25 µl). The reaction mixture was incubated for 5 min, before the addition of 13 µl 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm in a spectrophotometer. The percentage Fe^{2+} chelating ability was calculated as follows:

$$Fe^{2+}\ \text{chelating ability}(\%) = \left(\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}}\right)/\text{Abs}_{\text{ref}}*100$$

where, Abs_{ref} is the absorbance without sample and Abs_{sam} is absorbance of the samples extract

Preparation of rat's pancreas homogenates for lipid peroxidation assay

Male adult albino rats were immobilized by cervical dislocation and the pancreas was rapidly isolated, rinsed with cold saline, placed on ice and weighed. This tissue was

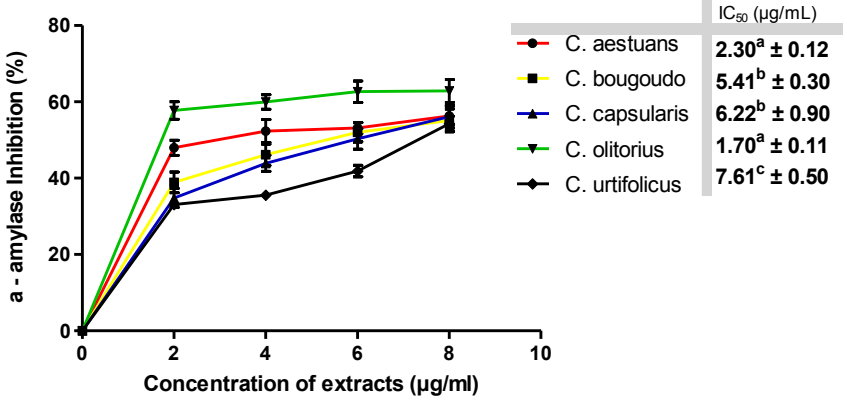

Figure 1: α-amylase inhibitory effects of the aqueous extracts from the leaves of five *Corchorus species*. IC_{50} values with the same superscript letter are not significantly different ($P > 0.05$).

Table 1: Pearson correlation coefficients for the five *Corchorus species*.

	TP	VC	AMY	AGLUC
TP	1	−.414	.047	−.762
VC	−.414	1	.512	.048
AMY	.047	.512	1	−.296
AGLUC	−.762	.048	−.296	1
N	5	5	5	5

TP = Total phenolic content; VC = Vitamin C content; AMY = α-amylase inhibitory effect; AGLUC = α-glucosidase inhibitory effect
n = number of sample treatment.

subsequently homogenized in 0.1M phosphate buffer (pH 7.4) (1:10 w/v) with about 10-up-and −down strokes at approximately 1200 rev/min in a Teflon glass homogenizer. The homogenate was centrifuged for 10 min at 3000 × g to yield a pellet that was discarded, and the low-speed supernatant (S1) was kept for lipid peroxidation assay.[18]

Lipid peroxidation and thiobarbituric acid reactions assay

Lipid peroxidation, induced by Fe^{2+} in isolated rat pancreas homogenates was carried out using the modified method of Okhawa et al.[19] The reaction mixture containing 100 µl of the supernatant fraction of tissue homogenate, 30 µL of 0.1 M pH 7.4 Tris-HCl buffer, extracts at different concentrations and 30 µL of 250 µM freshly prepared $FeSO_4$. The volume was made up to 300 µL with water before incubation at 37 °C for 1hr. Thereafter, 300 µL 8.1% SDS (Sodium dodecyl sulfate) and 500 µL of acetic acid/HCl (pH 3.4) were added. The color reaction was developed by adding 500 µL 0.8% TBA (Thiobarbituric acid) to the mixture and incubated at 100 °C for 1hr. TBARS (Thiobarbituric acid reactive species) produced was measured at 532 nm in a spectrophotometer. The absorbance was compared with that of standard curve using malondialdehyde.

Free radical scavenging ability assay

The free radical scavenging ability of the extracts against 1,1-diphenyl−2 picrylhydrazyl (DPPH) free radical was evaluated as described by Gyamfi et al.,[20]. Briefly, appropriate dilution of the extracts solution (1 ml) was mixed with 1 ml, 0.4 mM DPPH in methanolic solution. The mixture was left in the dark for 30 min and the absorbance was taken at 516 nm in a spectrophotometer. The DPPH free radical scavenging ability was subsequently calculated thus:

$$\text{DPPH scavenging ability}(\%) = \left(\text{Abs}_{\text{ref}} - \text{Abs}_{\text{sam}}\right)/\text{Abs}_{\text{ref}} * 100$$

where Abs_{re} is the absorbance without samples extract and Abs_{sam} is the absorbance of Samples extract

Determination of reducing property

The reducing property of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu.[21] 2.5 ml aliquot was mixed with 2.5 ml 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min and then 2.5 ml 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min 5 ml of the supernatant was mixed with an equal volume of water and 1 ml 0.1% ferric chloride. The absorbance was measured at 700 nm in a spectrophottometer. The ferric reducing antioxidant property was subsequently calculated.

Data analysis

The results of three (3) replicate experiments were pooled and expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to analyze the mean and the post hoc treatment was performed using Duncan multiple test.[22] Significance was accepted at $P < 0.05$. IC_{50} (extract concentration causing 50% inhibition) were calculated with GraphPad Prism version 5.00 for Windows. The Pearson correlations were calculated according to statistical program for social science (SPSS version 21.0. Armonk, NY: IBM Corp).

Results

The extracts of the various *Corchorus species* inhibited α-amylase activity in a concentration dependent manner (0−8 µg/ml in Figure 1). Based on the IC_{50} values, C. *olitorius*

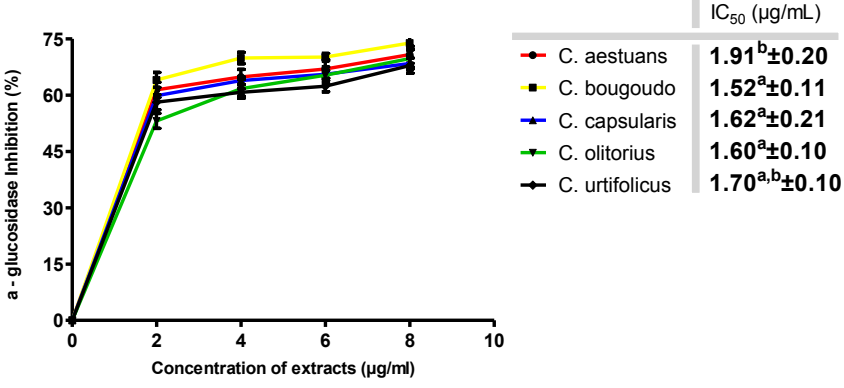

Figure 2: α-glucosidase inhibitory effects of aqueous extracts from the leaves of five *Corchorus species*. IC_{50} values with the same superscript letter are not significantly different ($P > 0.05$).

Table 2: Total phenol, total flavonoid and vitamin C contents of aqueous extracts from leaves of five *Corchorus species*.

Sample species	Total Phenol (mg/100 g)	Total Flavonoid	Vitamin C
Corchorus aestuans	325.00^{a} ± 0.10	78.30^{b} ± 1.00	101.80^{c} ± 0.90
Corchorus bougoudo	418.00^{c} ± 0.30	47.90^{a} ± 0.60	106.60^{d} ± 0.10
Corchorus capsularis	482.00^{d} ± 0.20	78.30^{b} ± 1.20	95.10^{a} ± 0.40
Corchorus olitorius	500.00^{e} ± 0.20	82.80^{b} ± 6.00	99.40^{b} ± 0.10
Corchorus urtifolicus	403.50^{b} ± 0.10	82.70^{b} ± 6.00	115.60^{e} ± 1.30

Values represent means ± standard deviation of triplicate readings.
Values with the same superscript letter on the same column are not significantly different (P > 0.05).

had the highest inhibitory effect, but not significantly different (P > 0.05) from *C. aestuans*; while *C. urtifolicus* had the least inhibitory effect. The Pearson correlation coefficient (Table 1) however, showed that there was no correlation (r = 0.047) between the phenolic contents of the extracts and their α-amylase inhibitory potentials.

Figure 2 showed that the extracts also inhibited α-glucosidase activity in a concentration dependent manner (0−8 µg/ml). However, based on the IC_{50} values, *Corchorus bougoudo* had the highest inhibitory effect which was not significantly different (P > 0.05) from that of *C. olitorius*, C. *capsularis*, and C. urtifolicus. *C. aestuans* had the least inhibitory effect. The Pearson correlation coefficient (Table 1) showed that α-glucosidase inhibitory potential of the extracts correlated moderately (r = −0.762) with their phenolic contents.

Furthermore, in Table 2, the total phenol content of the extracts ranged from 325.00 mg/100 g (*C. aestuans*) to 500.00 mg/100 g (*C. olitorius*). The flavonoid content of the extracts ranged from 47.90 mg/100 g (*C. bougoudo*) to 82.80 mg/100 g (*C olitorius*).. The vitamin C content of the extracts also ranged from 95.10 mg/100 g (*Corchorus capsularis*) to 115.60 mg/100 g (*C. urtifolicus*).

The GC analysis of the phenolic constituents of the extracts (Table 3 and Figure 3a−e) revealed that *Corchorus olitoriu* had the highest significant (P < 0.05) distribution of Phenol, Vanillic acid, Kaempferol, Luteolin and Rutin. *C. urtifolicus* had the highest significant (P < 0.05) amount of Caffeic acid and Ferrulic acid while Apigenin and Myricetin had the highest distribution in *C. aestuans* but the highest amount of Quercetin was found in C. *capsularis*.

Figure 4 revealed that all the extracts chelated Fe^{2+} in a concentration dependent manner (0−14 µg/ml). Furthermore, as reported in Figure 5, the incubation of rats' pancreas homogenate with 250 µM Fe^{2+} caused a significant increase ($P < 0.05$) in the malondialdehyde (MDA) content (148.0%). However, all the extracts inhibited MDA production in the homogenates in a concentration dependent manner (1.6−6.3 µg/ml) with *Corchorus bougoudo* exhibiting the highest inhibitory effect as measured by the MDA produced (30.985%) while *Corchorus urtifolicus* had the least inhibitory effect because it produced the highest MDA (48.9%) at the highest concentration.

The concentration-dependent manner (0−40 µg/ml) of DPPH radical scavenging ability of *the* extracts (Figure 6) revealed that *C. bougoudo* (87.77 µg/ml) had the highest scavenging ability at the highest concentration while *C. capsularis* (79.79 µg/ml) had the lowest scavenging ability at the same concentration.

The result of the ferric reducing antioxidant power (FRAP); of the extracts was presented as ascorbic acid equivalent in Figure 7. All the extracts exhibited ferric reducing antioxidant power; *C. olitorius* (0.7 mg AAE/g) top the list while *C. capsularis* (0.5 mg AAE/g) had the least FRAP.

Discussion

The ability of all the extracts to inhibit α-amylase activity could be one of the mechanisms by which the extracts cause reduction in blood glucose as obtained in folklore medicine. Specifically, the inhibitory effect of *C. olitorius* recorded in

Table 3: GC-FID characterization of the various phenolic constituents of the five *Corchorus species*.

Phenolic compds	*C. urtifolicus* (mg/100 g)	*C. olitorius*	*C. capsularis*	*C. bougoudo*	*C. aestuans*
Phenol	0.62^{b}	0.88^{e}	0.74^{d}	0.60^{a}	0.64^{c}
Vnillic acid	0.11^{b}	0.16^{d}	0.07^{a}	0.08^{a}	0.14^{c}
Caffeic acid	1.86^{d}	1.58^{c}	1.45^{c}	0.91^{a}	1.21^{b}
Ferulic acid	1.79^{c}	1.54^{b}	1.48^{b}	1.12^{a}	1.12^{a}
Apigenin	2.04^{a}	2.24^{b,c}	2.01^{a}	2.14^{a,b}	2.36^{c}
Kaempferol	4.13^{c}	4.28^{c}	3.81^{b}	3.61^{a}	3.74^{a,b}
Luteolin	2.21^{a}	3.03^{c}	2.34^{a}	2.22^{a}	2.70^{b}
Quercetin	1.59^{a}	2.27^{a,b}	2.42^{b}	2.10^{a}	2.18^{a}
Myricetin	0.42^{a}	0.60^{a,b}	0.48^{b}	0.52^{a,b}	0.68^{b}
Rutin	2.44^{b}	2.79^{c}	2.76^{c}	2.04^{a}	2.07^{a}
Gingerol	1.2×10^{-2a}	5.0×10^{-2b}	5.7×10^{-2b}	2.1×10^{-2a}	9.0×10^{-2c}

Values represent mean of triplicate experiment.
Values with the same superscript letter on the same row are not significantly different (P > 0.05).

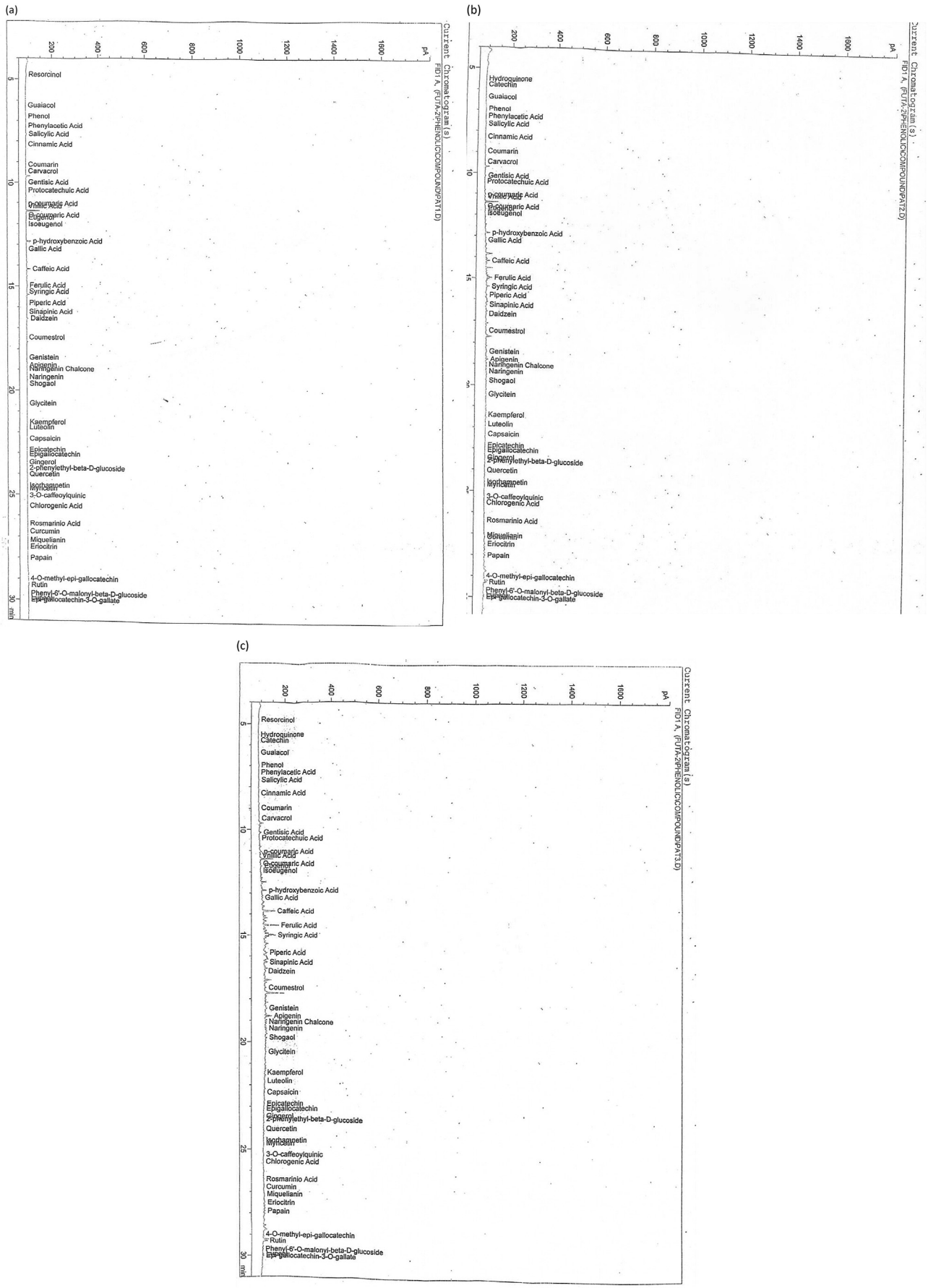

Figure 3: GC-FID chromatograms for the characterization of phenolic constituents of aqueous extracts from leaves of *C. urtifolicus* (a), *C. olitorius* (b), *C. capsularis* (c), *C. bougoudo* (d) and *C. aestuans* (e).

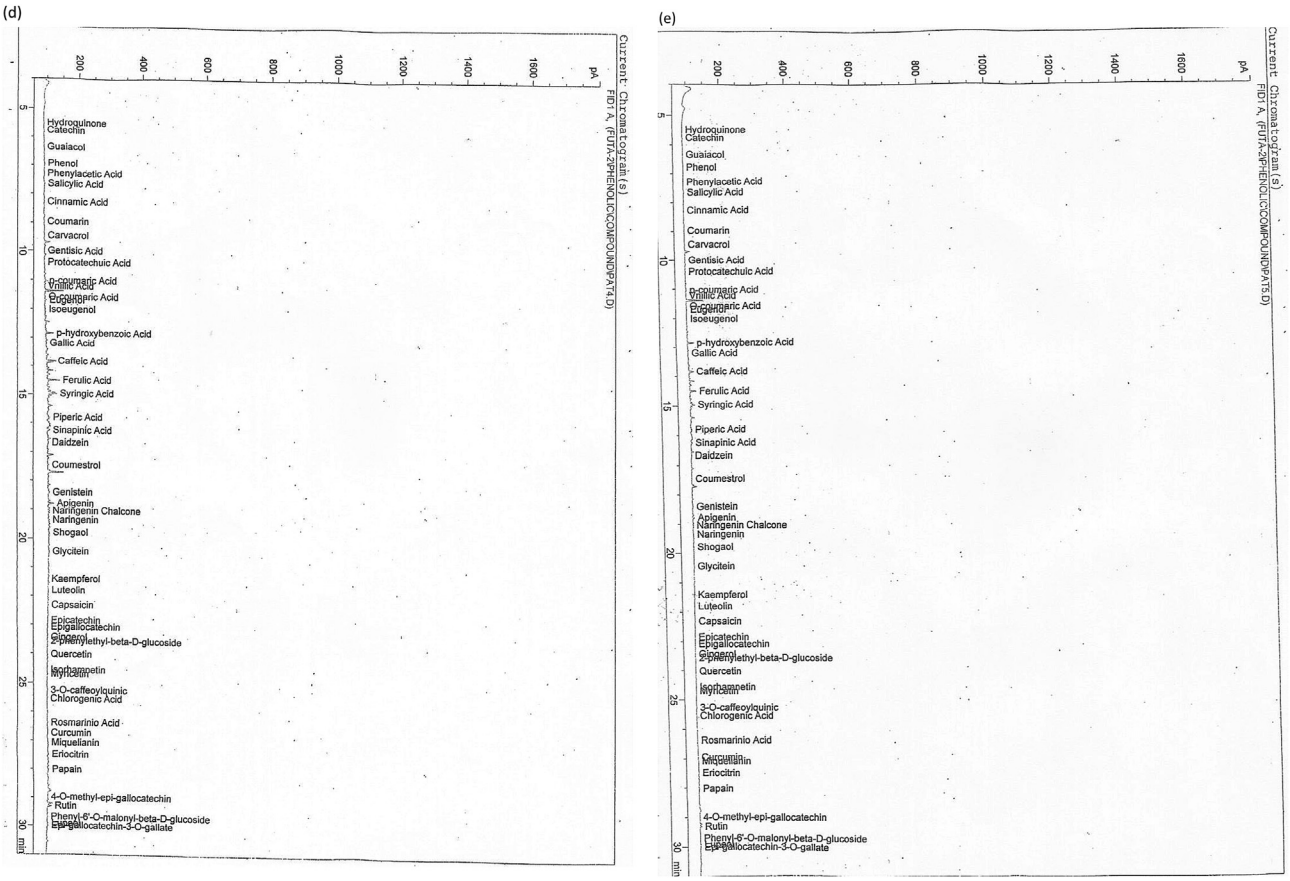

Figure 3: (*continued*).

this study, agreed with earlier report from our laboratory on α-amylase inhibitory effect of *C. olitorius*.[7] Therefore, the highest α-amylase inhibitory effect shown by *C. olitorius* in this study can be attributed to its highest total phenol and total flavonoid contents. However, it is noteworthy that there was no correlation between the α-amylase inhibition and the phenolic contents of the extracts. Therefore, we hypothesize that the α-amylase inhibitory effect by the extracts could be due to interactions between phenolic and non-phenolic phytochemicals present with the enzyme. Ranilla et al.[23] had also reported a similar non-correlation between the α-amylase inhibitory effect of some medicinal plants commonly used in Latin America and their total phenolic contents.

Similarly, the α-glucosidase inhibitory effect exhibited by all the extracts indicates their potential effectiveness at managing T2DM. The moderate correlation between the extracts' α-glucosidase inhibitory effect and their respective total phenol content could be as a result of interactions among individual phenolics and/or the presence of other non-phenolic phytochemicals which could produce synergistic inhibitory effects. It is also noteworthy that all the extracts significantly ($P < 0.05$) inhibited α-glucosidase more than α-amylase and agrees with earlier findings that plant phenolic extracts inhibits α-glucosidase better than α-amylase[24]. This can be of pharmacological importance at reducing the complications associated with use of synthetic α-amylase inhibitors.

Furthermore, the extracts were shown to be rich in phenolics content. This was substantiated by the GC-FID analysis of their phenolic constituents which revealed the presence of monophenols such as Vanillic acid, Caffeic acid, Ferrulic acid as well as flavonoids such as Kaempferol, Luteolin, Rutin and Quercetin. Hence, the abundant distribution of phenolics in the different *Corchorus* species could be largely responsible for their numerous medicinal properties including blood glucose lowering effect as reported in folklore medicine. Similarly, the rich vitamin C content of the extracts is worth noting as vitamin C has been shown to prevent the onset of some degenerative diseases including diabetes mellitus.[25]

Fe^{2+} is a powerful pro-oxidant, capable of inducing generation of ROS and initiating lipid peroxidation chain reaction.[26] This study has shown that all the extracts were able to prevent the progression of lipid peroxidation by inducing a reduction in the MDA content of the pancreatic tissue in a concentration dependent manner. Dewanjee et al.[27] had earlier reported the ability of Corchorus leaf extract to significantly attenuate the progression of lipid peroxidation in liver of Wister rats exposed to lead toxicity. Phenolic compounds can participate in coordinated chemical reactions by forming complexes with ligand molecules like Fe^{2+} and this can therefore prevent Fe^{2+}-induced ROS generation and lipid peroxidation. Hence, the inhibition of iron induced pancreatic lipid peroxidation by the extracts could be as a result of Fe^{2+}

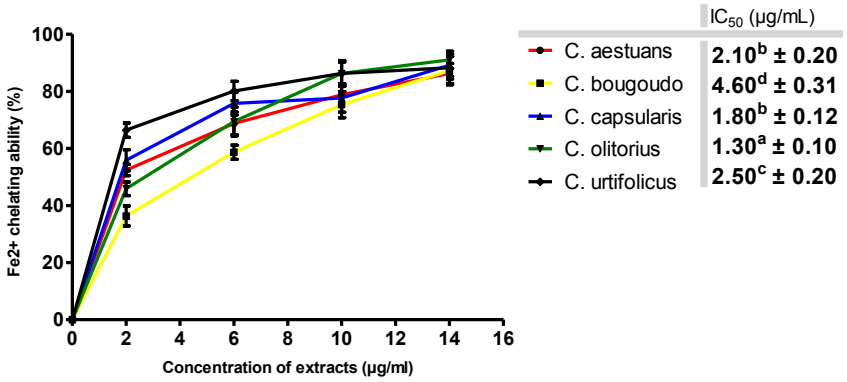

Figure 4: Percentage Fe^{2+} chelating ability of the aqueous extracts from the leaves of five *Corchorus species* IC_{50} values with the same superscript letter are not significantly different ($P > 0.05$).

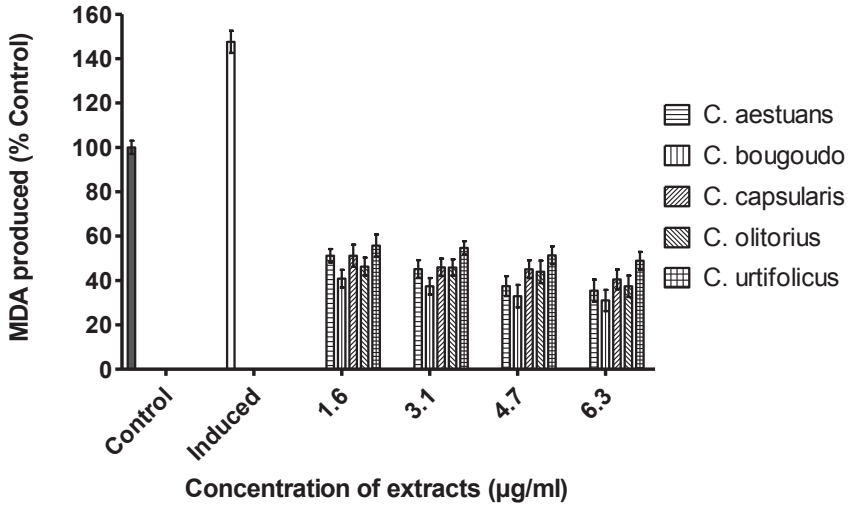

Figure 5: Inhibition of Fe^{2+} − induced lipid peroxidation in rat pancreatic tissue homogenate by the aqueous extracts from the leaves of five *Corchorus species*.

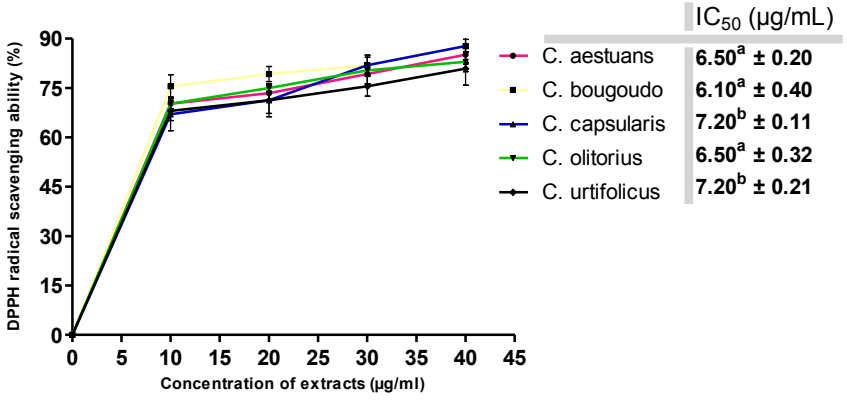

Figure 6: Percentage (1,1-diphenyl−2 picrylhydrazyl) DPPH radical scavenging of the aqueous extracts from the leaves of five *Corchorus species*. IC_{50} values with the same superscript letter are not significantly different ($P > 0.05$).

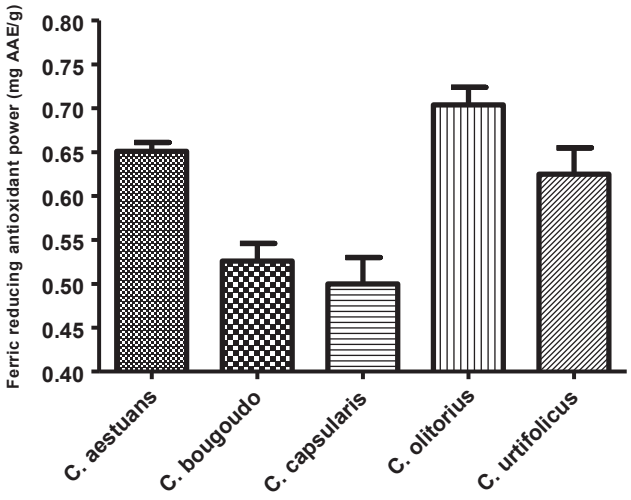

Figure 7: Ferric reducing antioxidant power (FRAP) of the aqueous extracts from the leaves of five *Corchorus species*.

chelating ability and/or radical scavenging ability of the phenolic and non-phenolics (especially vitamin C) phytochemicals present in the extracts.

Previous study have shown that hydrophilic extracts of *Corchorus oloitorius* significantly scavenged DPPH free radicals and this property was attributed to their high total phenol, total flavonoid and ascorbic acid contents.[28,29] In like manner, all the *Corchorus* extracts studied significantly ($P < 0.05$) scavenged DPPH free radicals; this scavenging ability of the extracts showed their antioxidant properties, which is of importance in scavenging excessive free radicals in cells. This is more so because excessive free radical generation has been linked to the pathogenesis and progression of T2DM.[6]

The antioxidant properties of phytochemicals have been linked to their ability to reduce oxidative species.[30] In this study, the reducing power of the extracts was measured by their ability to reduce Fe^{3+} to Fe^{2+}. Spectrophotometric quantification of the Fe^{2+} produced gave the rate of the reduction reaction and thus determine their reductive power. All the extracts were able to reduced Fe^{3+} to Fe^{2+}; an attribute linked to their rich phenolic and vitamin C contents, which have been shown to possess antioxidant properties.

Conclusion

The α-amylase and α-glucosidase inhibitory effects and antioxidant properties of all *Corchorus spp*. extracts can help explain the biochemical rationale underlying their use in the management of T2DM as obtained in folklore medicine. These therapeutic potentials can be ascribed to their phenolic constituents; although, other phytochemicals could have acted synergistically.

Conflict of interest

The authors have no conflict of interest to declare.

Authors' contributions

AAO was involved in the experimental design, data interpretation and preparation of this manuscript, OG was involved in the experimental design and data interpretation, AF was involved in the experimental design and data collection, OSI was involved in the experimental design and preparation of this manuscript and OOB was involved in experimental design and preparation of this manuscript.

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