Anti-α-glucosidase and antiglycation activities of galls from Guiera senegalensis J.F. Gmel (combretaceae)

Pierre AED Sombié1*, Rahman M Hafizur2, Moussa Compaoré3, Martin Kiendrébéogo3, M. Iqbal Choudhary2 and Odile Germaine Nacoulma3

Abstract
The hypoglycemic activity of Guiera senegalensis used in Burkinabe folk medicine has been already reported. The aim of this study was to investigate the in vitro antidiabetic activity from galls of G. senegalensis. The extracts and methanol fractions from galls of G. senegalensis showed strong α-glucosidase inhibitory activity compared with acarbose. The ethyl acetate fraction from methanol extract (EA/ME) showed potent antiglycation activity in an in vitro assay system. The galls did not show inhibition activity against α-chymotrypsin. The α-glucosidase inhibitory activity along with its antiglycation activity may open a new perspective for the use of G. senegalensis for the diabetic subject. The data suggests that consumption of G. senegalensis galls as an infusion or in food and pharmaceutical preparations may be useful for the management of diabetes and its complications.

Keywords: Guiera senegalensis; Galls; α-Glucosidase; Antiglycation; α-chymotrypsin; α-chymotrypsin

Introduction
The World Health Organization estimates that almost 3 million deaths occurring annually are as a result of diabetes and that there will be above 300 million cases of diabetes by the year 2025 [1,2]. The diabetes mellitus is a metabolic disorder of the pancreas in which blood glucose levels are abnormally high because either the body does not produce enough insulin (Diabetes Mellitus Type 1) or the insulin produced (Diabetes Mellitus Type 2) cannot be used by the body [3]. Diabetes Mellitus type 2 (DM 2), associated cardiovascular diseases and cancer are an increasing problem around the globe, especially in the developed countries [2]. One effective way to treat diabetes is by suppressing carbohydrate digestion due to the utilization of α-glucosidase inhibitors (AGIs) [4]. The inhibition of α-glucosidase, the most important enzyme in carbohydrate digestion leads to prevent excess glucose absorption at the small intestine [5]. The absorption of glucose obtained by α-glucosidase may result in a rapid rise in blood glucose levels in non-insulin dependent diabetes mellitus patients [6]. Moreover, hyperglycemia that increases free radicals production by the mitochondria may initiate diabetic complications [7]. Many reports indicate that hyperglycemia could induce non-enzymatic glycosylation of various macromolecules, generation of reactive oxygen species and alteration of endogenous antioxidants which could result in the development of chronic complications in diabetes [8]. Accumulated evidence has also suggested that diabetic patients are under oxidative stress, with an imbalance between free-radical-generating and radical-scavenging capacities [9].

The galls of G. senegalensis J.F. Gmel are rich in phytochemicals such as phenolics which have strong antioxidant properties has been reported to be good inhibitors of α-glucosidase [8] [10]. A polyphenol, 1,3-di-O-galloylquinic acid, and the quinic acid gallates, 3-O-, 4-O-, 5-O-, 3,4-di-O-, 4,5-di-O-, 3,5-di-O-, 3,4,5-tri-O- and 1,3,4,5-tetra-O-galloylquinic acids were isolated from the galls of G. senegalensis [11]. G. senegalensis is used orally for treatment of various illnesses such as hyperglycemia, malaria, cough, hypertension, diabetes and many microbial infections [12,13]. To the best of our knowledge,
no study has been conducted on the in vitro antidiabetic activity from galls of *G. senegalensis* and no literature was found regarding its potential clinical benefit. The main purpose of the present study was therefore to investigate the in vitro anti-α-glucosidase and antiglycation capacity from galls of *G. senegalensis*.

**Material and Methods**

**Extraction and fractionation procedures**

The galls of *G. senegalensis* were collected in Kadiogo (province of Burkina Faso). The extraction process was indicated in fig 1. Fifty grams of galls powder were extracted by using 500 ml of acetone/water (80/20) during 48 h under mechanical agitation at room temperature. Fifty grams powder was used for a decoction extraction (30 min, 95-98°C).

The filtrates obtained using What man filter paper were concentrated under reduced pressure in a rotary evaporator and lyophilized by using a freeze drying system to give the hydro-acetone extract (HAE) and aqueous decoction extract (ADE). Two kilogram of galls powder was extracted twice with methanol during 72 h at room temperature. The resulting extract was filtered and concentrated in rotavapour under reduced pressure. The concentrated extract was dried under hood to get the methanol extract (yield 23.8%, w/w). 2.5 grams of methanol extract was chromatographed over Sil-ica gel 60 (.040–0.063 mm, Merck) vacuum liquid chromatography (VLC) and successively eluted with hexane (100%), dichloromethane (100%), ethyl acetate (100%) and butanol (100%). Each fraction was taken to dryness under vacuum to give: hexane fraction (H/ME), dichloromethane fraction (DCM/ME), ethyl acetate fraction (EA/ME) and butanol fraction (B/ME).

**In vitro α-glucosidase inhibition assay**

α-glucosidase activity was assayed in 0.1 M sodium phosphate buffer (pH 6.8) with p-nitrophenyl-α-D-glucopyranoside as a substrate. The α-glucosidase enzyme (E.C.3.2.1.20) from Saccharomyces sp. was purchased from Wako Pure Chemical Industries Ltd. (Wako 076-02841). The concentration of α-glucosidase was 0.2 U/mL in each experiment. The enzyme (20 mL along with 100 mL of phosphate buffered saline) was incubated with various concentrations of tested extracts and fractions at 37°C. The pre-incubation time was 15 min, then 20 μL (0.7 mM) of p-nitrophenyl α-D glucopyranoside (PNP-G) as a substrate was added and reaction was carried out at 37°C for 30 min. Enzymatic activity was quantified by measuring the absorbance of p-nitrophenol at 400 nm on a microtitre plate spectrophotometer (Spectra Max, Molecular Devices, USA). One unit of α-glucosidase was defined as the amount of enzyme liberating 1.0 mmol of p-nitrophenol per minute under the conditions specified [14]. Acarbose was used as the positive control.

**In vitro antiglycation assay**

The Bovine Serum Albumin-methylglyoxal (BSA-MGO) assay was performed by using the method described by Pu et al. [15]. Triplicate samples of bovine serum albumin (BSA) at 10 mg/mL, 14 mM methylglyoxal (MGO), and 0.1 M phosphate buffer (pH 7.4) containing sodium azide (30 mM) were incubated under aseptic conditions, with each well containing 50 μL BSA, 50 μL MGO, and 20 μL test sample, at 37°C for 9 days in the presence or absence of various concentrations of the extracts and fractions. After 9 days of incubation, each sample was examined for the development of specific fluorescence (excitation 330 nm; emission 440 nm), against sample blank on a microtitre plate spectrophotometer (Spectra Max, Molecular Devices).

**Results**

**α-glucosidase inhibition**

The inhibitory activities of extracts and fractions of *G. senegalensis* galls were determined against α-glucosidase. Table 1 shows IC50 values against α-glucosidase. The α-glucosidase inhibitory activity ranged from 0.2 ± 0.001 to 88.4 ± 1.16 μg/mL with strong inhibition for hydroacetone extract (0.2 ± 0.001 μg/mL), followed by methanol extract (0.23 ± 0.01 μg/mL) and aqueous decoction extract (0.5 ± 0.006 μg/mL). The fractions of methanol extract (ME) showed lower inhibitory activity than the methanol extract. All the extracts and fractions from
galls of G. senegalensis showed strong inhibitory activity of α-glucosidase than acarbose, a reference compound. The IC₅₀ of acarbose against α-glucosidase is 840 ± 1.73 μM.

ADE: aqueous decoction extract, HAE: Hydroacetone ex-tract, ME: Methanol extract, H/ME: Hexane fraction from methanol extract, DCM/ME: Dichloromethane fraction from methanol extract, EA/ME: Ethyl acetate fraction from methanol extract, B/ME: Butanol fraction from methanol extract

Antiglycation activity
The protective effect of G. senegalensis galls extracts and fractions on the formation of advanced glycation end-products were evaluated using the BSA-glucose system, in which the BSA is used as a protein model and glucose as glycation agent. Our results showed that the extracts and fractions except the DCM/ME fraction have antiglycation activities compared to the control experiment (without the extracts added). From the results obtained, it was observed that the degree of antiglycation activities varies considerably from the different extracts/fractions tested. The EA fraction of methanol extract showed the strongest activity. The concentration of EA/ME fraction able to inhibit 50% of BSA glycation measured with fluorescence method is 0.41 ± mg/mL. All the extracts and fractions showed glycation inhibitory activities in a dose-dependent manner (Figure 2). The DCM/ME fraction showed a potential to increase the BSA glycation of 6% at the concentration of 0.125 mg/mL.

ADE: aqueous decoction extract, HAE: Hydroacetone extract, ME: Methanol extract, H/ME: Hexane fraction from methanol extract, DCM/ME: Dichloromethane fraction from methanol extract, EA/ME: Ethyl acetate fraction from methanol extract, B/ME: Butanol fraction from methanol extract

α-chymotrypsin inhibition activity
The percentage inhibition of α-chymotrypsin values varied from negative value to 65.2 % (Table 1). The DCM/ME fraction showed the high inhibition activity of α-chymotrypsin with an IC₅₀ value of 182.2 ± 7.1 μg/mL. All the extracts and fractions except DCM/ME fraction did not have good inhibition activities of α-chymotrypsin. ADE showed a potential to increase the activity of α-chymotrypsin at the concentration of 1.25 mg/mL.

Discussion
The α-glucosidase catalyzes the final step in the process of digestion of carbohydrates, may delay the absorption of carbohydrates and dietary postprandial hyperglycemia and could be useful for the treatment of diabetes. Inhibition of α-glucosidase is considered as an effective measure to control type 2 diabetes by controlling glucose uptake [9]. The galls of Guiera senegalensis effectively reduced the glucose level in α-glucosidase inhibition assay. The hydroxyl groups in polyphenolic compounds in plants have been shown to inhibit the activities of digestive enzymes due to their ability to bind with protein [17]. The activity of G. senegalensis could mainly due to the presence of polyphenolic compounds. In previous study, high polyphenols and flavonoids amount have been found in G. senegalensis galls extracts [10]. Disaccharidases are the targets of flavonoids in the regulation of glucose uptake and thus in glucose homeostasis [18]. Numerous studies have shown that some specific phytochemicals isolated from medicinal plants have antidiabetic effects [19]. The 5, 7, 30, 40-Tetramethoxyflavone and 5, 7, 40-trimethoxy isolated from Kaempferia parviflora showed strong inhibitory activities against α-glucosidase [20,21]. Prenylated flavonoids that have been shown to have great power inhibitory activity of α-glucosidase were isolated from some plants like Deriss scandens and Dorstenia psilurus [22, 23]. Acid 3, 4-di-O-cafeoyl quinic acid, quercetin, chlorogenic acid, kaempferol, rutin, vanillic acid identified in galls extracts of G. Senegalensis by Lamien et al. [24] have demonstrated inhibitory activity of α-glucosidase [25,26]. Palmitic acid identified by GC- FID/MS (data not shown) intensely stimulates glucose utilization through the activation of Akt and ERK1 / 2 in skeletal muscle cells [15]. The presence of these compounds could justify the strong inhibitory activity of the G. senegalensis galls observed. The anti-α-glucosidase extracts and fractions could also be related to their preventive effect of lipid peroxidation [10-27], which has been demonstrated to provide protection against diabetes by preventing the formation of endogenous aldehydes and removal of reactive oxygen species generated by auto-oxidation of glucose [25-28]. The glucosidase inhibitors such as acarbose and migliotol inhibit enzymes responsible for the breakdown of carbohydrates in the small intestine [29]. They were classified in the group of antihyperglycemic drugs that are often administered to patients with diabetes type 2 before meals to control postprandial glucose levels [30]. They are effective to prolong the digestion of carbohydrates and delay the absorption of glucose in the blood. The galls of G. senegalensis could therefore be used before meals to reduce the blood glucose level for the treatment of type 2 diabetic patients.

The antiglycative potential of several plants is linked to their polyphenols content [31]. The phenolic compounds such as flavonoids, chalcones, stilbenes, isoflavones and phenolic acids are able to trap the reactive dicarboxyl compounds and to inhibit glycation reaction [32]. However, it is also possible that non-phenolic antioxidants compounds contained in EA/ME fraction of G. senegalensis galls might display its antiglycative activity.
Table 1  α-glucosidase and α-Chymotrypsin inhibition activities

<table>
<thead>
<tr>
<th>Sample</th>
<th>α-glucosidase inhibition IC50 (µg/mL)</th>
<th>α-Chymotrypsin Inhibition percentage for 500 µg/ml of sample</th>
<th>IC50 ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous decoction extract</td>
<td>0.5 ± 0.006</td>
<td>Non detected</td>
<td></td>
</tr>
<tr>
<td>Hydroacetone extract</td>
<td>0.2 ± 0.001</td>
<td>Non detected</td>
<td></td>
</tr>
<tr>
<td>Methanol extract</td>
<td>0.23 ± 0.01</td>
<td>32 %</td>
<td></td>
</tr>
<tr>
<td>Hexane fraction</td>
<td>6.8 ± 0.16</td>
<td>2.5 %</td>
<td></td>
</tr>
<tr>
<td>Dichloromethane fraction</td>
<td>88.4 ± 1.16</td>
<td>65.2 %</td>
<td>182.2 ± 7.1 µg/mL</td>
</tr>
<tr>
<td>Ethyl acetate fraction</td>
<td>19.3 ± 0.33</td>
<td>6.2 %</td>
<td></td>
</tr>
<tr>
<td>Butanol fraction</td>
<td>0.8 ± 0.01</td>
<td>5.4 %</td>
<td></td>
</tr>
<tr>
<td>Acarbose</td>
<td>840 ± 1.73 µM</td>
<td>Non tested</td>
<td>5.7 ± 0.1 µM</td>
</tr>
<tr>
<td>Chymostatin (0.125 mM)</td>
<td>Non tested</td>
<td>98.6 %</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1 Extraction Process

Figure 2 Dose dependant manner of BSA glycation
The antiglycative compounds contained in this fraction could be important in the prevention of diabetes complications.

Protease inhibitors are considered as anti-nutritional factors that can interfere with the digestion and absorption of nutrients [33]. The presence of protease inhibitors (trypsin, chymotrypsin and other intestinal proteases inhibitors) results in impaired growth, poor food utilization and interference with digestion, causing pancreatic hypertrophy and metabolic disturbance of sulphur and amino acid utilization [34]. The extracts and fractions of galls showed weak inhibition against α-chymotrypsin. They don’t contain anti-nutritional factors and could be consumed regularly as part of the diet.

Conclusions

The galls of G. senegalensis could be used as functional food ingredients for the prevention and management of type 2 diabetes. The extracts of G. senegalensis showed a potent ability to inhibit α-glucosidase. The health and remedial benefits (anti-α-glucosidase and antiglycation activities) from galls of G. senegalensis indicate that these herbal medicines could be a natural source for diabetes management and prevention.

Authors’ contributions

PAEDS, RMH and MIC designed and performed the experiments about the inhibition on the activity of α-glucosidase and antiglycation potentials of G senegalensis

PAEDS wrote this manuscript, RMH, MC, Mk and OGN read and corrected the manuscript

All authors read and approved the final manuscript.

Acknowledgements

SOMBIE Pierre A.E. D is very grateful to the IFS (International Foundation of Sciences) for providing financial support (F/5091-1).

SOMBIE Pierre A.E. D also thanks the Academy of Sciences for the Developing World (TWAS) and International Center for Chemical and Biological Sciences (ICCBS) of the University of Karachi, Pakistan for their financial and technical support through the 2010

Author details

1Institute of Environment and Agricultural Research, BP 476 Ouagadougou 01, Burkina Faso. 2Institute of Chemistry, International Center for Chemical and Biological Sciences, University of Karachi, Karachi, Pakistan. 3University of Ouaga I Professor Joseph Ki-Zerbo, Laboratory of Biochemistry and Chemistry Applied (LABIOCA), Ouagadougou, 03 BP 7021 Ouagadougou 03, Burkina Faso.

References


