ORIGINAl RESEARCH ARTICLES

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 (DM2), 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 kilograms 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). 25 grams of methanol extract was chromatographed over Silica gel 60 (0.040–0.063 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 µL along with 100 µL 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 microtiter plate spectrophotometer (Spectra Max, Molecular Devices).

**In vitro α-chymotrypsin inhibition assay**

The inhibitory activity of α-chymotrypsin from bovine pancreas (Sigma-Aldrich) was performed in 50 mM Tris–HCl buffer pH 7.6 with 10 mM CaCl	extsubscript{2} as reported by Fouotsa et al. [16]. The enzyme; α-chymotrypsin (12 Units/mL prepared in buffer mentioned above) with the test compound (0.5 mM) prepared in DMSO (final concentration 7%) was incubated at 30°C for 25 min. The reaction was started by the addition of the substrate N-succinyl-L-phenylalalnine-p-nitroanilidile from Sigma-Aldrich (SPnNA; 0.4 mM prepared in the buffer as above). The change in absorbance by released p-nitroanilidile was continuously monitored at 410 nm. Positive controls without test compound and negative controls without enzyme or with standard inhibitors were run in parallel. The percent inhibition was calculated. Chymostatin was used as a positive control.

**Results**

**α-glucosidase inhibition**

The inhibitory activities of extracts and fractions of *G. senegalensis* galls were determined against α-glucosidase. Table 1 shows IC	extsubscript{50} 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 IC$_{50}$ 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 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

**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 IC$_{50}$ 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-Te’ramethoxylavone 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 *Derris 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 dia- betes 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 admin- istered 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 dicarbonyl 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

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

**Figure 1** Extraction Process

**Figure 2** Dosedependant 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

[1] George C, Lochner A, Huisamen B. The efficacy of Prosopis glandulosa as antidiabetic treatment in rat models of diabetes and insulin resistance. Journal of ethnopharmacology. 2011;137(1):298-304.
[2] Sabiu S, O’Neill FH, Ashafa AO. Kinetics of α-amylase and α-glucosidase inhibitory potential of Zea mays Linnaeus (Poaceae), Stigma maydis aqueous extract: An in vitro assessment. Journal of ethnopharmacology. 2016;183:1-8.
[3] Proença C, Freitas M, Ribeiro D, Oliveira EF, Sousa JL, Tomé SM, Ramos MJ, Silva AM, Fernandes PA, Fernandes E. α-Glucosidase inhibition by flavonoids: an in vitro and in silico structure–activity relationship study. Journal of enzyme inhibition and medicinal chemistry. 2017;32(1):1216-28.
[4] Yin Z, Zhang W, Feng F, Zhang Y, Kang W. α-Glucosidase inhibitors isolated from medicinal plants. Food Sci. Hum. Wellness. 2014; 3: 136–174.
[5] Jo SH, Cho CY, Lee JY, Ha KS, Kwon YI, Apostolidis E. In vitro and in vivo reduction of post-prandial blood glucose levels by ethyl alcohol and water Zingiber mioga extracts through the inhibition of carbohydrate hydrolyzing enzymes. BMC complementary and alternative medicine. 2016;16(1):111.
[6] Lin L, Dong Y, Zhao H, Wen L, Yang B, Zhao M. Comparative evaluation of rosmarinic acid, methyl rosmarinate and pedasin isolated from Rabdosia serra (MAXIM.) HARA as inhibitors of tyrosinase and α-glucosidase. Food chemistry. 2011;129(3):884-9.
[7] Behl T, Kaur I, Kotwani A. Implication of oxidative stress in progression of diabetic retinopathy. Survey of ophthalmology. 2016;61(2):187-96.
[8] Ademiluyi AO, Oboh G. Soybean phenolic-rich extracts inhibit key-enzymes linked to type 2 diabetes (α-amylase and α-glucosidase) and hypertension (angiotensin I converting enzyme) in vitro. Experimental and Toxicologic Pathology. 2013;65(3):305-9.
[9] Wang SY, Camp MJ, Ehlenfeldt MK. Antioxidant capacity and α-glucosidase inhibitory activity in peel and flesh of blueberry (Vaccinium spp.) cultivars. Food Chemistry. 2012;132(4):1759-68.
[10] Sombie PAED, Hilou A, Mounier C, Coulibaly AY, Kiendrebeogo M, Millogo JF, Nacoulma O.G. Antioxidant and anti-inflammatory activities from galls of Guiera senegalensis J.F. Gmel (Combretaceae). Research Journal of Medicinal Plant. 2011; 5: 448–461.
[11] Bouchet N, Levesque J, Blond A, Bodo B, Pousse J-L. 1,3-di-O-galloylquinic acid from Guiera senegalensis. Phytochemistry. 1996; 42: 189–190.
[12] Elrahman OF, Abuelgasim I, Galal M. Toxicopathological effects of Guiera senegalensis extracts in wistar albino rats. Journal of Medicinal Plants Research. 2009;3(10):699-702.
[13] Kankara SS, Mustafa M, Ibrahim HM, Nulit R, Go R. Effect of drying methods, solid-solvent ratio, extraction time and extraction temperature on phenolic antioxidants and antioxidant activity of Guiera senegalensis J.F.Gmel (Combretaceae) Leaves Water Extract. American Journal of Phytomedicine and Clinical Therapeutics. 2014;12: 1378-1392

[14] Choudhary MI, Shah SA, Khan SN, Khan MT. Alpha-glucosidase and tyrosinase inhibitors from fungal hydroxylation of tibolone and hydroxytibolones. Steroids. 2010;75(12):956-66.

[15] Pu J, Peng G, Li L, Na H, Liu Y, Liu P. Palmitic acid acutely stimulates glucose uptake via activation of Akt and ERK1/2 in skeletal muscle cells. J. Lipid Res. 2011;52(7): 1319–1327.

[16] Fouotsa H, Lannang AM, Mbazoa CD, Rasheed S, Marasini BP, Ali Z, Devkota KP, Kengfack AE, Shaheen P, Choudhary MI, Sewald N. Xanthones inhibitors of α-glucosidase and glycation from Garcinia nobilis. Phytochemistry letters. 2012;5(2):236-9.

[17] Zhang H, Tsao R. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Current Opinion in Food Science. 2016;8:33-42.

[18] Pereira DF, Cazarolli LH, Lavado C, Mengatto V, Figueiredo MS, Guedes A, Pizzolatti MG, Silva FR. Effects of flavonoids on α-glucosidase activity: potential targets for glucose homeostasis. Nutrition. 2011;27(11-12):1161-7.

[19] Revathi P, Jayaseelan S, Thirumalaikolundu Subramanian P, Manickavasagam S, Prabhu N. A Comparative Mechanism of Antidiabetic Role of Various Extracts of Bruguiera cylindrica L. Leaves. World Journal of Pharmacy and Pharmaceutical Sciences. 2015;4(05):1168-76.

[20] Leyama T, Gunawan-Puteri MDPT, Kawabata J. α-Glucosidase inhibitors from the bulb of Eleutherine americana. Food Chem. 2011; 128 (2): 308–311.

[21] Azuma T, Kayano S-I, Matsumura Y, Konishi Y, Tanaka Y, Kikuzaki H. Antimutagenic and α-glucosidase inhibitory effects of constituents from Kaempferia parviflora. Food Chem. 2011; 125 (2): 471–475.

[22] Tabopda TK, Ngoupayo J, Awoussong PK, Mitaine-Offre AC, Ali MS, Ngadjui BT, Lacaille-Dubois MA. Triprenylated flavonoids from Dorstenia psilurus and their α-glucosidase inhibition properties. Journal of natural products. 2008;71(12):2068-72.

[23] Rao SA, Srinivas PV, Tiwari AK, Vanka UM, Rao RV, Dasari KR, Rao MJ. Isolation, characterization and chemobiological quantification of α-glucosidase enzyme inhibitory and free radical scavenging constituents from Derris scandens Benth. Journal of chromatography B. 2007;855(2):166-72.

[24] Lamien CE, Meda A, Mans J, Romito M, Nacoulma OG, Viljoen GJ. Inhibition of fowlpox virus by an aqueous acetone extract from galls of Guiera senegalensis JF Gmel (Combretaceae). Journal of ethnopharmacology. 2005;96(1-2):249-53.

[25] Ooi KL, Muhammad TS, Tan ML, Sulaiman SF. Cytotoxic, apoptotic and anti-α-glucosidase activities of 3, 4-di-O-cafeoyl quinic acid, an antioxidant isolated from the polyphenolic-rich extract of Elephantopus mollis Kunth. Journal of ethnopharmacology. 2011;135(3):685-95.

[26] Xiao-Ping YE, Chun-Qing SO, Ping YU, Ren-Gang MA. α-Glucosidase and α-amylase inhibitory activity of common constituents from traditional Chinese medicine used for diabetes mellitus. Chinese Journal of Natural Medicines. 2010;8(5):349-52.

[27] Sombié PAED, Hilou A, Coulibaly AY, Tibiri A, Kiendrebeogo M, Nacoulma OG. Brain Protective and Erythrocytes Hemolysis Inhibition Potentials. J. Pharmacol. Toxicol. 2011; 6 (4): 361–370.

[28] Aslan M, Orhan N, Orhan DD, Ergun F. Hypoglycemic activity and antioxidant potential of some medicinal plants traditionally used in Turkey for diabetes. Journal of Ethnopharmacology. 2010;128(2):384-9.

[29] Jdir H, Khemakhem B, Chakroun M, Zouari S, Ali YB, Zouari N. Diplotaxis simplex suppresses postprandial hyperglycemia in mice by inhibiting key-enzymes linked to type 2 diabetes. Revista Brasileira de Farmacognosia. 2015;25(2):152-7.

[30] Hyun TK, Eom SH, Kim JS. Molecular docking studies for discovery of plant-derived α-glucosidase inhibitors. Plant Omics Journal. 2014;7(3):166–170.

[31] Bi X, Soong YY, Lim SW, Henry CJ. Evaluation of antioxidant capacity of Chinese five-spice ingredients. International journal of food sciences and nutrition. 2015;66(3):289-92.

[32] Navarro M, Morales FJ. Mechanism of reactive carbonyl species trapping by hydroxytyrosol under simulated physiological conditions. Food chemistry. 2015;175:92-9.

[33] Nikmaram N, Leong SY, Koubaa M, Zhu Z, Barba FJ, Greiner R, Oey I, Roohinejad S. Effect of extrusion on the anti-nutritional factors of food products: An overview. Food Control. 2017;79:62-73.

[34] Adeyemo SM, Onilude AA. Enzymatic reduction of antinutritional factors in fermenting soybeans by Lactobacillus plantarum isolates from fermenting cereals. Nigerian Food Journal. 2013;31(2):84-90.