South African Journal of Botany 113 (2017) 239–247

Contents lists available at ScienceDirect

South African Journal of Botany

journal homepage: www.elsevier.com/locate/sajb

a-Glucosidase inhibition, anti-glycation and antioxidant activities of *Liquidambar formosana* Hance leaf, and identification of phytochemical profile

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ARTICLE INFO

Article history:
Received 31 May 2017
Received in revised form 7 August 2017
Accepted 21 August 2017

Edited by V Kumar Minor

Keywords:
Liquidambar formosana Hance leaf
antioxidant activity
anti-glycation
a-glucosidase inhibition
HPLC-QTOF-MS/MS

ABSTRACT

This study firstly investigates the ability of *Liquidambar formosana* Hance leaf (LFHL) against diabetes and diabetic complications. The bio-active constituents in LFHL extract were fractionated with solvents at various polarities. The antioxidant, hypoglycemic and anti-glycation activities, and the content of phenolics, flavonoids, hydrolysable tannins and condensed tannins were measured. The phytochemical profile of which gave the highest activity was identified by using HPLC-QTOF -MS/MS. Results revealed that the ethyl acetate fraction (EAF) gave the richest phenolics, condensed tannins and hydrolysable tannins, which was 5.06, 8.75 and 3.57 fold of that of crude extract, respectively. The highest total flavonoids content was detected in *n*-butanol fraction. The EAF also exhibited the strongest antioxidant, hypoglycemic and anti-glycation activity. The DPPH·scavenging ability, *a*-glucosidase and advanced glycation end-products formation inhibitory percentage was 1.74, 168.19 and 2.51 fold of that of individual positive control. Totally, 22 compounds were identified or tentatively identified from the EAF of LFHL extract, including 7 phenolic acids, 4 flavanols, 9 flavonols, 1 tannin, and 1 lignan. Above results indicated that LFHL can be a potentially alternative source of products against diabetes and diabetic complications.

1. Introduction

Diabetes is a chronic metabolic syndrome characterized by permanent hyperglycaemia. These with diabetes are much easier to suffer from glucose oxidation, non-enzymatic glycation of proteins, and subsequently oxidative degradation of glycated proteins, resulting to the over formation of free radicals (Hunt et al., 1988; Mullarkey et al., 1990). Meanwhile, the increased oxidative stress and decline antioxidant defense mechanism can promote the development and progression of diabetes mellitus and its complications, since it can activate transcription factors and protein kinase C, promote the formation of advanced glycated end-products (AGEs) (Maritim et al., 2003; Giacco and Brownlee, 2010). In addition, protein glycation will lead to the accumulation of AGEs, which have been recognized as a major factor in the pathogenesis of diabetic complications, such as neuropathy, retinopathy, nephropathy and cataract (Goh and Cooper, 2008). It is reported that AGEs can alter the structure and function of plasma and extracellular matrix proteins, increase the release of pro-inflammatory cytokines and adhesion molecules (Wendt et al., 2002). Therefore, the natural products that can both release oxidative stress and inhibit the formation of AGEs, are considered as a potential therapeutic strategy for the prevention of diabetic complications.

a-Glucosidase is an important carbohydrate hydrolase located in the brush-border surface membrane of the small intestine, it can greatly increase postprandial blood glucose level by catalyzing the hydrolysis of oligosaccharide and disaccharide into absorbable monosaccharide (Kumar et al., 2011). The liberation of *D*-glucose from dietary carbohydrates will be retarded and the adsorption of glucose will be delayed when the activity of *a*-glucosidase is inhibited, resulting in reduced postprandial and fasting plasma glucose levels. Effective *a*-glucosidase inhibitors (AGIs) have been considered as an effective strategy for controlling blood glucose level, and for preventing diabetes and diabetic complications (Kalra, 2014). But synthetic AGIs, such as acarbose, usually have the side effect of bloating, diarrhea and angina abdominis, even lead to hepatotoxicity (Hsiao et al., 2006; Zhang et al., 2016a). At this point, screening of natural, effective and non-toxic AGIs from plants is meaningful for promoting the development of novel strategies against diabetes and diabetic complications.

Abbreviations: LFHL, *Liquidambar formosana* Hance leaf; LFH, *Liquidambar formosana* Hance; AGEs, advanced glycated end-products; AGIs, *a*-glucosidase inhibitors; CE, crude extract; DCF, dichloromethane fraction; EAF, ethyl acetate fraction; *n*BuF, *n*-butanol fraction; WF, water fraction.

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http://dx.doi.org/10.1016/j.sajb.2017.08.010
0254-6299/

Liquidambar formosana Hance (LFH) is a deciduous and ornamental tree belonging to the family of *Hamamelidaceae*, which widely distributed in China, Laos, North Korea, Vietnam and Japan. As a folk medicine, each part of this plant has certain medicinal values, the leaf has been used to treat hemostasis, dysentery, diarrhea, and detoxification (Zhong et al., 2013), the bark is used to cure diarrhoea, the fruit has been used as an analgesic, diuretic, anticonvulsant and hepatic protectant (Dat et al., 2004), and the root is used to treat inflammation, diarrhoea and indigestion (Yu et al., 2012). Up to date, several researches have been performed on the chemical constituents of LFHL. Zhong et al. (2015) isolated nine flavonoids from the ethyl acetate fraction of LFHL water decoction. Liao et al. (2014) purified and identified six phenolic acids, one flavonoids and one triterpene saponins. Hatano et al. (1986) revealed the presence of 8 tannins in LFHL. But no research is available about the capacity of LFHL extract against diabetes and diabetic complications.

This study was conducted to evaluate the potential of LFHL against diabetes and diabetic complications by fractionating the active constituents, testing their activities and identifying the functional compounds. Content of phenolics, flavonoids, condensed tannins and hydrolysable tannins of obtained extract and fractions was measured by UV–Vis spectrophotometer. The anti-diabetic potential of LFHL extracts were investigated by analyzing their radical scavenging ability, inhibition on the activity of α-glucosidase enzyme and the formation of AGEs. The functional compounds were identified by using high performance liquid cinematographer coupled to quadrupole time-of-flight tandem mass spectrometry (HPLC-QTOF-MS/MS).

2. Material and methods

2.1. Reagents and materials

Fresh *Liquidambar formosana* Hance leaf (LFHL) was collected from the campus of Jiangxi Agricultural University on 28th July, 2016, and identified by Prof. Min-fei Jian, who majors in plant classification and works at Jiangxi Normal University. The leaves were washed with tap water and freeze dried. Then the dried leaves were powdered, passed through 200 mesh and stored at 4 °C for further use.

Bovine serum albumin (BSA), 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), acarbose, α-glucosidase (yeast, EC 3.2.1.20) and *p*-nitrophenyl-α-D-glucopyranoside were purchased from Sigma (St. Louis, MO, USA). Gallic acid, quercetin, fructose, aminoguanidine hydrochloride and all other reagents were from Aladin (Shanghai, China). Formic acid and acetonitrile used for HPLC analysis were HPLC grade and purchased from Merck (New York, USA). All other reagents were of analytical grade.

2.2. Extraction and fraction of bio-active constituents

In this research, ultrasound-assisted extraction was employed to recover the bio-active constituents present in LFHL according to references (Yan and Gao, 2010) with minor modifications. The LFHL powder (200 g) was mixed with 80% ethanol at a solid/liquid ratio of 1:20 (*w*/*v*, g/mL). The mixtures were filtered after 60 min of ultrasound at 400 W and 60 °C. The supernatant was collected and the residue was re-extracted for another two times following the same conditions. Finally, all supernatant were combined, concentrated under reduced pressure to yield 39.29 g of crude extract.

The prepared crude extract (35.0 g) was dissolved in 100 mL distilled water, followed by partition with dichloromethane, ethyl acetate and *n*-butanol, successively. Each partition was repeated for 3–5 times with 200 mL of solvent until the color of solvent became colorless or very light. Finally, the same fractions were combined, concentrated under reduced pressure and lyophilized to obtain 13.86 g of dichloromethane fraction (DCF), 14.29 g of ethyl acetate fraction (EAF), 2.53 g of *n*-butanol fraction (*n*BuF) and 2.77 g of water fraction (WF). The concentration of all fractions was adjusted to 2.0 mg/mL with 80% ethanol for further analysis.

2.3. Determination of total phenolics and total flavonoids

The Folin-Ciocalteau method was employed to measure the total phenolics content, the results were expressed as milligram of gallic acid equivalents per gram of extract (mg GAE/g E.) (Zhang et al., 2016c). The total flavonoids content was tested by $AlCl_3$ colorimetric method following the procedures described by Zhang et al. (2016b), and expressed as milligram of quercetin equivalents per gram of extract (mg QuE/g E.). All experiments were run in triplicate.

2.4. Determination of hydrolysable tannins

Content of hydrolysable tannins in the crude extract and fractions was determined according to reference (Willis, 1998) with minor modifications. Samples with proper concentration (500 μL) were reacted with 750 μL saturation KIO_3 solution for 15 min at room temperature. The mixtures were centrifuged at 10,000 rpm for 2 min, then 200 μL of supernatant was transferred to 96-well micro-plate. Absorbance at 550 nm was read with a micro-plate reader. Gallic acid was used as standard, the results were presented as milligram gallic acid equivalents per gram of extract (mg GAE/g E).

2.5. Determination of condensed tannins

The condensed tannin content was measured following the procedures of El Euch et al. (2015) using a UV-3200 UV–vis spectrophotometry (Mapada, Shanghai, China). The results were calculated according to the standard curve plotted with catechin and expressed as mg catechin equivalents per gram of extract (mg CE/g E.).

2.6. DPPH·and $ABTS^+$·scavenging ability

The DPPH·and $ABTS^+$·scavenging ability assays were performed to evaluate the radical scavenging ability of samples (Zhang et al., 2016b). Quercetin was used as positive control. The IC_{50} value (μg/mL), the concentration required to scavenge 50% of radical, was calculated with polynomial fit of the scatter diagram of sample concentration *vs* inhibition percentage by using Origin 8.0 (OriginLab Co., USA).

2.7. a-Glucosidase inhibitory assay

The *a*-glucosidase activity inhibition of all samples was investigated following the procedures reported by Zhang et al. (2016c). The α-glucosidase inhibition was calculated as follows:

$$\text{Inhibition}(\%) = \frac{(A_c - A_b) - (A_i - A_j)}{(A_c - A_b)} \times 100\% \quad (1)$$

where, A_c is the absorbance of control group with sample replaced with 80% ethanol; A_i is the absorbance of sample group (with all reagents); A_j is the absorbance of group with α-glucosidase solvent replaced with 0.1 M pH 6.9 PBS. A_j is the absorbance of negative control without sample and α-glucosidase solvent. Acarbose was used as positive control, the IC_{50} value (μg/mL) was calculated by polynomial fit using origin 8.0.

2.8. Anti-glycation assay

The BSA-fructose model was carried to assess the ability of LFHL on inhibiting the formulation of AGEs (Sri Harsha et al., 2013). Frutose (625 mM) and BSA (25 mg/mL) solution was prepared using 0.2 M, pH 7.4 PBS containing 0.02% sodium azide. Then, 0.5 mL of 0.2 mg/mL