JOURNAL OF FOOD AND DRUG ANALYSIS XXX (2017) 1–8

Available online at www.sciencedirect.com

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journal homepage: www.jfda-online.com

Original Article

The inhibition of advanced glycation end-products by five fractions and three main flavonoids from *Camellia nitidissima* Chi flowers

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ARTICLE INFO

Article history:
Received 21 February 2017
Received in revised form
22 March 2017
Accepted 26 March 2017
Available online xxx

Keywords:
Camellia nitidissima Chi flowers
Advanced glycation end-products
Flavonoid glycosides and flavanol
Scavenging methylglyoxal
HPLC-ESI-MS/MS

ABSTRACT

Camellia nitidissima Chi (CNC), belonging to *Camellia* genus (Theaceae family), is a medicinal and edible plant in China. Among the whole plant, the CNC flowers are especially precious, but the biological activities and the compositions of the CNC flowers are unknown. In this study, inhibiting effects on the formation of advanced glycation end-products (AGEs) of five CNC flowers fractions and three isolated compounds were investigated, these three compounds are two flavonoid glycosides and one flavanol, namely kaempferol 3-O-[2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)-2,4-di-O-acetyl-α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside, kaempferol 3-O-[2,3,4-Tri-O-acetyl-α-L-rhamnopyranosyl-(1→3)-4-O-acetyl-α-L-rhamnopyranosyl-(1→6)]-β-D-glucopyranoside and catechin. Among these five fractions, the ethyl acetate fraction showed the highest total phenolic contents and inhibiting effects on AGE formation. Bovine serum albumin (BSA)-glucose and BSA-methylglyoxal assay showed that the ethyl acetate fraction inhibited AGE formation by 74.49% and 34.3% at 1 mg/mL, respectively. As the main components, these three compounds also showed remarkable inhibiting effects on AGE formation by scavenging methylglyoxal, next two catechin-carbonyl adducts were identified using HPLC-ESI-MS/MS. The results showed that the CNC flowers had remarkable inhibiting effects on the formation of AGEs. The primary structure-activity relationship showed (1) the glycosides could reduce the inhibiting effects compared to kaempferol and (2) the acetyl at position 2‴ in compound **1** had no remarkable influence of the inhibiting effects on AGE formation compared to compound **2**.

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http://dx.doi.org/10.1016/j.jfda.2017.03.007

Please cite this article in press as: Yang R, et al., The inhibition of advanced glycation end-products by five fractions and three main flavonoids from *Camellia nitidissima* Chi flowers, Journal of Food and Drug Analysis (2017), http://dx.doi.org/10.1016/j.jfda.2017.03.007

1. Introduction

Advanced glycation end-products (AGEs) are the nonenzymatic reaction final products between reducing sugars and amino groups in proteins, lipids, and nucleic acids [1]. AGEs and reactive carbonyl species play an important role in many human diseases such as diabetic complications [1], Alzheimer's disease [2], aging [3] and atherosclerosis [4]. Thus, it is a potential therapeutic method for the diabetic prevention or other pathogenic complications to search AGE inhibitors. Comparing with synthetic compounds, natural products have been proven relatively safe for human. Hence, some plant extracts have been evaluated for their inhibiting effects on the formation of AGEs in recent years [5–9]. Most of these plant extracts with the inhibiting effects on the AGE formation mainly contained the large amount of phenolic compounds in their phytochemicals [10–12]. As a major kind of phenolic compounds, flavonoid and flavonoid glycosides have been isolated from many plants, especially from some kinds of teas [13–15]. And the flavonoid and flavonoid glycosides show many biological activities in *vivo* and in *vitro*, especially flavonoid and flavonoid glycosides are regarded as AGEs inhibitors to represent a potential therapeutic target to prevent and treat diabetic complications [10,16,17].

Yellow *Camellia* includes over 42 species and 5 variants and they are mainly distributed in a narrow region of Guangxi province in Southern China and Northern Vietnam. The yellow petals of the flowers are rarely found in the world, so it is called "the pandas in plant kingdom" [14]. *Camellia nitidissima* Chi (CNC) belongs to *Camellia* genus and is regarded as a medicinal and edible plant in China. CNC plays an important role in human health [18], and the CNC leaves have the ability of inhibiting the formation of AGEs [8]. Since the CNC flowers are more rare and precious than the leaves, most of the studies about CNC are on the leaves, and the biological activities and the compositions of the CNC flowers are unknown. In this study, we isolate the major flavonoid glycosides and flavanol from the CNC flowers fractions, and evaluate the inhibiting effects on the AGE formation of CNC flowers fractions and the major flavonoid glycosides and flavanol.

2. Materials and Methods

2.1. Instruments and reagents

The nuclear magnetic resonance data (^{1}H-NMR and ^{13}C-NMR) were recorded on Bruker AV-500 (Bruker Inc., Germany). The mass spectrometry (MS) spectra were performed on Agilent 1100 Series LC-MSD-Trap/SL and Thermo TSQ Quantum LC/MS spectrometers. Silica gel (100–200 mesh, 200–300 mesh), which was used for silica gel column chromatography and thin-layer chromatography was purchased from Qingdao Marine Chemical Factory (Qingdao, China). Sephadex LH-20 (GE Healthcare Bio-sciences AB, Uppsala, Sweden), C18 (YMC, Japan) and RP-18 F254 plates (0.25 mm, Merck, Germany) were used. Methylglyoxal was purchased from Wuhan Huameihua Co. (Wuhan, China). Aminoguanidine was purchased from Dulai Biotechnology Co. (Nanjing, China). Bovine serum albumin (BSA) was purchased from Beijing Solarbio Science and Technology Co. (Beijing, China). HPLC grade methanol was purchased from Tedia (Fairfield, USA). All other chemicals were analytical grade and purchased from Shuangling Chemical Reagent Co. (Nanjing, China).

2.2. Plant materials

The *C. nitidissima* Chi (CNC) flowers were collected in July 2013 from Fangchenggang, Guangxi Province, China. The flowers were air-dried and coarsely powdered (ca. 40 mesh).

2.3. Determination of total phenolic contents

The total phenolic contents were determined by the Folin–Ciocalteu method following the literature [19].

2.4. The isolation of phytochemicals

The CNC flowers (6 kg) were refluxed with 95% ethanol for 3 times (3, 2, and 1 h, respectively). Ethanolic extract (1200 g) was obtained through a rotary evaporator at 45 °C. Then the ethanolic extract was suspended in water and partitioned by dichloromethane (3 × 4.5 L), ethyl acetate (3 × 4.5 L) and n-butanol (3 × 4.5 L), respectively, to yield the dichloromethane (52 g), ethyl acetate (256 g) and n-butanol (560 g) fractions. The water phase was dried at 50 °C to yield the water fraction (300 g). The dichloromethane fraction was subjected to silica gel column chromatography eluted with dichloromethane-methanol (1:0, 49:1, 25:1, 15:1, 9:1, 5:1, 0:1) gradient system to yield 4 subfractions on the basis of thin-layer chromatography analysis. The subfraction 3 was subjected to silica gel column chromatography, Sephadex LH-20 (dichloromethane-methanol 1:1) and C18 (methanol-water 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, 10:0) repeatedly, to yield compound **1** (3.25 g). Then the compound **2** (2.16 g) and compound **3** (2.57 g) were obtained from the ethyl acetate fraction through the similar methods.

2.5. Antiglycation assay in BSA-glucose model

The antiglycation assay in BSA-glucose model was tested using the published method [8] with minor modifications. The final concentrations of catechin were 5 mg/mL, 2.5 mg/mL, 1 mg/mL and 0.2 mg/mL. Fluorescence intensity (excitation wavelength was monitored at 340 nm and emission wavelength was monitored at 420 nm) of the test solution was measured on a microplate reader (TECAN Infinite 200 Pro., Austria).

2.6. Antiglycation assay in BSA-methylglyoxal model

The antiglycation assay in BSA-methylglyoxal model was carried out based on the published method [8] with minor modifications. The final concentrations of catechin were 5 mg/mL, 2.5 mg/mL, 1 mg/mL and 0.2 mg/mL. Fluorescence intensity (excitation wavelength was monitored at 340 nm and emission wavelength was monitored at 420 nm) of the test solution was measured on a microplate reader (TECAN Infinite 200 Pro., Austria).

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