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Profiling hydroxycinnamic acid glycosides, iridoid glycosides, and phenylethanoid glycosides in baobab fruit pulp (*Adansonia digitata*)

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ABSTRACT

The baobab (*Adansonia digitata* L.) is a magnificent tree revered throughout Africa and is becoming recognized for its high nutritional and medicinal values. Despite numerous reports on the pharmacological potential, little is known about its chemical compositions. In this study, four hydroxycinnamic acid glycosides (**1**–**4**), six iridoid glycosides (**5**–**10**), and three phenylethanoid glycosides (**11**–**13**) were isolated from the dried baobab fruit pulp. Their structures were determined by means of spectroscopic analyses, including HRMS, ^{1}H and ^{13}C NMR and 2D experiments (COSY, HSQC, HMBC, and ROESY). All 13 compounds isolated were reported for the first time in the genus of *Adansonia*. An ultra high-performance liquid chromatography high-resolution accurate-mass mass spectrometry (UHPLC HRAM MS) method was used to conduct further investigation of the chemical compositions of the hydro-alcohol baobab fruit pulp extract. Hydroxycinnamic acid glycosides, iridoid glycosides and phenylethanoid glycosides were found to be the main components in baobab fruit pulp.

1. Introduction

The genus *Adansonia* L. (Malvaceae) is well known as baobab, upside-down tree, Monkey-bread tree, among several other names. This genus comprises nine species and most of them occur naturally in Africa (Kamatou, Vermaak, & Viljoen, 2011). *Adansonia digitata* commonly known as 'Baobab' is found primarily in the Sahelian, Soudano-Sahelian, and Soudanian zones. It is a massive deciduous tree easily distinguishable by its huge trunk and can grow up to 25-meter or more in height, 12-meter in diameter and may live for several hundred years (Chadare, Linnemann, Hounhouigan, Nout, & Van Boekel, 2009). Some baobabs bear leaves only for three months per year. And most of the biosynthetic processes of secondary metabolites take place in the trunk and branches during the long leafless period (Gebauer, El-Siddig, & Ebert, 2002).

The baobab is considered bewitched by some indigenous people throughout Africa. It has multi-purpose uses and every part of the plant is reported to be useful. The products of baobab such as bark, leaves, fruits and seeds contribute to the livelihood of many populations in Africa as a source of food or medicine (Chadare et al., 2009; De Caluwe, Halamova, & Van Damme, 2010). The leaves, bark and fruit pulp have been traditionally used as immunostimulants, analgesics etc. in the treatment of diseases such as fever, diarrhea, cough, dysentery, haemoptysis, tuberculosis, microbial infection and worms (De Caluwe et al., 2010; Denloye, Teslim, & Fasasi, 2006; Kamatou et al., 2011; Rahul et al., 2015; Yusha'u, Hamaza, & Abdullahi, 2010). The seeds and oil are used as medicines in the treatment of muscle wounds, dandruff and other skin ailments (Kabore et al., 2011; Kamatou et al., 2011). Thus the tree is nick named as 'The small pharmacy tree' or 'Chemist tree'. Baobab fruit pulp is low in protein and fat, but rich in pectins, calcium, minerals, vitamin B, and it contains seven to ten times higher content of vitamin C than oranges. It can be dissolved in water or milk and used as a drink and sauce for food or as a substitute for cream in baking. Recently, baobab has been referred to as a 'super fruit' because

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of its nutritional profile (Rahul et al., 2015).

Baobab fruit pulp has been approved by statutory bodies for use in certain nutritional products. In 2008 the European Commission authorized the dried fruit pulp of baobab as a novel food (Buchmann, Prehsler, Hartl, & Vogl, 2010). Baobab fruit pulp was also approved as a food ingredient in the United States of America in 2009 (FDA, 2009). The products derived from fruit pulp have been exported to European and USA markets and the demand for these products are increasing. In order to meet the demands of the new commercial markets, studies were undertaken to determine factors that are important to the cultivation of baobab. In some areas of Burkina Faso and India, the planting of baobab trees has been started (Kamatou et al., 2011; Sanchez, Osborne, & Haq, 2010).

It is evident that the iconic African tree, baobab, is an important nutritional and medicinal resource. In the past decade, it has attracted the interest of a lot of scientists and pharmaceutical companies. And numerous studies have been conducted on the biological activities of baobab. This paper will present a systematic structural characterization of the constituents in baobab fruit pulp. Data for hydroxycinnamic acid glycosides (HAGs), iridoid glycosides (IGs), and phenylethanoid glycosides (PGs) are presented. This information is of great significance for its nutritional and medicinal applications.

2. Materials and methods

2.1. Chemicals and materials

HPLC grade methanol, acetonitrile and formic acid were purchased from VWR International, Inc. (Clarksburg, MD). HPLC water was purchased from Sigma-Aldrich (St. Louis, MO).

The fruits of *A. digitata* were collected from Abagana, Anambra State, Nigeria in July 2013, and identified by Mr. Ozioko A. A voucher specimen (No INTERCEDD0613) has been deposited in Food Composition and Methods Development Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service.

2.2. Extraction process for isolation and analysis

The air-dried fruits of *A. digitata* (0.8 kg) were powdered and extracted with 70% (v/v) EtOH-H_2O for three times (1 h for each time) to give 75 g of crude extract, which was dissolved in 750 mL of H_2O to form a suspension and successively partitioned with ethyl acetate (750 mL × 3) and *n*-butanol (750 mL × 3).

The fruits were ground into powder and passed through a 60 mesh sieve. Five hundred milligram fruit powders were extracted with 5.00 mL of methanol-water (60:40, v/v) with sonication for 30 min at ambient temperature. The slurry mixture was centrifuged at 5000*g* for 15 min. The supernatant was filtered through a 17 mm (0.20 μm) PVDF syringe filter (VWR Scientific, Seattle, WA, USA) and stored at 4 °C before analysis. All analyses were done within 24 h of extraction. The injection volume for all samples was 1 μL.

2.3. NMR

^{1}H and ^{13}C NMR were recorded on a Bruker AVIII-600 MHz spectrometer (Bruker, Rheinstetten, Germany) and a Varian VNMRS-600 MHz spectrometer (Agilent Technologies, Santa Clara, CA) operating at 600 MHz for ^{1}H NMR and 150 MHz for ^{13}C NMR at ambient temperature in methanol-d_4. The chemical shifts (δ) are reported in ppm referenced to the residual solvent peak. The coupling constants (J) are quoted in hertz.

The conditions of UHPLC-DAD-HRMS^n are presented in the Supporting information.

The process of isolation is presented in the Supporting information.

^{1}H and ^{13}C data for hydroxycinnamic acid glycosides (HAGs), iridoid glycosides (IGs), and phenylethanoid glycosides (PGs) are presented in the Supporting information.

3. Results and discussion

3.1. Chemical constituents obtained from baobab

The current study isolated four hydroxycinnamic acid glycosides (HAGs): 1-*O*-(*E*)-feruloyl-*β*-D-glucose (**1**) (Jaiswal & Kuhnert, 2014; Jaiswal, Matei, Glembockyte, Patras, & Kuhnert, 2014), 1-*O*-(*E*)-caffeoyl-*β*-D-glucose (**2**) (Jaiswal & Kuhnert, 2014; Jaiswal et al., 2014), 6-*O*-(*E*)-caffeoyl-*β*-D-glucose (**3**) (Jaiswal & Kuhnert, 2014; Jaiswal et al., 2014), 6-*O*-(*E*)-caffeoyl-*α*-D-glucose (**4**) (Jaiswal & Kuhnert, 2014; Jaiswal et al., 2014), six iridoid glycosides (IGs): (−)-specioside (**5**) (Sha'aban, El-Naggar, & Doskotch, 1980), verminoside (**6**) (Sticher & Afifi-Yazar, 1979), 6-*O*-(*E*)-feruloylcatalpol (**7**) (Young, Kim, Park, Chung, & Choi, 1992), 6-*O*-*p*-coumaroylajugol (**8**) (Nishimura, Sasaki, Morota, Chin, & Mitsuhashi, 1989), 6-*O*-(*E*)-caffeoylajugol (**9**) (Harinantenaina Liva, Kasai, Rakotovao, & Yamasaki, 2001), 6-*O*-(*E*)-feruloylajugol (**10**) (Li et al., 2011), and three phenylethanoid glycosides (PGs): martynoside (**11**) (Li et al., 2011; Sasaki, Nishimura, Chin, & Mitsuhashi, 1989), acteoside (**12**) (Li et al., 2011; Sasaki et al., 1989), isoacteoside (**13**) (Kim, Kim, Jung, Ham, & Whang, 2009) from the baobab fruit pulp (Fig. 1 and Table 1). Their structures were established on the basis of spectroscopic data, particularly the 1D NMR and several 2D shift-correlated NMR pulse sequences (^{1}H-^{1}H COSY, HSQC, HMBC and ROESY). All of the compounds were obtained from genus of *Adansonia* for the first time.

3.2. Putative identification of HAGs, IGs, and PGs using UHPLC-DAD-HRMS^n

HAGs, IGs, and PGs were studied using an UHPLC-DAD-HRMS^n method (Figs. 2 & 3).

3.2.1. Identification of HAGs

Most HAGs in baobab fruit pulp are formed from hydroxycinnamic acid (*p*-coumaroyl, caffeoyl, feruloyl, etc.) and mono-, di-, and trisaccharides. These compounds are found as the primary phenolic compounds in many common plant derived foods (Lin, Harnly, Zhang, Fan, & Chen, 2012).

Compound **1**–**4** are the HAGs in baobab fruit pulp (Fig. 2). They were isolated and their identifications were confirmed by NMR spectroscopic data.

As shown in Table 1, compounds **2**, **3**, and **4** displayed $[M-H]^-$ ion at *m/z* 341 with the same elemental composition of $C_{15}H_{18}O_9$. They showed the similar fragment ions at *m/z* 281, 251, 221, 179 and 161 in MS/MS. The diagnostic ions at *m/z* 179 and 161 suggested the presence of the caffeoyl moiety. The loss of 162 mass unit indicated the existence of a hexose. Based on the MS^2 spectra and the information obtained from the literatures (Jaiswal & Kuhnert, 2014; Jaiswal et al., 2014), the fragment ions at *m/z* 281, 251 and 221 were obtained through the ring fission fragmentation of the hexose (Scheme 1). Compounds **3** and **4**, which are the *α/β* anomers of 6-caffeoylglucose, can be distinguished from **2** based on the base peak of **3** and **4** at *m/z* 281 and that of **2** at *m/z* 179. The intensities of specific fragment ions were the key evidence for the identification of the linkage position between the hydroxycinnamic acid and the saccharide in HAGs. In MS/MS of 1-*O*-(*E*)-feruloyl-*β*-D-glucose (**1**), fragment ions at *m/z* 295, 265, 235, 193 and 175 were the characteristic ions for the feruloyl group and the hexose. And the base peak at *m/z* 193 provided the evidence for the identification of 1-*O*-feruloyl-hexose.

3.2.2. Identification of IGs

Based on our phytochemical investigation, IGs were the predominant constituents in baobab and most of them were the derivatives of catalpol and ajugol, in which the cyclopentanoid unit of the