CHARACTERIZATION OF NARINGENIN FROM THE FRUIT PULP EXTRACT OF *Parkia biglobosa* (FABACEAE)

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Abstract: The cold macerated portion of the fruit pulp extract was concentrated and subjected to partitioned process using standard protocol. The n-BuOH soluble portion of the partition fraction of MeOH extract was chromatographed over silica gel and eluted with solvent of increasing polarity, i.e. Pet – ether– EtOAC $CHcl_3$ – MeOH and MeOH to afford 18 fractions (F_1–F_{18}). F_{12} (78 mg) was repeatedly subjected to gel filtration techniques to afford a pale yellow Compound B (29 mg) R_f 0.6 in (B.A.W/2:2:6). Compound B was elucidated using UV, FTIR, FABMS and combinations of 1D and 2D (^{1}H NMR, ^{13}C NMR, COSY, HMBC, HSQC, DEPT, and NOESY. Compound B (mp. 210-212) was determined as *Naringenin*- 7-4'-*di- O-β-D-glucopyranoside*.

Keywords: *Parkia biglobosa*, *Flavonoid glycosides*, *Naringeni*, Fabaceae, *Spectral* data

Introduction

Flavonoids are known to be associated with reduced risk for certain chronic diseases (Kris Etherton *et al*., 2004). These include the prevention of cardiovascular disorder (Yochum *et al*., 1999) and other kinds of cancerous processes (Nichenametla *et al*., 2006). Flavonoids exhibit antiviral properties (Ares *et al*., 2006), anti-microbial (Cushinie & Lamb, 2005), anti-inflammatory activities (Kim *et al*., 2004), anti-ulcer (Borelli & 1ZZO, 2000) and anti -allergic properties (Middleton & Kandaswani, 1992). Flavonoids are mostly found in fruits (Moutida & Marzouk, 2003), vegetables and cereals (Hollman & Arts, 2000). The role of flavonoids in biological system appears yet to be far from definitively determined, involving a large number of research groups all over the world. In fact, many new actions of flavonoids *in-vivo* have been put forward. The previously actions are never dismissed, only relegated to secondary ways of flavonoids action, usually considered to be important in pathological conditions (Gomes *et al*., 2008). Flavonoids can prevent injury caused by free radicals in various ways. Flavonoids are oxidized by free radicals resulting in more stable less reactive radicals. In order words, they stabilized the reactive oxygen species by reacting with the reactive compound of the radical. Flavonoid glycosides are those that usually contain one or more glycosides but molecules with more residues that have been identified in nature. By definition *digycosides* can have the residue attached at different positions (di-O-glycosides and di-C, O-glycosides) or at the same (O-*diglycosides and C, O-diglycosides* (Cushinie & Lamb, 2005; Watt & Breyer, 1963). *Parkia biglobasa* also known as the African locust beans or *dodongba* and *Dorowa* (Hausa, Nigeria) is a perennial deciduous tree of the family *fabaceae* . It is found in a wide range of environments in Africa and is primarily grown for its foods that contains both the sweet pulp and valuable seeds. Where the tree is grown, the crushing and fermenting of the seeds constitutes an important economic activity while the various parts of the plant are used for medical purposes (Watt & Breyer, 1963; Sofowora, 2008).

Experiment

General experimental procedure: All melting points (mp) were determined on Gallenkamp melting point apparatus and results are uncorrected. All solvents of analytical grade were purchase from BDH chemical Ltd. Poole England. TLC analysis was carried out using cellulose (TLC) and Silica gel 60 F_{254} (TLC) plates (Merck, Darmstadt, Germany). Polyamide (Roth, England) and Sephadex LH_{20} (Fluka, Switzerland) were used for open column chromatography (Cc). Chromatographic system: TLC : n-BuOH: HOAC: H_2O (2:2:6) Upper phase, HOAC: HCl Conc: H_2O (30:3:10 V/V/V), n -BuOH: Pyridine: H_2O (6:4:3 V/V/V) and EtOAC: HCOOH: H_2O (18:1:1-V/V/V) were used as solvent system for sugars. Visualization of the plate were performed using visible light U.V fluorescence and or spraying with the following reagent i=2% $AlCl_3$, ii= 10% H_2SO_4, iii = Aniline phthalate by heating at 110 ℃ for (5–10 min) for sugars identification. Column chromatography (Cc): Chloroform, Chloroform/Ethylacetate mixture, Ethylacetate, Ethylacetate/methanol and methanol 100% was used base on increasing gradient polarity system. Compounds (1 and 2) were treated with 10 mg and refluxed with 5% H_2SO_4 for 2 h. UV spectra were recorded on specord 40 UV –VIS spectrophotometer (Jena Analytik AG Germany) and FAB-MS was measured on a mass Autospec-ultima-TOF spectrometer. ^{1}H NMR and ^{13}C NMR experiments were performed on Bruker spectrometer 500 MHZ for ^{1}H and 125MH_Z for ^{13}C NMR. Spectra were referenced to the CD_3OD solvent, signals at δ3.30 (`H) and 49.00 (^{13}C) with TMS as an internal solvent standard. Chemical shift – values (δ) were reported in parts per million (ppm) in relation to the appropriate internal solvent standard (TMS). The coupling constants (J – values) were given in Hertz – TOF spectrometer.

Plant material

Fruit pulp of *P. biglobosa* (Fabaceae) was collected from a farmland in Basawa, a village outskirt of Zaria in Kaduna State of Nigeria in the month of October, 2017. Botanical identification of the plant was performed at the herbarium section of the Department of biological science, Ahmadu Bello University, Zaria, Nigeria and voucher V/No.7709 was obtained.

Extraction and isolation

Fruit pulp of *Parkia biglobosa* was obtained, cut and sliced into pieces. This was then air dried at room temperature for 4 days to afford 870 g after which the material was crushed into powder. The powdered material was then subjected to cold maceration at room temperature with MeOH for 48 h with intermittent agitation. The extract was removed under pressure; this was then partitioned with n – hexane, Chloroform, EtOAC and n- BuOH. The various partitioned portions were concentrated using rotary evaporator glass wares to afford n – hexane (3 g), chloroform (5 g), EtOAC (2.3 g) and n –Butanol (6.2 g), respectively. The n-BuOH – soluble portion was subjected to D101 resin column chromatography and eluted with H_2O 20%, MeOH, 50% MeOH and MeOH 100%. The 20% MeOH eluted portion was

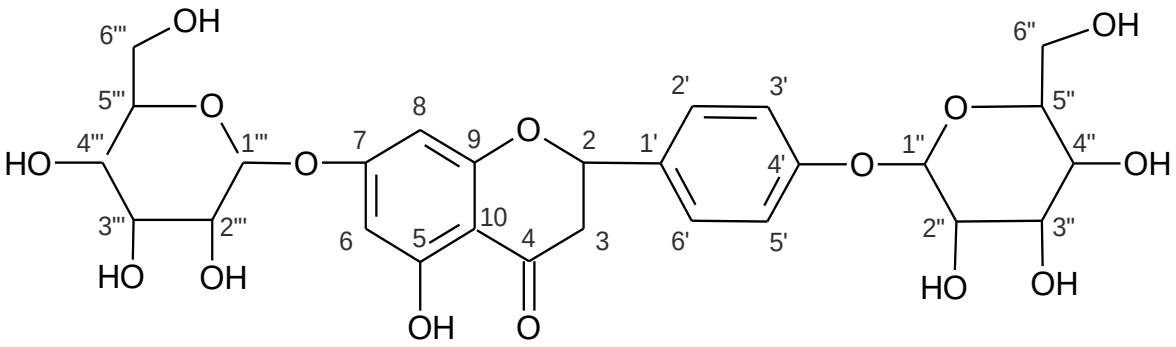
concentrated at room temperature. This was further resubmitted to silica gel column chromatography eluted with $CHCl_3$ –MeOH – H_2O in gradient manner to afford 25 fractions ($B_1 – B_{25}$). Similar fractions were pooled together on the basis of TLC analysis to afford 18 fractions ($F_1 – F_{18}$). Fractions (F_8= 150mg) was further re-subjected to repeated gel filtration techniques using sephadex LH-20 and RP – 18 column chromatography to obtain compound B (26 mg, R_f 0.6), respectively. The progress of elution was monitored by TLC analysis using precoated plate in different solvent system of EtOAC : MeOH: water (100:13:10), chloroform: MeOH: water (65:45:12) and n – BuOH: AcOH: water (2:2:6) upper layer. The chromatogram were spread with 10% H_2S04 and kept in an oven at temperature of 105 °C for 5 min after which it was removed to ascertain the compound on the plate.

Acid hydrolysis

Compound (B) 5 mg in a mixture of 8% HCl (2 mL) and CH_3OH (20 mL) was separately reflux for 2 h (Scheme 1). The reaction mixture was reduced *in vacuo* to dryness, dissolved in H_2O (3 mL) and neutralized with NaOH. The neutralized products was subjected to TLC analysis using eluent: (EtOAC: MeOH: H_2O: HOAC. 6:2:1:1). Paper chromatography was carried out on what man No 1 paper using solvent systems: (i) n – BuOH – HOAC – H_2O (2:2:6), (ii) HOAC – H_2O (3: 17) (iii) C_6H_6 - n - BuOH - H_2O – pyridine 1:5:3:3 (16).

Chemical test

The chromatogram was prayed with aniline hydrogen phthalate followed by heating in the oven at 105 °C for 5 min. The sugar was identified after comparison with authentic sugar samples (Andersen *et al.*, 2006).



Scheme 1: Compound B

Results and Discussion

Compound B was isolated as a yellow amorphous powder mp. 210 – 212℃. This was also obtained as a yellow powder on an acid hydrolysis, which also yield glucose sugar residues as identified by TLC and PC co–chromatography with the authentic samples. The FAB- MS of compound B was calculated at 596.1668 with formula as $C_{27}H_{32}O_{15}$ (Scheme 1 and Table 1) Compound B was reorganized as a glycosyl flavonoid from the positive test with Hcl Mg powder and the molish reagent (Miroslav *et al.*, 2010). The UV spectrum of compound B in MeOH showed an absorption maximum at 283 nm signifying compound B to a flavonoid (Hollman & Arts, 2000). An absence of bathochromic shift observed in band 11 with NaOAC (in MeOH) indicates no free hydroxyl group at position C – 7 (Richard, 1998; Emad *et al.*, 2013). The IR spectrum absorption showed V^{KBR} 3374 cm^{-1} (OH) 1642 cm^{-1} (C = 0), 1135, -1050 cm^{-1} (glycosidic linkage).

The ^1H NMR spectrum of compound B indicated the presence of a glycosidic moiety from the appearance of well separated two anomeric proton signal at δ_H 5.36 (1H, d, J = 6.9Hz) and δ_H 5.07(1H, d, J = 7.4Hz). Considering the coupling constants, the two sugar moieties are confirmed to be β – D glucopyranosyl (Brown, 2003). The composition of the sugar moieties were also determined by 2D NMR techniques. In the HMBC spectrum, the anomeric proton δ_H 5.36 ppm and δ_H 5.07 ppm were found to be correlated with the carbon at δ_c 167.0 (C – 7) and δ_H 158.6 ppm (C -4`), respectively (Table 1). Therefore, the sugar moieties were determined to be linked to the aglycone through C – 7 and C – 4' (Mohammed *et al.*, 2015). The chemical ionization mass spectrum (CIMS) portrays a molecular $[M+H]^+$ at m/z 596]. Fragments observed at m/z 434 could be attributed to loss of sugar $[M-162+H]^+$, while m/z 272 [M-2x162+H] $^+$ represents the loss of two sugar molecules, respectively (Brown, 2003; Samarya & Sarin, 2013).

Table 1: 'H NMR and ^{13}C NMR Spectrum data of compound B in CD_3OD (500.^{13}MHZ -`H and 125.77MHz for ^{13}C NMR). Multiplicity and coupling constant (J, Hz) δ ppm

Position	Dept	δ_C	δ_H	HMBC
2	C	157.50	-	H 2'
3	C	133.2	-	
4	C	177.4	-	
5	C	160.5	-	H – 6
6	CH	99.2	6.48 (d, 2.0)	H – 8
7	C	162.3	-	H – 6, H – 8
8	CH	93.1	6.70 (d, 2.0)	H – 6
9	C	156.5	6.70 (d, 2.0)	H – 8
10	C	105.2	-	H – 6, H – 8
1'	C	136.3	-	H – 2',5'
2'	CH	129.07	(d, 1.4)	H – 2', 5'
3'	C	144.5	-	H – 2', 5'
4'	C	148.6	-	H – 6'
5'	CH	116.2	6.88 (d, 7.6)	
6'	CH	129.3	7.4(dd 1.4, 7.4)	
1"	CH	101.1	5.36 (d, 6.9)	H"
2"	CH	74.02	3.30	
3"	CH	76.5	3.40	
4"	CH	69.6	3.14	
5"	CH	76.8	3.60	
6"	CH_2	60.5	3.84(m), 3.61(m).	
1'''	CH	99.8	5.07 (d,7.4)	
2'''	CH	73.5	4.1(m)	
3'''	CH	76.3	3.7(m)	
4'''	CH	69.7	3.4(m)	
5'''	CH	77.2	3.2(m)	
6'''	CH_2	60.6	3.75(m)	

The ^{1}H NMR spectrum of compound B displayed two anomeric proton signals at δ_H 5.36 (d, J=6.9$_{HZ}$) and δ_H 5.07 (d, J=7.4$_{HZ}$). In ^{13}CNMR spectrum, the inner glucose was shown to be linked to another terminal sugar through the (1 →2) bond linkage on the bases of glucosyl C- 2" down field shift of (+4 ppm) at δc 81.2 ppm, indicating that the bios is at β – glucosyl (1→2) – glucoside (Harborne, 1993; Harborne & Baxter, 1999).

In conclusion, on the basis of spectral analysis (FTIR, ABMS, ID and 2D NMR) and comparison with 1H NMR and ^{13}C NMR for reference data, compound B was determined as Naringenin 7-4'-di-O-β-D-glucopyranoside.

Conflict of Interest

Authors declare that there are no conflicts of interest.

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