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Chemical constituents of fruits of *Khaya senegalensis* A. Juss growing in Vietnam

Vu Thi Hien^{1,2}, Vu Dinh Hoang^{3*}

¹Department of Meteorology, Ho Chi Minh City University of Natural Resources and Environment

²Faculty of Chemistry, Vinh University

³School of Chemical Engineering, Hanoi University of Science and Technology

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Abstract

Four limonoids, seneganolide (**1**), khayanone (**2**), khayanolide B (**3**), 6-acetoxy-methyl angolensate (**4**), and two flavonoids, (-)-epicatechin (**5**) and quercitrin (**6**), were isolated from the ethyl acetate extract of fruits of *Khaya senegalensis* A. Juss. Their structures were elucidated by MS and NMR spectroscopic methods as well as comparison with literature data.

Keywords. *Khaya senegalensis*, seneganolide, khayanone, khayanolide B, 6-acetoxy-methyl angolensate, epicatechin, quercitrin.

1. INTRODUCTION

Khaya senegalensis widely distributed in Africa, Australia, Cuba, India, Indonesia, Puerto Rico, Singapore and Vietnam [1]. It is used as a fodder, timber, fuel, fiber and medicinal purposes. In traditional medicine, its bark and bark extract have a considerable reputation as a fever remedy, vermifuge, taenicide, depurative and for treating syphilis, jaundice, dermatoses, scorpion bite, allergies, gum infection, hookworm, bleeding wounds (disinfectant), and as a laxative. Its seeds and leaves are also used for treating fever, headache; its roots are used against sterility, for the treatment of mental illness, syphilis, leprosy and as an aphrodisiac [2, 3]. The previous reports have shown that *K. senegalensis* contains quinones, tannins, saponins, phenolics and especially, phragmalins limonoids having antifeedant properties [4, 5].

In this paper chemical study of ethyl acetate extract of fruits of *Khaya senegalensis* was described resulting in the identification of six compounds, seneganolide (**1**), khayanone (**2**), khayanolide B (**3**), 6-acetoxy-methyl angolensate (**4**), catechin (**5**), and quercitrin (**6**).

2. MATERIALS AND METHODS

2.1. Plant materials

The fruits of *Khaya senegalensis* A. Juss were collected in Nghean in January 2012. They were identified by Dr. Tran Huy Thai (Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology). Voucher specimen (number DHV 2014) is kept at the Faculty of Biology, Vinh University.

2.2. General experimental procedures

The melting points were determined on Boetius microscope. All NMR spectra were recorded on a Bruker AM500 FT-NMR spectrometer (500 MHz for ¹H and 125 MHz for ¹³C-NMR), chemical shifts are reported in ppm using TMS as an internal standard. The electrospray ionization mass spectra (ESI-MS) were determined using an Agilent 1200 LC-MSD Trap spectrometer. Column chromatography (CC) was performed on silica gel (Kieselgel 60, 70-230 mesh and 230-400 mesh, E. Merck). Thin layer chromatography (TLC) was conducted on precoated Kieselgel 60 F₂₅₄ plates (Merck) and the compounds were visualized by spraying with 10 % (v/v) H₂SO₄ followed by heating at 110 °C for 10 min.

2.3. Extraction and isolation

The dried powdered fruits of *Khaya senegalensis* A. Juss (4.0 g) were extracted three times with

methanol at room temperature. The methanol extract was evaporated under vacuum to dryness as a dark brown mass (285.0 g). The methanol residue (285.0 g) was suspended in water and partitioned with *n*-hexane and ethyl acetate to afford *n*-hexane (55.0 g) and ethyl acetate (95.0 g) extracts, respectively.

The ethyl acetate extract (95.0 g) was applied to silica gel column chromatography with a *n*-hexane:acetone stepwise gradient system (100:0; 50:1; 39:1; 30:1; 20:1; 15:1; 9:1; 4:1; 2:1; 1:1) to afford minor fractions which were combined into seven fractions. Fraction 3 was subjected to silica gel column chromatography (200 g, 60 × 3 cm) eluting with *n*-hexane:acetone (20:1; 15:1; 9:1; 2:1) to afford five subfractions. Sub-fraction 3.2 was purified with silica gel column chromatography (200 g, 60 × 5 cm) eluting with *n*-hexane:acetone (20:1) to yield compound **2** (23 mg). Sub-fraction 3.3 was purified with silica gel column chromatography (100 g, 60 × 2 cm) eluting with a *n*-hexane:acetone stepwise gradient system (9:1; 4:1) to yield compounds **1** (15 mg) and **3** (29 mg). Fraction 4 was subjected to silica gel column chromatography (200 g, 60 × 3 cm) eluting with chloroform:methanol (9:1) to afford compound **4** (12.5 mg). Fraction 5 was subjected to silica gel column chromatography (200 g, 60 × 3 cm) eluting with *n*-hexane:acetone (15:1; 9:1; 4:1; 2:1) to afford seven subfractions. Sub-fraction 5.2, eluting with chloroform:methanol (20:1), was further fractionated and purified with silica gel column chromatography (100 g, 60 × 3 cm) to afford compound **5** (53 mg). Sub-fraction 5.4, eluting with chloroform: methanol (15:1, 9:1), was further fractionated and purified with silica gel column chromatography (100 g, 60 × 3 cm) to afford compound **6** (71.5 mg).

Seneganolide (1): white powder, m.p. 276-277 °C; ESI-MS m/z 471 [M+H]⁺ (C₂₆H₃₁O₈); ¹H-NMR (CDCl₃&CD₃OD, 500 MHz), ¹³C-NMR (CDCl₃&CD₃OD, 125 MHz) δ (ppm), see table 1.

Khayanone (2): colorless prisms, m.p. 170-171 °C; ESI-MS m/z 503 [M+H]⁺ (C₂₇H₃₅O₉); ¹H-NMR (CDCl₃, 500 MHz), ¹³C-NMR (CDCl₃, 125 MHz) δ (ppm), see table 1.

Khayanolide B (3): colorless needles, m.p. 303-304 °C; ESI-MS m/z 519 [M+H]⁺ (C₂₇H₃₅O₁₀); ¹H-NMR (CDCl₃, 500 MHz), ¹³C-NMR (CDCl₃, 125 MHz) δ (ppm), see table 1.

6-acetoxy methyl angolensate (4): white powder, m.p. 208-209 °C; ESI-MS m/z 529 [M+H]⁺ (C₂₇H₃₇O₉); ¹H-NMR (acetone-*d*₆, 500 MHz) and ¹³C-NMR (acetone-*d*₆, 125 MHz) δ (ppm), see table 1.

(-)-Epicatechin (5): yellow needles, m.p. 177-178 °C, ESI-MS m/z 291 [M+H]⁺ (C₁₅H₁₅O₆); ¹H-NMR (DMSO-*d*₆, 500 MHz) δ (ppm): 6.89 (*s*, H-2'),

6.67 (1H, *d*, *J* = 8.0 Hz, H-6'), 6.65 (1H, *d*, *J* = 8.5 Hz, H-5'), 5.89 (1H, *d*, *J* = 2.0 Hz, H-8), 5.72 (1H, *d*, *J* = 2.0 Hz, H-6), 4.66 (1H, *d*, *J* = 4.5 Hz, H-2), 4.01 (1H, *m*, H-3), 2.68 (1H, *dd*, *J* = 16.5, 4.0 Hz, H-4a), 2.46 (1H, *dd*, *J* = 16.5, 3.0 Hz, H-4b); ¹³C-NMR (DMSO-*d*₆, 125 MHz), δ: 156.5 (C-7), 156.2 (C-5), 155.8 (C-9), 144.5 (C-3'), 144.4 (C-4'), 130.6 (C-1'), 118.0 (C-6'), 114.9 (C-5''), 114.8 (C-2''), 98.5 (C-10), 95.1 (C-6), 94.1 (C-8), 78.1 (C-2), 65.0 (C-3), 28.2 (C-4).

Quercitrin (6): bright yellow precipitate, m.p. 181-182 °C, ESI-MS m/z 449 [M+H]⁺ (C₂₁H₂₁O₁₁); ¹H-NMR (DMSO-*d*₆, 500 MHz), δ (ppm): 7.32 (1H, *dd*, 2.0, 8.0 Hz, H-6'), 7.35 (1H, *d*, 2.0 Hz, H-2'), 6.93 (1H, *d*, 8.0 Hz, H-5'), 6.21 (1H, *d*, 2.0 Hz, H-6), 6.37 (1H, *d*, 2.0 Hz, H-8), 5.37 (1H, *d*, 1.5 Hz, H-1''), 4.24 (1H, *d*, 8.0 Hz, H-5''), 3.77 (1H, *br d*, 3.5 Hz, H-2''), 3.44 (1H, *m*, H-3''), 3.36 (1H, *m*, H-4''), 0.97 (3H, *d*, 6.5 Hz, H-6''); ¹³C-NMR (DMSO-*d*₆, 125 MHz), δ (ppm): 179.5 (C-4), 165.7 (C-7), 163.1 (C-9), 159.2 (C-5), 158.4 (C-2), 149.7 (C-4'), 146.3 (C-3'), 136.2 (C-3), 122.9 (C-1'), 122.8 (C-6'), 116.9 (C-2'), 116.3 (C-5'), 105.8 (C-10), 103.5 (C-1''), 99.7 (C-6), 94.7 (C-8), 73.2 (C-4''), 72.1 (C-3''), 71.9 (C-2''), 71.8 (C-5''), 17.6 (C-6'').

3. RESULTS AND DISCUSSION

Compound **1** was obtained as white powder. It gave a molecular formula of C₂₆H₃₀O₈ as established by an ESI-MS ion at m/z 471 [M+H]⁺ (C₂₆H₃₁O₈). The ¹H and ¹³C NMR spectra (table 1) indicated that five double bonds: two carbon-carbon (as a furan ring) and three CO (two esters and one ketone). The NMR data also showed three methyls, six methylenes, eight methines (three olefinic) and nine quaternary carbons (one olefinic, one keto and two ester carbonyls). Moreover, the spectra showed the presence of a β-furyl moiety. All of the proton-bearing carbons were assigned by the HSQC spectrum. Further extensive studies of ¹H-¹H COSY and HMBC spectra suggested that **1** was a limonoid of mexicanolide skeleton. The presence of the hemiacetal linkage of C-1 to C-8 was confirmed from the chemical shifts of C-1 (δ 107.5) and C-8 (80.1). The same acetal linkage of C-1 to C-8 has been observed in many mexicanolides. Further, a methine proton at 2.84 (H-2) attached to a carbon at δ 53.0 nearby a carbonyl at δ 213.5 (C-3) indicated HMBC correlations with the ¹³C-NMR signals at δ 213.5, 107.5, 31.4, 44.7, 46.8 and 80.1 and led to their assignments as C-3, C-1, C-30, C-10, C-4 and C-8, respectively. A methine proton at δ 2.32 (H-5) showed HMBC correlations with ¹³C-NMR signals at δ 29.5 (C-6), 23.8 (C-28), 19.6 (C-29), 44.7 (C-4),

213.5 (C-3), 174.1 (C-7), 46.8 (C-10), 74.1 (C-19) and 61.2 (C-9). In addition, ^1H - ^1H COSY spectrum showed that a methine proton at δ 2.01 (H-9) was coupled to a methylene proton at δ 1.41 (H-11 β) which in turn coupled with two protons adjacent to a methylene proton at δ 1.41 (H-12 α) and 1.76 (H-12 β). The presence of only three methyls at 4 α (29), 4 β (30) and 13 α (18) and HMBC analysis indicated that the remaining methyl group (19) in the basic mexicanolide skeleton was changed to a methylene group directly attached to an oxycarbonyl group. These analyses and the comparison of spectral data of **1** with literature data [6] confirmed **1** to be seneganolide.

Compound **2** was isolated as colorless prisms, and its molecular formula was established as $\text{C}_{27}\text{H}_{34}\text{O}_9$ by ESI-MS spectrum with m/z 503 $[\text{M}+\text{H}]^+$, and NMR data. The NMR spectra of compound **2** could be compared with those of

compound **1**. The ^1H and ^{13}C NMR data of **2** showed that six of the elements of unsaturation were present as double bonds: two $\text{C}=\text{C}$ bonds (as a furan ring) and four $\text{C}=\text{O}$ bonds (as two ketones and two esters). A β -furyl moiety and one methyl ester group were also apparent from the spectra. These NMR data studies indicated **2** also to be a mexicanolide. Based on the comparison of NMR data of compound **2** with the literature data [7], we can conclude that **2** is khayanone.

Compound **3** was isolated as colorless needles, and its molecular formula was established as $\text{C}_{27}\text{H}_{35}\text{O}_{10}$ by ESI-MS with m/z 519 $[\text{M}+\text{H}]^+$, and its NMR data. The NMR spectra of compound **3** are mostly similar to those of compounds **1** and **2** except some differences, especially at C-1, C-19, C-3 (see table 1), suggesting **3** to have phragmalin structure. The comparison of NMR data of compound **3** with the literature data [8] confirmed **3** to be khayanolide B.

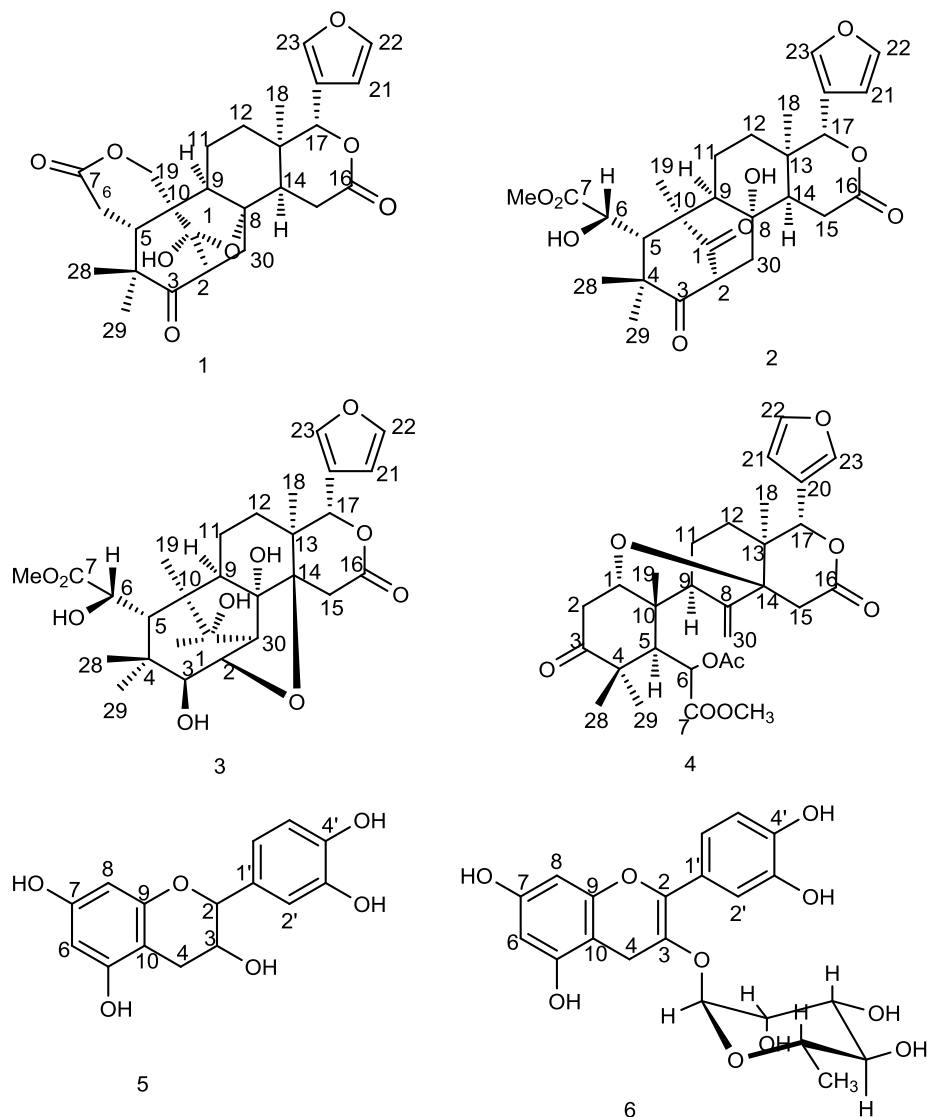


Figure 1: Chemical structures of compounds **1-6**

Compound **4** was obtained as a white powder. It gave a molecular formula of $C_{29}H_{36}O_9$ as established by an ESI-MS ion at m/z 529 $[M+H]^+$. It was also clear from its NMR data (Table 1) that **4** contained five methyl groups at δ_H 0.92 (3H, *s*); 1.16 (3H, *s*); 1.47 (3H, *s*); 1.06 (3H, *s*); 2.20 (3H, *s*), and a methyl ester at 3.77 (3H, *s*). The presence of a β -furyl moiety and one methyl ester group was also indicated from the spectra. The ^{13}C -NMR and DEPT of **4** showed signal of 29 carbons, including 6 methyl, 5 methylene, 8 methine groups and 10 carbons tertiary. Based on these data and the comparison with literature data [8], compound **4** is confirmed to be methyl 6-acetoxy angolensate.

Compound **5** was isolated as yellow needles. It had a pseudomolecular ion peak $[M+H]^+$ at m/z 291 in ESI-MS spectrum. The 1H -NMR spectra of **5** were very clear and understandable. The ^{13}C -NMR spectra of compound **5** showed the presence of 15 carbons, among them signals appeared at δ_C 28.2, 65.0, 78.1, 94.1, 114.8, 114.9, 118.0 were due to

C-4, C-3, C-2, C-8, C-2', C-5', C-6' carbons respectively, and other aromatic carbons showed peaks at δ_C 98.5, 130.6, 144.4, 144.5, 155.8, 156.2 and 156.5. These spectral data belongs to the group of flavan-3-ol. The comparison of spectral data of **5** with literature data [9] asserted **5** to be (-)-epicatechin.

Compound **6** was isolated as bright yellow precipitate, m.p. 181-182°C. The ESI-MS spectrum showed a pseudomolecular ion peak $[M+H]^+$ at m/z 449 ($C_{21}H_{21}O_{11}$). The 1H -NMR spectrum showed signals at δ_H 0.97 (3H, *d*, rhamnose-CH₃), 3.77-3.36 ppm multiplet (rhamnosyl Hs), 5.25 (1H, rhamnosyl H-1'), meta coupling related protons at δ_H 6.21 (1H, *d*, $J = 2.0$ Hz) and 6.37 (1H, *d*, $J = 2.0$ Hz) depicting substitution pattern of ring A. Ring B protons appeared at δ_H 6.93 (1H, *d*, $J = 8.0$ Hz), 7.38-7.35 (2H, *m*). The ^{13}C -NMR spectra data are in good agreement with the literature [10] and the compound was identified as quercetin-3-*O*- α -L-rhamnoside (quercitrin).

Table 1: 1H - and ^{13}C -NMR data of compounds 1-4

(1)	Compound 1		Compound 2		Compound 3		Compound 4	
	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
C	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)	δ_C	δ_H (J, Hz)
1	107.5		213.1		84.2		78.9	3.67 (<i>dd</i> , 5.5, 3.0)
2	53.0	2.84 (<i>dd</i> , 7.0, 13.5)	54.3	3.14 (<i>d</i> , 9.0)	72.2	4.50 (<i>dd</i> , 9.0, 7.0)	39.9	
3	213.5		214.2		78.5	3.40 (<i>d</i> , 7.0)	211.1	
4	44.7		50.2		42.6		49.4	
5	38.5	2.32 (<i>dd</i> , 10.5, 7.0)	46.0	2.78 (<i>m</i>)	40.7	3.06 (<i>d</i> , 7.0)	47.4	3.02 (1H, <i>s</i>)
6 α β	29.5	2.70 (<i>dd</i> , 11.0, 15.0) 2.56 (<i>dd</i> , 7.0, 15.0)	70.7	4.42 (<i>m</i>)	71.4	4.20 (<i>d</i> , 7.0)	73.0	
7	174.1		175.5		175.4		171.7	
8	80.1		72.9		86.9		146.9	
9	61.2	2.01 (<i>br d</i> , 11.5)	61.2	1.87 (<i>dd</i> , 13.0, 5.0)	56.0	2.09 (<i>d</i> , 8.0)	51.7	2.28 (<i>dd</i> , 14.0, 3.0)
10	46.8		50.2		59.3		45.2	
11 α B	20.8	1.61 (<i>m</i>) 1.41 (<i>m</i>)	22.6	1.19 (<i>m</i>) 1.82 (<i>m</i>)	16.4	1.86 (<i>m</i>) 1.77 (<i>m</i>)	24.8	
12 α β	34.9	1.41 (<i>br t</i> , 12.5) 1.76 (<i>dd</i> , 2.0, 11.0)	35.0	1.72 (<i>m</i>) 1.26 (<i>m</i>)	26.0	0.96 (<i>m</i>) 1.85 (<i>m</i>)	29.3	
13	35.3		35.4		37.6		41.9	
14	44.7	2.27 (<i>dd</i> , 2.0, 7.0)	51.1	1.75 (<i>dd</i> , 7.5, 2.0)	81.4		81.7	

(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
15 α β	27.5	2.78 (<i>dd</i> , 2.0, 19.5) 2.83 (<i>dd</i> , 7.0, 19.5)	27.1	2.82 (<i>dd</i> , 19.0, 2.0) 2.75 (<i>dd</i> , 19.0, 7.5)	32.0	3.16 (<i>d</i> , 19.0) 2.77 (<i>d</i> , 19.0)	34.3	
16	170.6		171.2		171.7		169.6	
17	78.1	5.28 (<i>s</i>)	76.8	5.60 (<i>s</i>)	81.2	5.64 (<i>s</i>)	79.9	
18	22.4	1.03 (<i>s</i>)	23.8	0.99 (<i>s</i>)	14.4	1.10 (<i>s</i>)	14.3	0.92 (<i>s</i>)
19 α β	74.1	4.45 (<i>d</i> , 11.5) 4.21 (<i>d</i> , 12.0)	25.5	1.36 (<i>s</i>)	17.6	1.20 (<i>s</i>)	23.0	1.16 (<i>s</i>)
20	120.7		120.9		120.6		122.3	
21	140.9	7.40 (<i>br s</i>)	141.1	7.45 (<i>br s</i>)	140.9	7.47 (<i>br s</i>)	141.7	7.58 (<i>m</i>)
22	109.6	6.33 (<i>t</i> , 1.0)	109.8	6.37 (<i>m</i>)	110.0	6.42 (<i>m</i>)	110.8	6.47 (<i>m</i>)
23	143.1	7.40 (<i>d</i> , 2)	143.1	7.43 (<i>t</i> , 2.0)	142.6	7.41 (<i>t</i> , 2.0)	143.9	7.55 (<i>m</i>)
28	23.8	1.19 (<i>s</i>)	23.8	1.27 (<i>s</i>)	19.2	1.07 (<i>q</i>)	25.2	1.47 (<i>s</i>)
29	19.6	1.32 (<i>s</i>)	26.7	1.28 (<i>s</i>)	44.6	1.89 (<i>d</i> , 11.5) 1.38 (<i>d</i> , 11.5)	24.4	1.06 (<i>s</i>)
30 α β	31.4	2.20 (<i>td</i> , 2.0, 13.5) 1.87 (<i>dd</i> , 7.0, 13.5)	39.0	2.36 (<i>ddd</i> , 15.0, 9.5, 2.0) 3.13 (<i>d</i> , 15.0)	63.2	2.60 (<i>d</i> , 9.5)	112.4	5.29 (<i>s</i>) 5.10 (<i>s</i>)
OMe			53.0	3.83 (<i>s</i>)	52.1	3.71 (<i>s</i>)	53.1	3.77 (3H, <i>s</i>)
OAc/ 2							170.4 20.9	2.20 (3H, <i>s</i>)
6-OH				2.89				
OH				2.78				

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Corresponding author: **Vu Dinh Hoang**

School of Chemical Engineering
Hanoi University of Science and Technology
No 1, Dai Co Viet, Hai Ba Trung, Hanoi
E-mail: hoang.vudinh@hust.edu.vn.