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A new flavanocoumarin glucoside from the roots of *Lannea kerstingii*

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ABSTRACT

An undescribed phyllocoumarin-3-*O*-*α*-*L*-rhamnopyranoside **8** together with catechin **1**, catechin-3-O-*α*-*L*-rhamnopyranoside **2**, catechin-[7,8-bc]-4*β*-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranon **3**, 5-*O*-*trans*-*p*-coumaroylquinic acid **4**, *trans*-*p*-coumaric acid **5**, catechin-[5,6-e]-4*β*-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranon **6**, catechin-[5,6-e]-4*α*-(3,4-dihydroxyphenyl)-dihydro-2(3H)- pyranon **7**, phyllocoumarin **9** and vaccinin A **10** were isolated from the roots of *Lannea kerstingii*. The structures of the compounds were established using NMR, IR and HR-ESI-MS spectroscopic analysis.

Keywords*:* *Lannea kerstingii*, Anacardiaceae, phyllocoumarin glucoside.

INTRODUCTION

The genus Lannea belongs to the family Anacardiaceae and consists of 77 genera with 600 speces (Eyana, 2007). Most of them are used to tread several ailments such as ulcers, enteritis, diarrhea, yellow fever, dysentery, wound healing, malaria, toothaches, blood pressure, rheumatism and diabetes (Sathish et al., 2010; Chegaing et al., 2020; Bharti et al., 2021; Islam et al., 2022). Pharmacological studies of some Lannea species have shown their anticancer, antibacterial and antioxidant activities (Sivaraj et al., 2018; Md. Hossain et al., 2018; Sabo et al., 2019; Mbaoji et al., 2020, Ombouma et al., 2022). The edible fruits of most of them are well known for their nutritional value in food or as a food supplement, and have enormous potential contribution in the economy and households (Atato et al., 2011; Sereme et al., 2014; Goudégnon et al., 2016; Diarra et al., 2016; Muhammad et al., 2018; Konaré et al., 2022).

The chemical investigation of Lannea genus has led to identification and isolation of various secondary metabolites, including: carbohydrates, alkylphenols, alkylhexanones, flavonoids, saponins steroid, coumarins, tannins (Muhaisen et al., 2013; Ibibia et al., 2016; Njinga et al., 2016; Md. Hossain et al., 2018; Kallo et al., 2018), sesquiterpenoids

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from essential oils (Bouaré et al., 2012; Ogundajo et al., 2021) and furane derivatives (Tameko et al., 2017).

Lannea kerstingii Engl et K. Krause (Anacardiaceae) is a deciduous tree that usually grows than 12 meters tall, and distributed throughout the Sudanese and Guinean Savannah (Arbonnier, 2009). *Lannea kerstingii* is used in traditional medicine to treat several pathologies such as anemia and liver diseases (Batawila et al., 2007; Allabi et al., 2011; Kpodar et al., 2016). In West Africa, *L. kerstingii* is used in traditional medicine to treat Buruli ulcer (Yemoa et al., 2008).

The use of herbal medicines in primary healthcare by African poeples, especially in Côte d'Ivoire, is tremendously increased. However, few data are available on the chemical composition of many of them. This work aims to the phytochemical investigation of *Lannea kerstingii* in order to contribute to its promotion and for its safety and efficacity uses in traditional medicine. Thus, in this study, we report isolation of new phyllocoumarin glucoside and nine known compounds from the roots of *Lannea kerstingii*.

MATERIALS AND METHODS

Plant material

The roots of *Lannea kerstingii* were collected in November 2018 at Flakièdougou (Bondoukou) in the Easten region of Côte d'Ivoire. The plant was identified at the floristic center of University Félix HOUPHOUËT- BOIGNY (Abidjan, Côte d'Ivoire), where a voucher specimen (UCJ 000967) was deposited.

General experimental procedure

NMR experiments were carried out in MeOH-d_4 on Bruker Avance DRX III 500 instruments. HR-ESI-MS experiments were performed using a Micromass Q-TOF micro instrument. Analytical TLC was performed on pre-coated silica-gel 60 F_{254} Merck and spots were observed under UV light at 254 and 365 nm or visualized by spraying the dried plates with 50% H_2SO_4, followed by heating. CC was carried out on Kieselgel 60 (63-200 mesh). Extracts were fractionned first on vaccumm liquid chromatography (VLC). HPLC was performed on a Dionex apparatus equipped with an ASI-100 autosampler, an Ultimate 3000 pump, a diode array detector UVD 340S and Chromeleon software. Interchim column (C_{18} -HQ, 5 µm, 250 x 10 mm) was used for semi-preparative HPLC or preparative HPLC (PLC) with binary gradient eluent (H_2O (filtred at 0.22 with TFA); CH_3CN) and a flow rate of 4 mL/min in semi-preparative HPLC and 20 mL/min in PLC; the chromatogram was monitored at 205, 210, 254 and 365 nm.

Extraction and isolation

Coarsely powdered root (1.5 Kg) of *L. kerstingii* was exhaustively extracted with 15 L of the mixture CH_3OH/H_2O (80/20). The hydromethanolic extract was dissolved in H_2O (500 mL) and the mixture serially extracted with cyclohexan and ethyl acetate (2×500 mL). The ethyl acetate fraction was dried under low temperature and pressure to obtain 16.0 g. This extract was separated on VLC C-18 (10 cm x 5 cm) eluting with EtOAc/MeOH/H_2O gradient to yield 5 fractions (AE-1 to AE-5).

Fraction AE-1 (3.59 g) was subjected to flash chromatography on normal silica with DCM/MeOH/H_2O (90/10/0; 70/30/5; 60/40/7) to give 12 fractions (F1 to F12). Fraction F4 (77.8 mg) was purified by preparative PLC with H_2O/ CH_3CN (15 to 25% of CH_3CN) as the mobile phase to afford compound **1** (3.20 mg).

Fractions 5 and 6 combinated (180.7 mg) was chromatographed on normal silica with Toluene/AcOEt/MeOH/H_2O (50/50/0/0; 50/30/20/0; 0/77/13/10) to give 8 sub-fractions. Sub-fractions 5 (23.34 mg) was purified on preparative PLC eluted with H_2O/CH_3CN (15 to 25% of CH_3CN) to afford compounds **2** (Rt 19.49 min, 10.0 mg) and **3** (Rt 19.51 min, 2.8 mg).

Fractions 11 (68.8 mg) was purified on semi-prep HPLC eluted with H_2O/CH_3CN gradient to give compounds **4** (Rt 19.19 min, 2.30 mg).

Fraction AE-3 (7.30 g) was subjected to flash chromatography on normal silica with DCM/MeOH/H_2O (90/10/0; 70/30/0; 70/30/5) to give 6 fractions.

Fraction 1 (544 mg) was purified using preparative PLC with H_2O/CH_3CN (15 to 30% of CH_3CN) as the mobile phase to afford compounds **5** (Rt = 19.50 min, 1.2 mg), **6** (Rt 19.59 min, 5.0 mg) and **7**, (Rt = 19.63 min, 5.0 mg). Fraction 3 (708.9 mg) was purified using preparative PLC with H_2O/CH_3CN (15 to 30% of CH_3CN) as the mobile phase to afford compounds **8** (Rt = 19.78 min, 1.4 mg)

Fraction AE-4 (100 mg) was purified on semi-preparative HPLC eluted with H_2O/CH_3CN (15 to 25% of CH_3CN) to yield compounds **9** (Rt 19.48 min, 1.2 mg) and **10** (Rt 19.52 min, 7.5 mg).

RESULTS AND DISCUSSION

Characterization of isolated molecules

The structures of the compounds were established using a combination of HR-ESI-MS and NMR spectroscopy, and comparing the spectral data with literature. In exception of compound **8**, all the other compounds including catechin **1**, catechin-3-O-*α-L*-rhamnopyranoside **2** (Kim et al., 2012), 5-*O-trans-p*-coumaroylquinic acid **4**, *trans-p*-coumaric acid **5** (Chen et al., 2008), catechin-[7,8-bc]-4*β*-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranon **3**, catechin-[5,6-e]-4*β*-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranon **6**, catechin-[5,6-e]-4*α*-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranon **7** (Hsue-Fen et al., 1993), phyllocoumarin **9** (Foo et al.,1989); and vaccinin A **10** (Matsuo et al., 2010) had been reported in the literature as referenced. The compounds isolated are mostly catechin as the basic skeleton. Catechins are known for their many biological properties on human health such as anti-oxidant, anti-microbial, anti-viral, anti-inflammatory, anti-allergenic, and anti-cancer activities (Bae et al., 2020).

Compound 8

Compound **8** was obtained as an amorphous powder. $[\alpha]_D^{20}$ was – 37.80 (c 0.737, MeOH).

The molecular formula was established as $C_{24}H_{24}O_{11}$ based on HR-ESI-MS and NMR data. The HR-ESI-MS in negative-ion mode showed a peak at *m/z* 487.1240 [M-H], (calcd. for $C_{24}H_{24}O_{11}$ [M-H], 487.1248), corresponding to the molecular formula $C_{24}H_{24}O_{11}$. The IR (ν^{KBr}, cm^{-1}: 1693), UV (λ_{max}^{MeOH} nm (log ε): 208, 289 and 332) and NMR spectra of compound **8** indicated the presence of a coumarin nucleus (Foo et al.,1989). The structure of compound **8** was deduced from that of the known flavanocoumarin (compound **9**) (Foo et al.,1989). The ^{1}H-NMR spectrum (Table 1) showed the presence of catechin and coumarin moieties. The 1, 2, 4- trisubstituted benzene ring of three aromatic signals typical of ABX spin system, obviously included the double doublet at δ_H 6.75 (*J* = 8.2 and 1.9 Hz, H-6'), coupling in *ortho* with a doublet at δ_H 6.80 (*J* = 8.0 Hz, H-5') and in *meta* with another doublet at δ_H 6.85 (*J* = 1.9 Hz, H-2'). The pyran ring (C) was caracterised by the presence of multiplet at 4.12 ppm (H-3), which coupled with a doublet at 4.99 ppm (J = 7.0 Hz ; H-2) and two double doublets at 2.92 (*J* = 16.5 and 5.3 Hz ; H-4b) and 2.85 ppm (*J* = 16.5 and 7.5 Hz ; H-4a). The presence of only one single signal at 6.39 ppm (H-6) showed that the ring A was pentasubtituated. A pair of double doublets at δ_H 8.09 (*J* = 9.6 Hz ; H-9) and δ_H 6.11 (*J* = 9.5 Hz ; H-10) was attributed to pyrone ring protons of coumarin moiety. The ^{1}H-NMR spectrum also exhibited a doublet at δ_H 4.47 (J = 1.2 Hz ; H-1"), attributed to an anomeric proton of *α-L*-rhamnopyranose (Table 1) by COSY and NOESY spectrum analysis. The link between the sugar and the pyran ring was showed from HMBC correlation through the cross-peak between H-1" (4.47 ppm) and C-3 (75.7 ppm) (Figure 1). Furthermore, the downfield shift of C-3 at δ_C 75.7 ppm of compound **8** compared to that of compound **9** (C-3 at 67. 5 ppm) (Table 1) confirmed the attachment of sugar at C-3 in compound **8**. Accordingly, compound 8 was assigned as phyllocoumarin-3-*O-α-L*-rhamnopyranoside. To the best of our knowledge and according to the available literature data, compound **8** (Figure 1) is a new compound isolated for the first time from *Lannea kerstingii*.

Catechin (1): ^{1}H-NMR (500 MHz, CD_3OD) δ_H: 4.59 (d; J = 7.59Hz, H-2); 3.99 (m; H-3); 2.89 (dd; J =16.16, 5.53; H-4b); 2.55 (dd; J =16.15, 8.20; H-4a); 5.95 (d; J = 2.32 H-6); 5.88 (d ; J = 2.32 H-8); 6.85 (d; J = 1.89 H-2'); 6.78 (d; J = 8.13; H-5'); 6.73 (dd; J = 8.13; 2.03; H-6').
^{13}C-NMR (125 MHz, CD_3OD) δ_c: 80.1 (C-2); 68.6 (C-3); 30.0 (C-4); 158.6 (C-5); 94.6 (C-6); 158.4 (C-7); 92.8 (C-8); 157.9 (C-9); 101.2 (C-10); 133.4 (C-1'); 116.2 (C-2'); 147.5 (C-3'); 147.3 (C-4'); 117.5 (C-5'); 121.2 (C-6').

Catechin-3-O-*α*-*L*-rhamnopyranoside (2) : ^{1}H-NMR (500 MHz, CD_3OD) δ_H : 4. 60 (d; J = 7.68; H-2); 3.99 (m; H-3); 2.89 (dd; J =16.66, 5.61 H-4b); 2.55 (dd; J =16.02, 8.4; H-4a); 5.92 (s; H-6); 5.85 (s; H-8); 6.83 (d; J =1.9 H-2'); 6.78 (d; J = 8.13; H-5'); 6.71 (dd; J = 8.1, 1.88; H-6'); 4.28 (d; J =1.06; H-1''); 3.46 (dd; J = 9.9, 1.48; H-2''); 3.56 (dd; J = 9.9, 3.40 H-3''); 3.30 (t; 9.50; H-4''); 3.96 (m; H-5''); 1.25 (d; J = 6.38; H-6'').
^{13}C-NMR (125 MHz, CD_3OD) δ_c : 81.1(C-2); 75.9 (C-3); 28.0 (C-4); 156.8 (C-5); 96.0 (C-6); 157.5 (C-7), 95.1 (C-8); 157.5 (C-9); 100.6 (C-10); 133.4 (C-1'); 116.2 (C-2'); 146.3 (C-3'); 146.2 (C-4'); 116.1 (C-5'); 120.0 (C-6'); 102.1 (C-1''); 71.9 (C-2''); 72.2 (C-3''); 73.9 (C-4''); 70.3 (C-5''); 17.0 (C-6'').

Catechin-[7,8-bc]-4*β*-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranon (3) : ^{1}H-NMR (500 MHz, CD_3OD); δ_H : 4.68 (d; J = 8,28 Hz, H-2); 3.96 (m; H-3); 2,60 (dd; J = 16.40, 7.20; H-4a); 2.80 (dd; J = 16.73, 6.56; H-4b); 6.20 (s; H-6); 6.64 (d; J = 2.43; H-2'); 6.58 (d; J = 8.36; H-5'); 6.38 (dd; 8.36, 2.16; H-6'); 6.52 (d; J = 2.43; H-2''); 6.66 (d; J = 7.82; H-5''); 6.44 (dd; J = 8.09, 2.43; H-6''); 4.40 (dd; J = 7.25, 1.48; H-7''); 2.80 (dd; J = 16.73, 5.58; H-8''a); 3.09 (dd; J = 15.74, 8.20; H-8''b). ^{13}C-NMR (125 MHz, CD_3OD) δ_c : 82.85 (C-2); 68.24 (C-3); 28.15 (C-4); 156.8 (C-5); 96.2 (C-6); 152.7 (C-7); 105.6 (C-8); 152.9 (C-9); 105.9 (C-10); 131.6 (C-1'); 114.8 (C-2'); 146.3 (C-3'); 146.0 (C-4'); 116.0 (C-5'); 119.5 (C-6'); 135.4 (C-1''); 115.1 (C-2''); 146.0 (C-3''); 145.5 (C-4''); 115.5 (C-5''); 119.5 (C-6''); 38.6 (C-7''); 35.4 (C-8''); 170.6 (C-9'').

5-*O*-*trans*-*p*-coumaroylquinic acid (4) : ^{1}H-NMR (500 MHz, CD_3OD), δ_H : 7.46 (d; J = 7.78 Hz; H-2, H-6); 6.81 (d; J = 8.18; H-3, H-5); 7.63 (d; J = 16.52; H-7); 6.33 (d; J = 16.36; H-8) 2.19 (m, H-2'); 4.16 (m, H-3'); 3.74 (dd; J = 8.73, 3.66; H-4'); 5.33 (m, H-5'); 2.04 (m, H-6'); ^{13}C-NMR (125 MHz, CD_3OD) δ_c : 127.2 (C-1); 131.2 (C-2, C-6); 116.8(C-3, C-5); 161.2 (C-4); 146.6 (C-7); 115.4 (C-8); 168.6 (C-9); 115.7 (C-1'); 38.3 (C-2', C-6'); 71.4 (C-3'); 73.8 (C-4'); 72.0 (C-5'); 177.4 (C-7').

***Trans*-*p*-coumaric acid (5)** ^{1}H-NMR (500 MHz, CD_3OD) δ_H : 7.45 (d; J = 8.52; H-2, H-6); 6.80 (d; J = 8.92; H-3, H-5); 7.58 (d; J =15.91; H-7); 6.73 (d; J =15.52; H-8).

Catechin-[5,6-e]-4*β*-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranon (6) : ^{1}H-NMR (500 MHz, CD_3OD) δ_H : 4.71 (d; J = 6.97; H-2); 4.09 (m; H-3); 2.63 (dd; J = 16.45, 7.71; H-4a); 2.95 (dd; J = 16.42, 5.20; H-4b); 6.22 (s; H-8); 6.84 (d; J = 1.97; H-2'); 6.78 (d; J = 3.78; H-5'); 6.73 (dd; J = 5.26; 1.97; H-6'); 6.54 (d; J = 2.12; H-2''); 6.67 (d; J = 8.31; H-5''); 6.45 (dd; J = 8.15, 2.09; H-6''); 4.43 (s; H-7''); 3.02 (m; H-8''). ^{13}C-NMR (125 MHz, CD_3OD) δ_c : 82.8 (C-2); 68.0 (C-3); 27.6 (C-4); 151.8 (C-5); 107.2 (C-6); 154.7 (C-7); 99.7 (C-8); 157.7 (C-9); 101.4 (C-10); 131.8 (C-1'); 115.0 (C-2'); 146.3 (C-3'); 146.3 (C-4'); 116.1(C-5'); 119.7(C-6'); 134.8 (C-1''); 115.1(C-2''); 145.1(C-3''); 145.1(C-4''); 116.4(C-5''); 119.2 (C-6''); 27.7(C-7''); 38.4(C-8''); 170.3(C-9'').

Catechin-[5,6-e]-4*α*-(3,4-dihydroxyphenyl)-dihydro-2(3H)- pyranon (7) : ^{1}H-NMR (500 MHz, CD_3OD) δ_H : 4.71 (d; J = 5.49; H-2); 4.07 (m; H-3); 2.70 (dd; J = 16.43, 7.67; H-4a); 2.88 (dd; J = 16.41, 5.62; H-4b); 6.23 (s; H-8); 6.83 (d; J = 1.95; H-2'); 6.76 (d; J = 3.47; H-5'); 6.71 (dd; J = 5.27, 1.95; H-6'); 6.56 (d; J = 2.12; H-2''); 6.66 (d; J = 8.15; H-5''); 6.45 (dd; J = 8.15, 2.09; H-6''); 4.42 (s; H-7''); 3.02 (m; H-8''). ^{13}C-NMR (125 MHz, CD_3OD) δ_c : 82.7 (C-2); 68.0 (C-3); 27.7 (C-4); 151.8 (C-5); 107.2 (C-6); 154.7 (C-7); 99.7 (C-8); 157.7 (C-9); 101.5 (C-10); 131.8 (C-1'); 115.0 (C-2'); 146.3 (C-3'); 146.3 (C-4'); 116.2 (C-5'); 119.7 (C-6'); 134.9 (C-1''); 115.1 (C-2''); 145.1 (C-3''); 145.1 (C-4''); 116.5 (C-5''); 119.2 (C-6''); 27.6 (C-7''); 38.3 (C-8''); 170.3 (C-9'').

Vaccinin A (10) : ^{1}H-NMR (500 MHz, CD_3OD) δ_H : 4.84 (d; J = 6.7; H-2); 4.18 (m; H-3); 2.80 (dd; J = 16.37, 7.59; H-4a); 3.03 (dd; J = 16.17, 4.99; H-4b); 6.61 (s; H-8); 6.85 (d; J = 1.46; H-2'); 7.79 (d; J = 7.94; H-5'); 6.75 (dd; J = 7.94, 1.85; H-6'); 6.69 (s; H-3"); 7.19 (s; H-6"); 6.00 (s; H-8"). ^{13}C-NMR (125 MHz, CD_3OD) δ_c : 84.4 (C-2); 68.2 (C-3); 28.0 (C-4); 154.0 (C-5); 102.5 (C-6); 151.6 (C-7); 99.7 (C-8); 160.7 (C-9); 105.6 (C-10); 132.0 (C-1'); 115.8 (C-2'); 147.3 (C-3'); 147.4 (C-4'); 117.1 (C-5'); 120.6 (C-6'); 149.9 (C-1"); 154.9 (C-2"); 105.2 (C-3"); 145.8 (C-4"); 145.4 (C-5"); 110.0 (C-6"); 108.7 (C-7"); 93.2 (C-8"); 166.1 (C-9").

Table 1: ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data of 8 and 9 in CD_3OD.

	8		**9**	
Position	**δ_H m (*J* in Hz)**	**δ_c**	**δ_H m (*J* in Hz)**	**δ_c**
2	4.99 d (7.0)	82.5	4.99 d (7.0)	82.5
3	4.12 m	75.7	4.15 m	67.5
4a	2.85 dd (16.5 ; 7.5)	27.8	2.85 dd (16.5 ; 7.5)	25.4
4b	2.92 dd (16.5 ; 5.3)	27.8	2.92 dd (16.5 ; 5.3)	25.4
5	-	161.6	-	161.6
6	6.39 s	94.1	6.39 s	94.1
7	-	157.2	-	157.2
8	-	105.8	-	105.8
9	8.09 d (9.6)	139.5	8.09 d (9.6)	139.5
10	6.11d (9.5)	108.8	6.11d (9.5)	108.8
11	-	164.7	-	164.7
12	-	-	-	-
13	-	153.1	-	151.1
14	-	102.9	-	102.9
1'	-	129.5	-	129.5
2'	6.85 d (1.9)	113.2	6.85 d (1.9)	113.2
3'	-	145.2	-	145.2
4'	-	145.0	-	145.0
5'	6.80 d (8.0)	114.8	6.80 d (8.0)	114.8
6'	6.75 dd (8.2 ; 1.9)	118.0	6.75 dd (8.2 ; 1.9)	118.0
	***α-L*-rhamnopyranose**			
1"	4.47 d (1.2)	103.8	-	-
2"	3.59 m	73.1	-	-

3"	3.57 dd (9.4 ; 3.3)	74.7	-	-
4"	3.35 t (9.3)	72.4	-	-
5"	3.64 dd (9.5 ; 6.2)	69.0	-	-
6"	1.12 d (6.38)	16.5	-	-

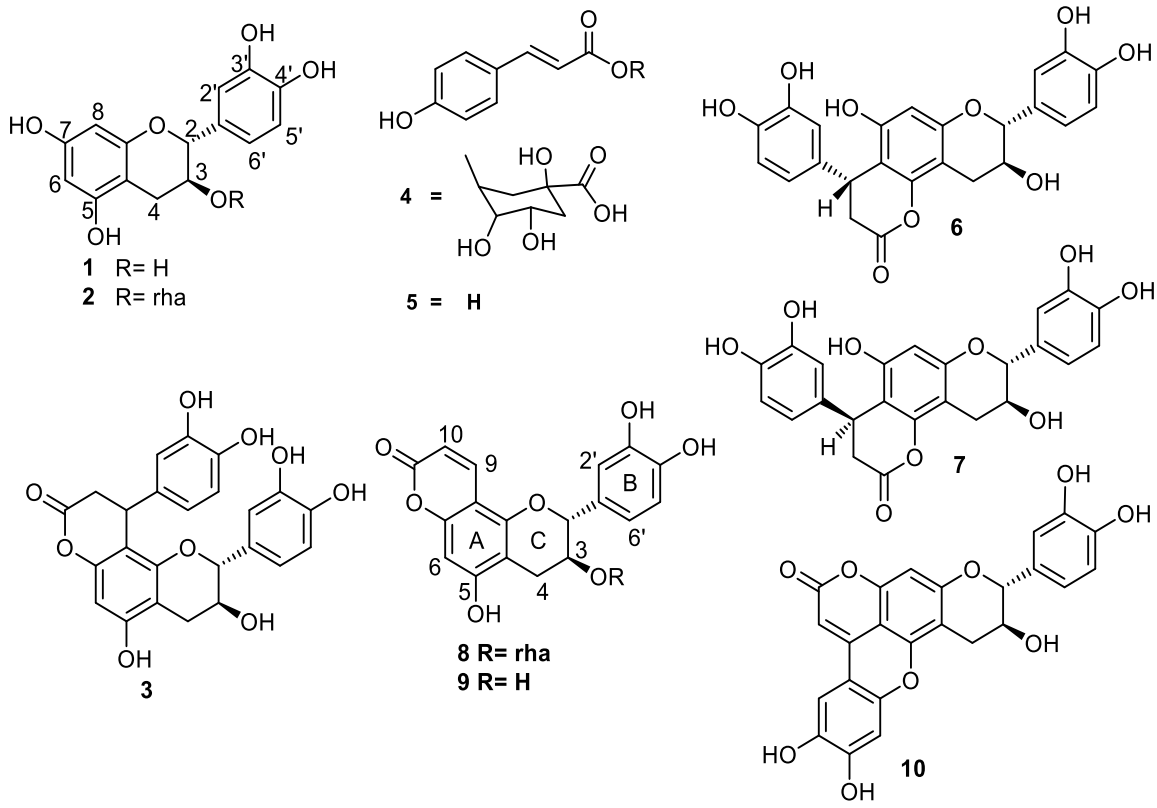

Figure 1: Isolated compounds from the roots of *L. kerstingii*.

Conclusion

This study is part of the promotion of medicinal plants of Côte d'Ivoire. Few details studies were traced on the chemical composition of *L. kerstingii*. A study of the roots of this plant has been investigated. Ten compounds including a new phyllocoumarin-3-*O*-*α*-*L*-rhamnopyranoside were isolated. Eight of the isolated compounds were derivated from catechin. For our knowledge, these molecules are isolated for the first time from the roots of *L kerstingii* and their presence could explain the different biological activities of this plant.

COMPETING INTERESTS

The authors declare that there is no competing interest related to this manuscript.

AUTHORS' CONTRIBUTIONS

PAY-K directed the project and wrote the article. AAM, LV-N, J-MKK and EZLA conducted all experiments and analyzed the RMN data. J-MKK collected the plant material, isolated compounds. All authors

discussed the results and commented on the manuscript.

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