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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.

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

INTRODUCTION

The genus *Lannea* belongs to the family Anacardiaceae and consists of 77 genera with 600 species (Eyana, 2007). Most of them are used to treat 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

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 peoples, 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 efficacy 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 Flakiédougou (Bondoukou) in the Eastern 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 F₂₅₄ Merck and spots were observed under UV light at 254 and 365 nm or visualized by spraying the dried plates with 50% H₂SO₄, 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 (C₁₈-HQ, 5 μm, 250 x 10 mm) was used for semi-preparative HPLC or preparative HPLC (PLC) with binary gradient eluent (H₂O (filtered at 0.22 with TFA); CH₃CN) 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 CH₃OH/H₂O (80/20). The hydromethanolic extract was dissolved in H₂O (500 mL) and the mixture serially extracted with cyclohexan and ethyl acetate (2x500 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/H₂O 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/H₂O (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 H₂O/ CH₃CN (15 to 25% of CH₃CN) as the mobile phase to afford compound **1** (3.20 mg).

Fractions 5 and 6 combined (180.7 mg) was chromatographed on normal silica with Toluene/AcOEt/MeOH/H₂O (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 H₂O/CH₃CN (15 to 25% of CH₃CN) 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 H₂O/CH₃CN 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/H₂O (90/10/0; 70/30/0; 70/30/5) to give 6 fractions.

Fraction 1 (544 mg) was purified using preparative PLC with H₂O/CH₃CN (15 to 30% of CH₃CN) 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 H₂O/CH₃CN (15 to 30% of CH₃CN) 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 H₂O/CH₃CN (15 to 25% of CH₃CN) 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 C₂₄H₂₄O₁₁ 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 C₂₄H₂₄O₁₁ [M-H], 487.1248), corresponding to the molecular formula C₂₄H₂₄O₁₁. 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 ¹H-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 characterised 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 pentasubstituted. 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 ¹H-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): ¹H-NMR (500 MHz, CD₃OD) δ_H: 4.59 (d; J = 7.59 Hz, 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 Hz, H-6); 5.88 (d; J = 2.32 Hz, H-8); 6.85 (d; J = 1.89 Hz, H-2'); 6.78 (d; J = 8.13; H-5'); 6.73 (dd; J = 8.13; 2.03; H-6').

¹³C-NMR (125 MHz, CD₃OD) 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): ¹H-NMR (500 MHz, CD₃OD) δ_H: 4.60 (d; J = 7.68; H-2); 3.99 (m; H-3); 2.89 (dd; J = 16.66, 5.61 Hz, 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 Hz, 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 Hz, H-3'"); 3.30 (t; 9.50; H-4'"); 3.96 (m; H-5'"); 1.25 (d; J = 6.38; H-6'"). ¹³C-NMR (125 MHz, CD₃OD) 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]-4β-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranon (3): ¹H-NMR (500 MHz, CD₃OD); 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). ¹³C-NMR (125 MHz, CD₃OD) 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): ¹H-NMR (500 MHz, CD₃OD), 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'); ¹³C-NMR (125 MHz, CD₃OD) 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) ¹H-NMR (500 MHz, CD₃OD) 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]-4β-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranon (6): ¹H-NMR (500 MHz, CD₃OD) 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'"). ¹³C-NMR (125 MHz, CD₃OD) 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]-4α-(3,4-dihydroxyphenyl)-dihydro-2(3H)-pyranon (7): ¹H-NMR (500 MHz, CD₃OD) 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'"). ¹³C-NMR (125 MHz, CD₃OD) 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) : ¹H-NMR (500 MHz, CD₃OD) 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''). ¹³C-NMR (125 MHz, CD₃OD) 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: ¹H (500 MHz) and ¹³C (125 MHz) NMR data of 8 and 9 in CD₃OD.

Position	8		9	
	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	-	-

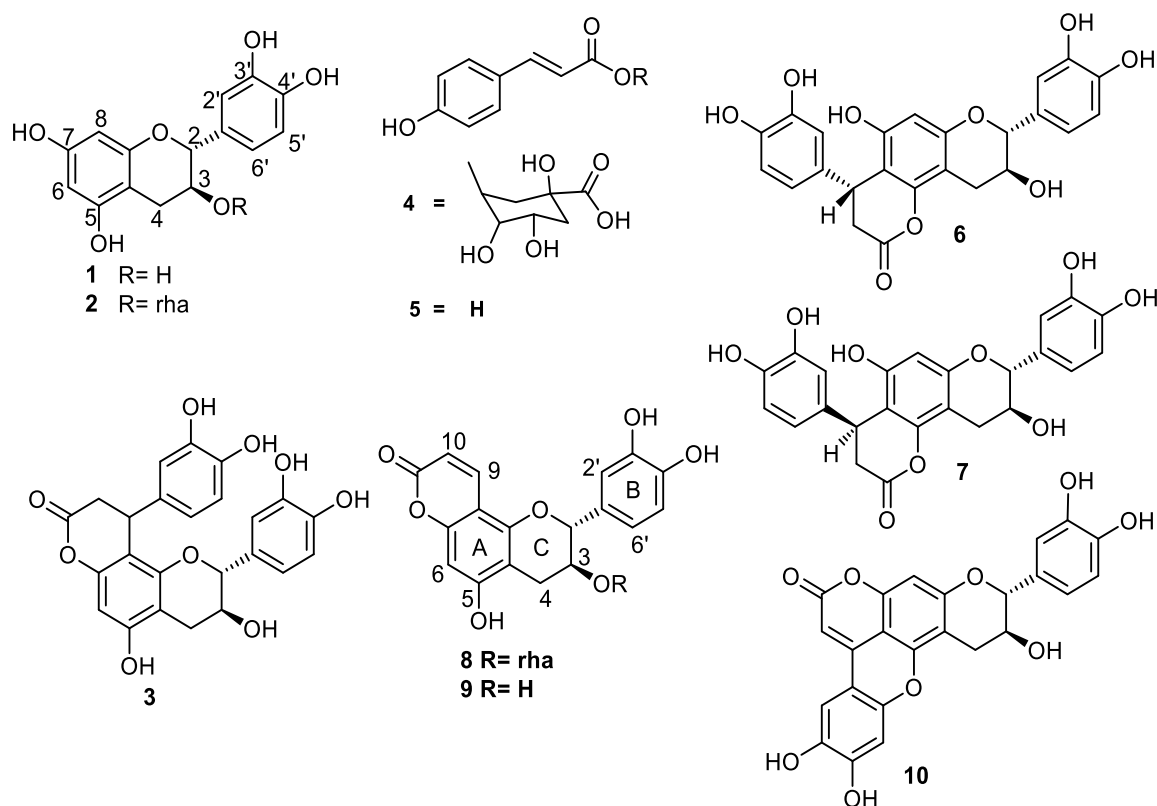


Figure 1: Isolated compounds from the roots of *L. kerstingii*.

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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