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New C-13 Norisoprenoids and Flavonoids from *Lannea kerstingii*

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Abstract

Lannea kerstingii is known for its multiple therapeutic and biological activities. Despite of many traditional uses of this plant, scientific research on the content of its chemical compounds is still limited. This study aims to isolate the chemical compounds contained in the n-butanol fraction of *Lannea kerstingii* leaves. The chemical investigation of the leaves of *Lannea kerstingii* led to isolation of three undescribed C-13 norisoprenoids, lankerstinol **A-C** (**1-3**), together with six (**4-9**) known flavonoid glycosides. The structures of these compounds were established by spectroscopic analyses.

Keywords

Lannea kerstingii, Anacardiaceae, Flavonoids, C-13 Norisoprenoids, Structure Elucidation

1. Introduction

Plant natural products have an important biomedical source playing a major role in drug discovery. The Anacardiaceae family, especially Lannea species are the site of multiple sources of secondary metabolites such as terpenes, polyphenol [1] [2] [3] [4] alkenyl phenols and alkenyl cyclohexenones [5], steroid [6] [4] and ceramide [7]. Lannea species have numerous pharmacological properties such as antibacterial [4] [5] [8], antoxidant [7] [8] [9], anticancer [10] [11] [12], antihypertensive [13].

Lannea kerstingii Engl et K. Krause (Anacardiaceae) is a deciduous tree distri-

buted throughout the Sudanese and Guinean savannahs [14]. Its leaves and roots have been employed in folk medicine to treat anemia, malaria, liver diseases [15] [16], and Buruli ulcer [17]. According to prior investigations, *Lannea kerstingii* possesses various pharmacological properties such as anticancer, hepatoprotective [10] [18], antioxidant, and antimicrobial [19] [20]. The major classes of molecule reported from *Lannea kerstingii* included furan derivatives [21], flavonoid and sterol [4] [6].

Despite the multiple uses of *L. kerstingii*, very few information about the chemical study of the leaves of *L. kerstingii* is available, so it is necessary to isolate and identify the chemical compounds contained in its leaves to further promote its medicinal uses. Thus, the present work aims to investigate the phytochemical study of *L. kerstingii*.

Nine (**1-9**) naturally-occurring compounds including three undescribed (**1-3**) were characterized from n-butanol fraction of *L. kerstingii* leaves.

2. Experimental

2.1. General Experimental Procedures

Nuclear magnetic resonance (NMR) experiments were carried out in MeOH-d_4 on Bruker Avance DRX III 500 instruments (Karlsruhe, Germany). HR-ESI-MS experiments were performed using a Micromass Q-TOF micro instrument (Manchester, UK). Analytical TLC was performed on pre-coated silica-gel plates Merck Kieselgel 60 F254 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. Column chromatography (CC) was carried out on silica-gel Kieselgel 60 (63 - 200 mesh). Extracts were fractionned first on vaccumm liquid chromatography (VLC). Analytical and semi-preparative HPLC were performed on a Dionex apparatus equipped with an ASI-100 autosampler, an Ultimate 3000 pump, a diode array detector UVD 340S and Chromeleon software. A prepacked RP-C_{18} column (Phenomenex 250 × 10 mm, Luna 5 µ, Interchim, France) was used for semi-preparative HPLC with binary gradient eluent (H_2O (filtred at 0.22 with TFA 99%, Sigma Aldrich); CH_3CN) and a flow rate of 4 mL/min; the chromatogram was monitored at 205, 210, 254 and 365 nm.

2.2. Plant Material

The leaves 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 Université Félix HOUPHOUËT-BOIGNY (Abidjan, Côte d'Ivoire), where a voucher specimen (UCJ 000967) was deposited.

2.3. Extraction and Isolation

The dried powdered leaves of *L. kerstingii* (0.9 Kg) were macerated of *L. kerstingii* with 9 L of the mixture CH_3OH/H_2O (80/20). The hydromethanolic solution was concentrated to obtain a crude extract (10.4 g), which was dissolved in water

(200 mL) and was sequentially partitioned with dichloromethane (DCM) (3 × 200 mL), ethyl acetate (EtOAc) (3 × 200 mL) and n-butanol (n-BuOH) (3 × 200 mL). The fractions were dried under low temperature and pressure to obtain 1.49 g of DCM, 1.17 g of EtOAc and 3.50 g of n-BuOH fractions.

The n-BuOH fraction (3.50 g) was subjected to flash chromatography on C_{18} silica with H_2O/CH_3CN (15/85; 25/75) to generate ten fractions (F1-F10).

Fraction F2 (256.8 mg) was purified using semi-preparative HPLC with H_2O/acétonitrile (CH_3CN) (15% to 25% of CH_3CN) as the mobile phase to afford compound **2** (Rt 20.09 min; 1.0 mg) and **3** (Rt 20.33 min; 2.2 mg).

Fraction F3 (120.3 mg) was purified using semi-preparative HPLC with H_2O/CH_3-CN (15% to 20% of CH_3CN) as the mobile phase to obtain compound **4** (Rt 8.76 min; 6.7 mg).

Fraction F4 (165.3 mg) was subjected to flash chromatography on normal silica with DCM/CH_3OH (90/10; 70/30) to produce seven sub-fractions (F_{4a}-F_{4g}). Sub-fraction F_{4b} (25.80 mg) was purified using semi-preparative HPLC with H_2O/CH_3CN (15% to 20% of CH_3CN) as the mobile phase to afford compound **1** (Rt 20.67 min; 2.40 mg).

Fraction F5 (267.8 mg) was subjected to flash chromatography on normal silica with DCM/CH_3OH (90/10; 70/30) to give eight sub-fractions (F_{5a}-F_{5h}). Sub-fraction F_{5d} (31.4 mg) was purified using semi-preparative HPLC with H_2O/CH_3-CN (15% to 25% of CH_3CN) as the mobile phase to yield compounds **5** (Rt 18.95 min; 1.0 mg), **6** (Rt 23.85 min; 1.50 mg), **7** (Rt 27.90 min; 1.30 mg) and **8** (Rt 29.20 min; 2.0 mg). The mixture of sub-fractions F_{5e}-F_{5f} (44.70 mg) was purified on HPLC semi-préparative, eluated with H_2O/CH_3CN from 15% to 25% of CH_3CN in 30 min to give **9** (Rt 18.82 min; 1.40 mg).

2.4. Sugar Analysis and Determination of Absolute Configuration

A part of the fraction F5 (100 mg), from which compounds **5-9** were purified, was refluxed with TFA 2N (15 mL) for 4 h. After filtration, the mixture was extracted with CH_3Cl (3 × 25 mL) and the acid aqueous layer was evaporated. The monosaccharides glucose, xylose, galactose and rhamnose were separated by semi-preparative HPLC using an isocratic of H_2SO_4 2.5 µM 35%. The configuration D for glucose, galactose and xylose and L for rhamnose were established after chiral analytical HPLC using an isocratic of *n*-hexane/EtOH/TFA (80/20/1), in comparison with authentic monosaccharide samples.

3. Results and Discussion

Phytochemical analysis of n-butanol fraction led of isolation of flavonoids and C-13 norisoprenoids. The structure of the compounds (**1-9**) (**Figure 1**) was determined mainly on the basis of NMR spectral data and by comparing with those described in literature.

Lankerstinol A (1) was obtained as a brown amorphous powder. The molecular formula was determined to be $C_{13}H_{24}O_4$, according to the peak of its

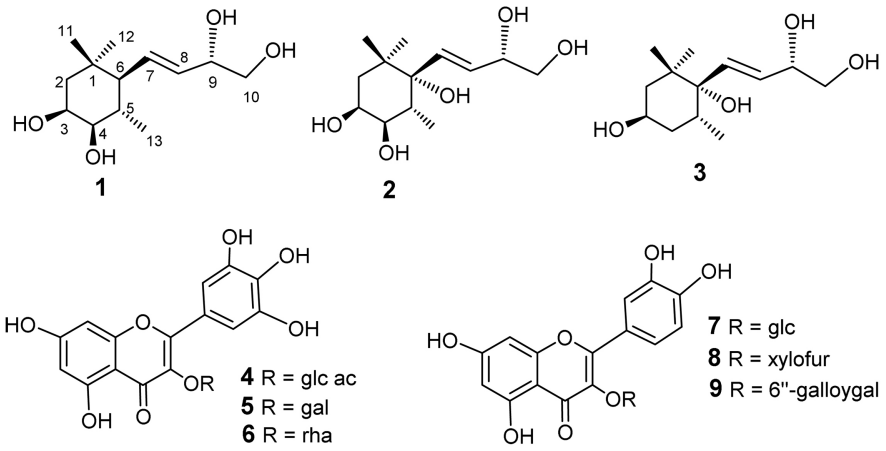

Figure 1. Isolated compounds from the leaves of *L. kerstingii.*

HRESI-MS at m/z 267.1571 $[M+Na]^+$ (calcd for $C_{13}H_{24}O_4Na$ 267.1572), indicating two degrees of unsaturation.

The ^1H NMR and HSQC (*J-mod*) spectrum, (**Table 1**), showed three methyl groups. Two of them displayed as singlets at δ_H 0.79/δ_C 32.4, (Me-11) and δ_H 1.02/δ_C 24.1 (CH_3-12), and the other one displayed as doublet at 0.94 ppm (J = 6.45 Hz, CH_3-13). Seven methine groups were ascribed at δ_H: 3.91 (m, H-3), 3.06 dd (J = 10.59; 3.23 Hz, H-4), 1.80 m (H-5), 1.48 t (J = 10.03 Hz, 6-H), 5.47 dd (J = 15.43; 9.65 Hz, H-7), 5.38 dd (J = 15.43; 6.43 Hz, H-8) and 4.11 m (H-9). Among them two were olefinic protons (H-7) and (H-8), and three were connected to OH group (H-3, H-4 and H-9) according to their deshielded values. The same spectra displayed two methylene groups at 1.41 dd (J = 14.52; 3.03 Hz, H-2a), 1.74 dd (J = 14.52; 3.13, H-2b) and 3.46 dd (J = 16.33; 4.50 Hz, H-10). H-10 was an oxygenated methylene group according to their deshielded values.

The ^{13}C NMR spectrum displayed 13 signals (**Table 1**), including three methyls, two methylenes, seven methines and one quaternary carbone.

According to the two degrees of unsaturation and the presence of one sp2 carbon, compound **1** was confirmed to have one cyclic ring in its skeleton [22].

A spin system of 11 protons, ranging from H2 to H10, coupling each other successively (**Figure 2**) was recognized on the COSY spectrum.

On the HMBC spectrum, signal at δ_H 0.94 (H-13) had HMBC correlation with δ_C 33.8 (C-5), indicating the methyl 13 substitution at C-5 (**Figure 2**). The correlations of H-11, H-12 with C-1 suggested that the two methyl groups were substituted at C-1. Another correlation between the protons H-2 and H-6 with C-1 indicated the direct link of C-1 to C-2 and C-6.

The above informations suggested that compound **1** possessed a nor-sesquiterpene ionone-derivative skeleton.

The relative stereostructure of **1** was determined by the NOESY experiment. On the NOESY spectrum, the strong cross peak between the proton H-5 and H-11 indicated the β-orientation of methyl-11 and H-5 (**Figure 2**). The correlations between H-3, H-4 and H-6 indicated their α-orientation. Also the

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR spectroscopic data of 1, 2 and 3 in CD_3OD.

N°	1		2		3	
	δ_H m (*J* in Hz)	δ_c	δ_H m (*J* in Hz)	δ_c	δ_H m (*J* in Hz)	δ_c
1	-	33.9	-	36.9	-	37.2
2a **2b**	1.41 dd (14.52; 3.03) 1.74 dd (14.52; 3.13)	46.1	1.52 dd (14.60; 3.03) 1.89 dd (14.60; 3.13)	40.6	1.40 d (14.76) 1.81 dd (14.76; 3.16)	41.4
3	3.91 m	71.3	3.95 m	71.3	4.03 m	66.7
4	3.06 dd (10.59; 3.23)	78.2	3.06 dd (10.59; 3.23)	78.2	1.70 t (13.12), 1.53 d (13.89)	35.8
5	1.80 m	33.8	2.12 m	33.8	2.28 m	29.6
6	1.48 t (10.03)	58.1	-	79.7	-	78.1
7	5.47 dd (15.43; 9.65)	133.7	5.75 dd (15.43; 9.65)	135.2	5.78 d (15.12)	135.1
8	5.38 dd (15.43; 6.43)	133.2	5.66 dd (15.43; 6.43)	128.6	5.69 dd (15.12; 5.04)	128.7
9	4.11 m	74.4	4.21 m	74.4	4.21 m	72.9
10	3.46 dd (16.33; 4.50)	67.6	3.50 dd (11.96; 4.50)	67.6	3.46 ddd (11.96; 4.50)	66.3
11	0.79 s	32.4	0.81 s	24.9	0.82 s	26.3
12	1.02 s	24.1	1.15 s	26.3	1.158 s	24.9
13	0.94 d (6.45)	17.2	0.98 d (6.45)	10.6	0.84 d (6.48)	15.0

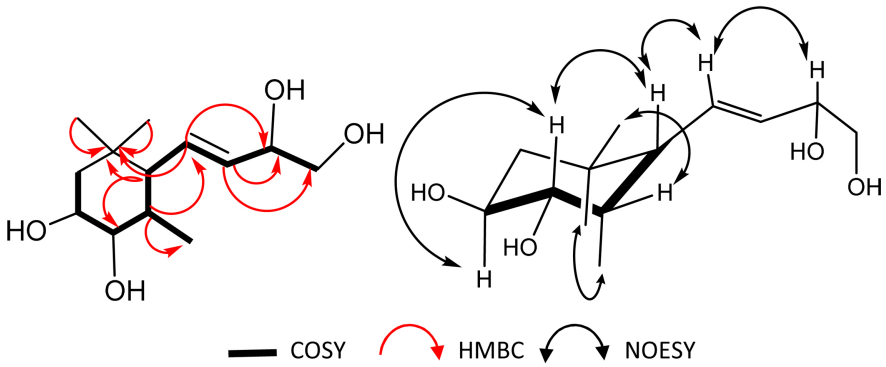

Figure 2. Key HMBC, COSY, NOESY correlations of **1**.

β-orientation of the 3,4-dihydroxy-1-butenyl chain and H-9 was determined by the strong correlation between H-7 and H-5*β*, H-11*β*, and H-9. Therefore, **1** was identified as (1S, 2R, 3S, 4S)-4-[(S, E)-3,4-dihydroxybut-1-en-1-yl]-3,5,5-trimethylcyclohexane-1,2-diol and named Lankerstinol A.

Interestingly, to our knowledge the hydroxy group attached to C-10 of the butenyl chain has not been previously found in the sesquiterpoid ionone skeleton.

Lankerstinol B (2) was also obtained as a brown amorphous powder, and had the molecular formula $C_{13}H_{24}O_5$, as deduced from ESI-MS (negative mode) data (m/z 259 $[M-H]^-$).

The ^{1}H NMR spectrum of **2** showed strong similarity to that of **1** except for the replacement of the proton H-6 by a hydroxy group, confirming by the down-

field shift of C-6 at 79.7 ppm and HMBC correlations between C-6 and H-12, H-11, H-2, H-5 and H-7.

On the NOESY spectrum of compound **2**, the strong correlation between the proton H-5 and the methyl group H-11 indicated a β orientation of H-5. By reasoning analogous to that of **1**, the absolute configuration of **2** is determined. Accordingly, compound **2** was elucidated as (1S, 2R, 3R, 4R)-4-[(S, E)-3,4-dihydroxybut-1-en-1-yl]-3,5,5-trimethylcyclohexane-1,2,4-triol named Lankerstinol B.

Lankerstinol C (3) was isolated as a brown amorphous powder. Its molecular formula was established as $C_{13}H_{24}O_4$ by means of the ESI-MS (negative mode) $[M-H]^-$ peak at m/z 243.

The NMR spectra of **3** were showed very similarity to those of **2**. Compounds **3** and **2** were distinguished from each other by the absence of the hydroxy group at C-4 in **3**. Moreover, characteristic signals protons at 1.70 (t, J = 13.12 Hz) and 1.53 (d, J = 13.89 Hz) and their HMBC correlations with the C-5 and C-6 confirmed the methylene group at C-4. The absolute configuration of **3** was given by the NOESY spectrum as similary described for **1** and **2** (Figure 2). Obviously, compound **3** was identified as (1S, 4R, 6R)-1-[(S, E)-3,4-dihydroxybut-1-en-1-yl]-2,2,6-trimethylcyclohexane-1,4-diol named Lankerstinol C.

Several Ionone derivative compounds has been shown to exert a variety of pharmacological effects, including anticancer, chemopreventive, cancer-promoting, melanogenesis, anti-inflammatory and antimicrobial activity [23] [24] [25].

According to literature the known isolated are reported as followed: myricetin-3-*O*-β-D-glucuronide **4** [26]; myricetin-3-*O*-β-D-galactopyranoside **5** [27]; myricetin-3-*O*-α-L-rhamnopyranoside **6** [28]; quercetin-3-*O*-β-D-glucopyranoside **7** [29]; quercetin-3-*O*-β-D-xylofuranoside **8** [30] and myricetin 3-*O*-β-D-(6''-galloyl)glucopyranoside **9** [31].

Myricetin-3-*O*-β-D-glucuronide (4): ^{1}H-NMR (500 MHz, CD_3OD); δ_H: 6.23 (d; J = 2.03; H-6); 6.42 (d; J = 2.03; H-8); 7.39 (s; H-2' and H-6'); 5.49 (d; J = 7.9; H-1''); 4.24 (dd; J = 8.9; 7.9; H-2''); 3.81 (dd; J = 8.9; 8.09; H-3''); 3.84 (dd; J = 8.9; 8.09; H-4''); 3.78 d (8.9; H-5''). ^{13}C-NMR (125 MHz, CD_3OD); δ_c: 157.5 (C-2); 134.1 (C-3); 177.8 (C-4); 161.6 (C-5); 93.2 (C-6); 164.6 (C-7), 98.4 (C-8); 157.0 (C-9); 104.1 (C-10); 120.3 (C-1'); 108.5 (C-2'); 145.0 (C-3'); 136.7 (C-4'); 145.0 (C-5'); 108.5 (C-6'); 104.2 (C-1''); 71.5 (C-2''); 76.3 (C-3''); 73.9 (C-4''); 75.5 (C-5''); 171.0 (C-6'').

Myricetin-3-*O*-β-D-galactopyranoside (5): ^{1}H-NMR (500 MHz, CD_3OD); δ_H: 6.21 (d; J = 2.05; H-6); 6.40 (d; J = 2.05; H-8); 7.38 (s; H-2' and H-6'); 5.22 (d; J = 7.8; H-1''); 3.83 (dd; J = 7.8; 9.0; H-2''); 3.67 (dd; J = 3.11; 9.0; H-3''); 3.87 (d; J = 3.11; H-4''); 3.51 (dd; J = 6.35; 11.0; H-5''), 3.64 (dd; J = 2.74; 10.96; H-6a''), 3.73 (dd; J = 5.98; 10.96; H-6b''). ^{13}C-NMR (125 MHz, CD_3OD); δ_c: 152.5 (C-2); 137.2 (C-3); 179.3 (C-4); 162.4 (C-5); 94.6 (C-6); 167.5 (C-7), 99.8 (C-8); 158.4 (C-9); 107.5 (C-10); 121.6 (C-1'); 109.8 (C-2'); 146.4 (C-3'); 137.5 (C-4'); 146.4 (C-5'); 109.8 (C-6'); 105.4 (C-1''); 75.1 (C-2''); 73.2 (C-3''); 77.2 (C-4''); 70.0 (C-5''); 61.9 (C-6'').

Myricetin-3-*O*-*α*-L-rhamnopyranoside (6): ^{1}H-NMR (500 MHz, CD_3OD); δ_H: 6.05 (d; *J* = 2.04; H-6); 6.15 (d; *J* = 2.05; H-8); 6.79 (s; H-2' and H-6'); 5.45 (d; *J* = 1.07; H-1''); 3.84 (m; H-2''); 3.42 (dd; *J* = 9.9; 3.35 H-3''); 3.09 (m; H-4''); 3.19 (m; H-5''); 0.98 (d; *J* = 6.11; H-6''). ^{13}C-NMR (125 MHz, CD_3OD); δ_c: 158.3 (C-2); 135.6 (C-3); 178.3 (C-4); 162.0 (C-5); 99.4 (C-6); 164.9 (C-7), 94.3 (C-8); 156.9 (C-9); 104.7 (C-10); 134.5 (C-1'); 108.8 (C-2'); 146.5 (C-3'); 137.2 (C-4'); 146.5 (C-5'); 108.8 (C-6'); 102.4 (C-1''); 70.5 (C-2''); 70.8 (C-3''); 71.7 (C-4''); 71.0 (C-5''); 18.0 (C-6'').

Quercetin-3-*O*-*β*-D-glucopyranoside (7): ^{1}H-NMR (500 MHz, CD_3OD); δ_H: 6.22 (d; *J* = 2.07; H-6); 6.42 (d; *J* = 2.1; H-8); 7.72 (d; *J* = 2.20; H-2'); 6.75 (d; *J* = 8.26; H-5'); 7.61 (dd; *J* = 8.47; 2.25; H-6'); 5.25 (d; *J* = 7.87; H-1''); 3.50 (t; *J* = 8.17; H-2''); 3.45 (t; *J* = 9.03; H-3''); 3.37 (t; *J* = 9.65; H-4''); 3.26 (m; H-5''), 3.74 (dd; *J* = 11.96; 2.40; H-6b'') 7.61 (dd; *J* = 11.91; 5.37; H-6a''). ^{13}C-NMR (125 MHz, CD_3OD); δ_c: 159.8 (C-2); 136.2 (C-3); 178.0 (C-4); 164.2 (C-5); 100.8 (C-6); 167.2 (C-7), 95.5 (C-8); 159.3 (C-9); 106.7 (C-10); 123.9 (C-1'); 118.3 (C-2'); 146.8 (C-3'); 150.7 (C-4'); 116.9 (C-5'); 124.1 (C-6') 105.2 (C-1''); 76.3 (C-2''); 78.5 (C-3''); 73.9 (C-4''); 79.6 (C-5''); 64.3 (C-6'').

Quercetin-3-*O*-*β*-D-xylofuranoside (8): ^{1}H-NMR (500 MHz, CD_3OD); δ_H: 6.11 (d; *J* = 2.07; H-6); 6.31 (d; *J* = 2.03; H-8); 7.56 (d; *J* = 2.13; H-2'); 6.75 (d; *J* = 8.48; H-5'); 7.52 (dd; *J* = 8.19; 2.13; H-6'); 5.19 (d; *J* =7.54; H-1''); 3.57 (m; H-2''); 3.80 (dd; *J* = 11.48; 5.17; H-3''); 3.41 (t; *J* = 9.50; H-4''); 3.12 (dd; *J* = 11.48; 9.37; H-5''). ^{13}C-NMR (125 MHz, CD_3OD); δ_c: 158.5(C-2); 136.6 (C-3); 180.5 (C-4); 164.6 (C-5); 100.5 (C-6); 167.4 (C-7), 96.4 (C-8); 160.2 (C-9); 108.4 (C-10); 123.3 (C-1'); 118.3 (C-2'); 146.3 (C-3'); 150.7 (C-4'); 116.5 (C-5'); 124.8 (C-6') 104.5 (C-1''); 75.1 (C-2''); 77.6 (C-3''); 70.9 (C-4''); 67.2 (C-5'').

Myricetin-3-(6''-galloylglucopyranoside) (9): ^{1}H-NMR (500 MHz, CD_3OD); δ_H: 6.19 (d; *J* = 2.09; H-6); 6.33 (d; *J* = 2.09; H-8); 7.92 (s; H-2' and H-6'); 5.22 (d; *J* =7.73; H-1''); 3.58 (t; *J* = 8.28; H-2''); 3.42 (t; *J* = 8.55; H-3''); 3.44 (t; *J* = 9.08; H-4''); 3.48 (m; H-5''), 4.41 (dd; *J* = 11.80; 5.50; H-6b''); 4.28 (dd; *J* = 11.96; 1.78; H-6a''); 7.22 (s; H-2''' and H-6'''). ^{13}C-NMR (125 MHz, CD_3OD); δ_c: 158.9 (C-2); 135.8 (C-3); 179.9 (C-4); 163.4 (C-5); 99.8 (C-6); 167.2 (C-7), 95.1 (C-8); 158.2 (C-9); 106.4 (C-10); 122.5 (C-1'); 110.1 (C -2'); 146.8 (C-3'); 138.4 (C-4'); 146.8 (C-5'); 110.1 (C-6'); 104.9 (C-1''); 75.8 (C-2''); 72.6 (C-3''); 78.2 (C-4''); 76.5 (C-5''); 64.9 (C-6''); 122.3 (C-1'''); 110.1(C-2''' and C-6'''); 146.8(C-3''' and C-5'''); 140.0 (C-4''').

This study confirms the presence of flavonoid glycosides-rich extract of Lannea species collected in Côte d'Ivoire [2]. Interestingly, these molecules can be regarded as the characteristic compounds and as the chemotaxonomic marker of Lannea species. Glycosides derived from medicinal plants are described as promising good anti-analgesic components [32]. Our previous research on Lannea species has reported that compounds **4**, **6** and **7** possessed high antioxidant activity [2]. Thus, the presence of these compounds can justify the use of this species in traditional medicine.

4. Conclusion

Phytochemical study of the leaves of *L. kerstingii* has been investigated. Nine compounds including three new C-13 norisoprenoids and six known flavonoid glycosides were isolated. To the best of our knowledge, these compounds were isolated for the first time in this plant. Moreover, the hydroxy group attached to C-10 of the butenyl chain in compounds **1-3**, has not been previously found in the sesquiterpoid ionone skeleton. The great interest of this study is that it contributes to the knowledge of the molecules derived from *L. kerstingii* and therefore contributes to the promotion of this plant used in traditional therapy in West Africa. The presence of these molecules can explain the different activities of the leaves of *L kerstingii*. However, further chemical and biological studies are needed to make this plant to be an effective source of pharmaceuticals.

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Conflicts of Interest Statement

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

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