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NEW FLAVONOL GLYCOSIDE FROM *GUIBOURTIA EHIE* (FABACEAE)

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Keywords:

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ABSTRACT: *Guibourtia ehie* (A. Chev.) J. Leonard is a medicinal plant widely used in the Ivorian traditional medicine against stomach ulcers, sexual infections, diabetes, hypertension and microbial infections, particularly those caused by amoeba and fungi. Furthermore, this plant is widely known for its commercial interest due to economic value of its wood. Biological and phytochemical investigations also confirmed local medicinal uses of the plant and showed a various chemical composition including numerous polyphenols. In this article, we reported the results of leaves studies of *Guibourtia ehie*, harvested in Côte d'Ivoire, which resulted in the isolation of a new flavonol, 8-isopentenylkaempferol-7-*O*-*β*-D-(2'''-*O*-galloyl) glucopyranoside (1), that we named 2''-*O*-Galloyl-epimedoside C, along with two phenolic known compounds, orcinol (2) and methyl orsellinate (3). The structures of compounds where elucidated by spectroscopic data including HR-ESIMS, UV, NMR 1D (^{1}H and ^{13}C) and NMR 2D (COSY, HSQC and HMBC). These compounds were isolated for the first time from *Guibourtia ehie* (A. Chev.) J. Leonard.

INTRODUCTION: *Guibourtia ehie* (A. Chev.) J. Leonard is a copal tree of Fabaceae family up to 45–50 m tall, with a straight, cylindrical bole, branchless for up to 25 m. The leaves are spirally arranged, paripinnate with a pair of leaflets; the stipules are leaf-like, up to 2 cm long, and often persistent; the petioles are 0.5–1 cm long. The distribution area of *Guibourti ehie* extends from Liberia to Gabon where it used as medicinal plant against various ailments.

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It is also used for the treatment of gonorrhea, ulcers, high blood pressure and sexual infections [1]. The wood of the plant is also extensively commercialized in export [2]. Very few works in the literature deal with the phytochemistry of the *Guibourtia* genus and chalcone, stilbene and flavonoid derivatives were mainly isolated [1].

From leaves and trunk of *Guibourtia ehie*, rhaponticin, ellagic acid, 2,6-dimethoxy-benzoquinone, lupeol, taraxerol, friedelan-3-one, lanosterol, scopoletin and pilloin were recently isolated [3]. Antioxidant and antibacterial activities were attributed to this plant [3, 4]. In our ongoing to search novel bioactive compounds from Ivorian medicinal plants [5–7], *Guibourtia ehie* species was systematically investigated for its chemical constituents.

The present paper report the results of leaves studies of *Guibourtia ehie* which resulted in the isolation and structure elucidation of one new flavonol (1) together with two known compounds (2–3).

MATERIAL AND METHODS:

General Methods: Chromatography columns were carried out on silica gel (Merck, 40-230 mesh) or Sephadex® LH-20. Thin layer chromatography (TLC) were carried out on aluminum plates coated with silica gel 60 F_{254} (Merck) and revealed under UV light (254 and 366 nm) and/or with vanillin-H_2SO_4, Lieberman (Acetic anhydride-H_2SO_4) and Fast Blue B reagents. 1D-NMR spectra (^1H, ^{13}C) and 2D-NMR spectra (COSY, HSQC, HMBC and NOESY) were recorded in the CD_3OD on a Bruker AC-400 spectrometer operating at 400 MHz for ^1H spectra and 100 MHz for ^{13}C. Low resolution mass spectra, APCIMS and ESIMS, were acquired using a Bruker Esquire-LC_00040 spectrometer. HRESIMS spectra were recorded with a Bruker Esquire LC_00040 spectrometer.

Plant Material: Leaves of *Guibourtia ehie* were collected in April 2019 at Agbo 2 forest (6° 24' 6"N, 4° 5' 56"W), near Affery locality in Akoupé Department. The species was later authenticated by botanists at the Centre National de Floristique (CNF), Félix Houphouët-Boigny University (Abidjan, Côte d'Ivoire). A voucher specimen (OAT-Ge-2019) was also deposited in the Herbarium. The collected plant material was washed, cut into small pieces and dried during two weeks. Dry samples were crushed and stored at 25°C until use.

Extraction and Isolation of Compounds: The powder of *Guibourtia ehie* dried leaves (1kg) was extracted in a Soxhlet apparatus with methanol. After solvent removed, the crude extract (MeOH, 450 g) was suspended in water and then partitioned sequentially using hexane, dichloromethane and ethyl acetate to yield hexane (Hex), dichloromethane (Dic) and ethyl acetate (Ae) sub-extracts as well as the residual aqueous fraction (Aqr). The ethyl acetate sub-extract (6 g) was fractionated on a silica gel column, eluted with DCM/EtOAc (30:70 to 10:90), to give eight fractions (F_1 to F_8). Fraction F_8 (245.3 mg) was purified on Sephadex® LH-20 column (DCM/MeOH, 2:1) to yield compound **1** (40 mg). The dichloromethane sub-extract (Dic, 4.5 g) was fractionated on a silica gel column using DCM/MeOH elution (100:0 to 80:20) to yield thirteen fractions (F_1 to F_{13}). Fraction F_5 (340 mg) was further chromatographed on a silica gel using Hex/DCM (30:70) to provide thirteen sub-fractions ($F_{5\text{-}1}$ to $F_{5\text{-}13}$). Sub-fraction $F_{5\text{-}8}$ (115,3 mg) was successfully purified on silica gel (Hex/DCM, 95:5) and Sephadex® LH-20 (DCM/MeOH, 2:1) columns, yielding the compound **2** (10 mg). Fraction F_7 (620 mg) was successfully purified on silica gel (Hex/DCM/MeOH, 5:90:5 and Hex/EtOAc, 30:70) and Sephadex® LH-20 (CH_2Cl_2/MeOH, 2:1) columns to yield compound **3** (5 mg).

2''-*O*-Galloyl-epimedoside C (or 8-Isopentenylkaempferol-7-*O*-*β*-D-(2'''-*O*-galloyl) glucopyranoside) (1): Yellowish oil; UV (MeOH) λmax (log ε) 262 (4.5), 267 (4.0) nm; ^1H NMR (400 MHz, MeOD) and ^{13}C NMR (100 MHz, MeOD) see **Table 1**; HRESIMS *m/z* 669.1823 $[M+H]^+$ (calcd for $C_{33}H_{33}O_{15}$, 669.1819).

Orcinol (2): Colorless solids; ^1H NMR (400 MHz, MeOH); δ_H: 6.07 (dd; J = 0.40; 2.00; H-2); 6.12 (dd; J = 0.06; 2.8; H-4); 2.16 (s; H-5-CH_3); 6.11 (dd; J = 0.60; 2.80; H-6). ^{13}C-NMR (125 MHz, MeOH); δ_C: 159.3 (C-1); 100.7 (C-2); 159.3 (C-3); 108.6 (C-4); 141.1 (C-5); 21.6 (C-5-$\underline{C}H_3$), 108.6 (C-6).

Methyl Orsellinate (3): Yellow crystals; ^1H NMR (400 MHz, MeOH); δ_H: 6.14 (d; J = 0.80; H-3); 6.16 (d; J = 0.80; H-5); 2.27 (s; H-6-CH_3); 3.79 (s; H-7-OCH_3). ^{13}C-NMR (125 MHz, MeOH); *δc*: 107.42 (C-1); 161.1 (C-2); 100.4 (C-3); 161.0 (C-4); 110.6 (C-5); 140.7 (C-6); 21.9 (C-6-$\underline{C}H_3$); 170.1 (C-7); 51.7 (C-7-O$\underline{C}H_3$).

RESULTS AND DISCUSSION: The leaves crude MeOH extract of *Guibourtia ehie* was sequentially partitioned using hexane, dichloromethane and ethyl acetate to provide the corresponding sub-extracts. The dichloromethane and ethyl acetate sub-extracts, individually separated using various chromatographic techniques, resulted in the isolation of the new flavonol, 8-isopentenyl-kaempferol-7-*O*-*β*-D-(2'''-*O*-galloyl) gluco-pyranoside (1), together with two known

compounds, orcinol (2) and methyl orsellinate (3) [8] **Fig. 1**. The structures of the isolated compounds were elucidated by spectroscopic techniques including UV, NMR and MS analysis. These compounds were isolated for the first time from *Guibourtia ehie.*

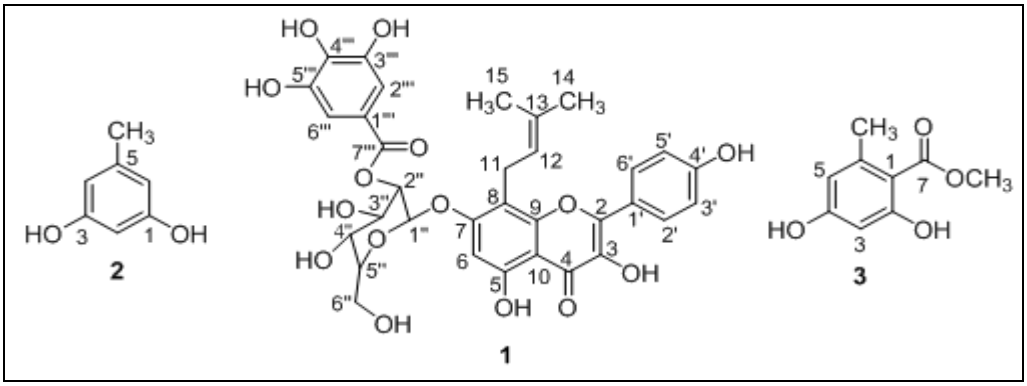

FIG. 1: STRUCTURE OF ISOLATED COMPOUNDS (1–3)

Compound 1 was obtained as yellow-colored oil. The HR-ESIMS spectrum exhibited an ion peak at *m/z* 669.1823 $[M+H]^+$ (calcd for $C_{33}H_{33}O_{15}$, 669.1819), indicated the molecular formula of $C_{33}H_{32}O_{15}$ corresponding to eighteen degree of hydrogen deficient. Fragment ions at *m/z* 517.1706 $[M-C_7H_5O_4+H]^+$ and 153.0185 $[C_7H_5O_4]^+$ were characteristic to the loss of a gallic acid ester moiety [9]. UV absorptions at 262 and 267 nm were typical of a flavonol skeleton [10]. The ^{13}C NMR spectrum of compound 1 **Table 1** showed 33 carbon resonances, among them 15 carbons were deduced to be assignable to a flavonoid skeleton, five to a prenyl moiety, seven to a galloyl group and six to one sugar unit. The ^1H NMR spectrum **Table 1** showed two pairs of coupled aromatic protons, at δ_H 8.04 and 6.86 (2H each, d, J = 8.9 Hz), which showed HSQC correlations to carbon signals at δ_C 130.9 and 116.2, respectively, suggesting a typical aromatic AA′BB′ NMR coupling system on the B ring [11].

In the HMBC spectrum, all these two proton signals (δ_H 8.04 and 6.86) also showed correlations to carbon signals at δ_C 123.8 and 160.7 **Fig. 2**, indicated that the first one was the chemical shift of C-1′, and the second, that of C-4′ [11]. The ^{13}C NMR spectrum showed characteristic resonances attributed to the A ring and C ring of a flavonol skeleton through the signals for a conjugated carbonyl group at δ_C 177.9 (C-4) [12], an aromatic non-protonated carbon at δ_C 106.3 (C-10) and five aromatic oxygenated carbons [δ_C 137.4 (C-3), 148.9 (C-2), 154.7 (C-9), 160.3 (C-5), 161.1 (C-7)]. 1D NMR (^1H and ^{13}C NMR) and 2D NMR (HSQC and HMBC) spectra also exhibited signals indicated the presence of galloyl group through a singlet aromatic proton at δ_H 6.62 (1H, s) correlating to δ_C 98.6 (C-6); two aromatic protons at δ_H 7.09 (2H, s), correlated to δ_C 110.5 (C-2’’’/C-6’’’), three aromatic oxygenated carbons [δ_C 146.4 (C-3’’’/C-5’’’), 139.9 (C-4’’’)], an aromatic non-protonated carbon at δ_C 121.4 (C-1’’’) and an ester carbonyl carbon (δ_C 167.4, C-7’’’) [13]. A serial proton signals at δ_H 4.86 (1H, m, H-12), 3.34 (2H, m, Ha-11/ H_b-11), 1.65 (3H, s, Me-14) and 1.39 (3H, s, Me-15), showed correlations with carbon signals at δ_C 123.4 (C-12), 22.9 (C-11), 25.5 (C-15) and 18.3 (C-14) in the HSQC spectrum, respectively. Among them, δ_H 4.86 (1H, m, H-12) showed HMBC correlations to δ_C 25.5 (C-15) and 18.3 (C-14), as well as δ_H 1.65 (3H; s) to δ_C 123.4 (C-12) in the HMBC spectrum, suggesting the presence of a prenyl group [12].

The location of the prenyl group at C-8 was supported by the HMBC correlation between H-11 (2H, m, 3.34) and C-7 (δ_C 161.1). The sugar moiety was identified as D-glucose by acid hydrolysis. δ_H 5.32 (1H, d, J = 8.2 Hz), as well as δc 100.3 was deduced to be the anomeric proton and carbon signal of the Glc moiety. The *β*-pyranosyl configuration of the glycosidic bond of Glc moiety was deduced from the coupling constant at J = 8.2 Hz of the anomeric proton [14]. The HMBC correlation between H-1’’ (1H, 5.32, d, J = 8.2 Hz) and C-7 (δ_C 161.1) indicated that the Glc group was attached to C-7 of the aglycone. In the ^1H-^1H COSY spectrum, δ_H 5.32 (H-1’’) was observed to be correlated with δ_H 5.25 (H-2’’) that was also

correlated to δ_H 3.77 (H-3″) **Fig. 2**. The galloyl group located at C-2'' of the Glc moiety was suggested by the HMBC correlation of H-2'' (1H, 5.25, dd, *J* = 8.4, 8.0 Hz) of the Glc to the galloyl carbonyl (δc 167.4) **Fig. 2**. Based on the above results, compound 1 was determined to be 8-isopentenylkaempferol-7-*O*-*β*-D-(2''-*O*-galloyl) glucopyranoside that we named 2''-*O*-galloyl-epimedoside C [15].

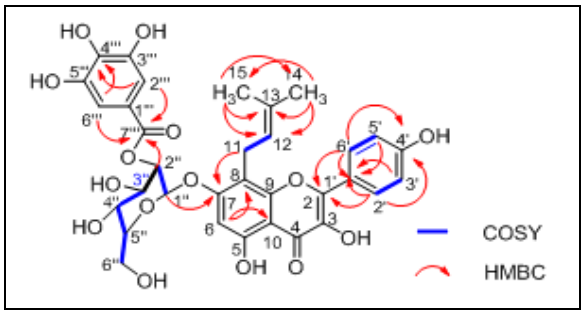

FIG. 2: IMPORTANT 2D CORRELATIONS OBSERVED IN COMPOUND 1

From a chemotaxonomic viewpoint, prenylated flavonoids were identified as active components in about twenty-six plant families, mainly in Fabaceae, Moraceae and Euphorbiaceae, and could be classified into 6 categories: prenylated-flavones, flavanones, chalcones, isoflavones, flavans and isoflavans [16]. Those with a 8-C-isopentenylflavonol skeleton as aglycone, such as 1, belonging to prenylated flavones group were mostly obtained in Rutaceae, Berberidaceae, Sapindaceae, Asclepiadaceae, Thymelaeaceae, Euphorbiaceae, Platanaceae and Fabaceae [17]. Nevertheless, those obtained in Fabaceae family with this type aglycone have been only reported from *Desmodium*, *Glycyrrhiza*, *Mundulea*, *Rhynchosia* and *Sophora* genus, all to Faboideae sub-family [18–20]. Orcinol (2) and methyl orsellinate (3), as well as derivatives, which are considered as ubiquitous of lichens [8, 21], were also found in bryophytes and marchantiophytes [22, 23].

In higher plants, these compounds have been identified in a few genera of Apocynaceae [24], Boraginaceae [25], Ericaceae [8], Fabaceae [26], Liliaceae [27], Myrtaceae [28], Nyctaginaceae [29], Piperaceae [30], Salicaceae [31] and Styracaceae [32].

To our knowledge, all these compounds are described here for the first time in *Guibourtia* genus that belonging to Caesalpinioideae sub-family. Therefore, they could be used to establish a relationship between these species.

TABLE 1: 1D- AND 2D-NMR OF COMPOUND 1 IN CD_3OD

Position	^{13}C (δ, ppm)	^{1}H (δ, ppm) (m; J, Hz)	COSY	HMBC
Flavonol aglycone				
2	148.0	-		
3	137.4	-		
4	177.9	-		
5	160.3	-		
6	98.6	6.67 (1H; s)		C-7; C-8; C-10
7	161.1	-		
8	110.3	-		
9	154.7	-		
10	106.3	-		
1’	123.8	-		
2’	130.9	8.04 (2H; d; 8.9)	H3’; H5’	C-4’; C-2; C-2’
3’	116.2	6.86 (2H; d; 8.9)	H2’; H6’	C1’
4’	160.7	-		
5’	116.2	6.86 (2H ; d; 8.9)	H2’; H6’	C1’
6’	130.9	8.04 (2H; d; 8.9)	H3’; H5’	C-4’; C-2; C-2’
Prenyl				
11	22.4	3.34 (2H; m)	H12	C-7
12	123.4	4.86 (1H; m)	H11	C-14 ; C-15
13	132.7	-		
14	18.3	1.65 (3H; s)		C-13; C-12; C-15
15	25.5	1.39 (3H; s)		C-13; C-12; C-14
β-D-Glucopyranose				
1’’	100.3	5.32 (1H; d; 8.2)	H2’’	C-7
2’’	74.9	5.25 (1H; dd; 8.2; 8.4)	H1’’; H3’’	C-1’’
3’’	76.8	3.77 (1H; d; 8.4)	H2’’; H4’’	

4''	71.4	3.59 (1H; d; 18.3)	H3''; H5''	
5''	78.5	3.63 (1H; dd; 2.3; 5.4)	H4''	
6''	62.3	3.98 (1H; Ha; dd; 8.0; 8.4) 3.80 (1H; Hb; t; 4.7)	H5''	
Gallic acid				
1'''	121.4	-		
2'''	110.5	7.09 (1H; s)		C-7'''; C-6'''; C-4'''
3'''	146.4	-	-	
4'''	139.9	-	-	
5'''	146.4	-	-	
6'''	110.5	7.09 (1H;s)	;	C-7'''; C-2'''; C-4'''
7'''	167.4	-		

CONCLUSION: This study demonstrated that *Guibourtia ehie* leaves are a natural source of bioactive compounds, particularly phenols. Among the phenolic compounds, 2''-*O*-galloyl-epimedoside C was isolated for the first time in this plant study. These isolated compounds were found to have an important chemotaxonomic significant.

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CONFLICT OF INTEREST: The authors declare that there are no conflicts of interest among themselves or with any public or private company in relation to this manuscript.

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