Inhibitory antibacterial activity of two novel dihydroxy fatty acids and other constituents from the stem bark of Trichilia gilgiana Harms (Meliaceae)

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Abstract

Two novel dihydroxy-fatty acids, gilgianic acid (**1**), phenylgilgianic acid (**2**) together with four known compounds: *ß*-sitosterol (**3**), stigmasterol (**4**), sitosterol-3-*O*-*ß*-D-glycoside (**5**) and lupeol (**6**) were isolated from the stem bark of *Trichilia gilgiana*. The structures of compounds **1**–**6** were elucidated based on spectroscopic (1 D and 2 D NMR) and HR-ESI-MS data as well as comparison with relevant literature data. This study reports for the first time the isolation of fatty acids from the genus *Trichilia*. Compounds **3**–**6** had already been isolated from *trichilia* genus. The crude extract and compounds **1**–**5** were tested for their inhibitory effect against a panel of bacterial strains using the microdilution technique with ampicillin, nalidixic acid and streptomycin as standard drugs. The crude extract and the tested compounds were found to exhibit significant activity against *Bacillus subtilis* and *Klebsiella aerogenes* with a MIC value of 2.3 *µ*g/mL. Compounds **3** and **5** were also found to be active against *Enterococcus faecalis* with a MIC value of 2.3 µg/mL.

Introduction

Antimicrobial resistance (AMR) has emerged as one of the principal public health problems of the 21st century resulting in high rates of morbidity and mortality, increased length of hospitalization and higher healthcare costs [1, 2]. For the past 30 years, regulatory authorities have not approved any new class of antibiotics and this has been termed the discovery void [3]. Therefore, there is a need for identifying novel molecules with unprecedented modes of action and improved efficacy. Such challenges have made it necessary to continuously search for alternative drugs with greater therapeutic efficiency for the prevention and treatment of infectious diseases. Natural products from microbes and plants have historically made a major contribution to pharmacognosy especially for infectious diseases and therefore remain a great source of secondary metabolites that could be used as lead for the development of new antibiotics [4, 5]. In this context, an investigation of the chemical components of a Cameroonian medicinal plant *Trichilia gilgiana* Harms and an evaluation of their antibacterial activity was undertaken.

Trichilia gilgiana Harms is one of the largest trees in the Meliaceae family, mainly distributed in the South West Region of Cameroon [6]. The stem bark of this plant is used in traditional phar for the treatment of typhoid fever, fever, and abdominal pains [6, 7]. Previous phytochemical investigations of some *Trichilia* species led to the isolation of cycloartanes [8], steromacopeiaids [9], coumarins [10], flavalignans [11] and limonoids [6, 12–14]. Recently, [15, 16] reported the isolation of a new phenyl alkene and limonoid from *Trichilia gilgiana* stem bark. Based on the traditional uses of *Trichilia gilgiana* Harms as well as the wide chemical diversity of the genus *Trichilia*, this plant was selected for chemical and biological investigation. This paper therefore reports the isolation and structural characterization of secondary metabolites including two novel dihydroxy fatty acids isolated from the stem bark of this plant and the results of their antibacterial activity.

Results

Isolation and structure elucidation

The dichloromethane-methanol (1:1) extract of T. gilgiana stem-bark was subjected to silica gel column chromatography to afford two novel dihydroxy fatty acids (**1**) and (**2**) together with four known metabolites *β*-

sitosterol (**3**) [17], stigmasterol (**4**) [18], *β*-sitosterol-3-O- β -D-glucopyranoside (**5**) [19] and lupeol (**6**) [18]. The structures of the isolated compounds are presented in Fig. 1.

Compound (**1**) was isolated as a white powder using a mixture of *n*-Hex/EtOAc (4:1, v/v) and it was soluble in chloroform. It's molecular formula $C_{22}H_{44}O_4$ was deduced from HR-ESI-MS (Fig. S8) analysis that displayed a pseudo-molecular ion peak at *m/z* 407.2899 $[M + Cl]^-$ (calcd for $C_{22}H_{44}O_4Cl$, 407.2928) containing one double bond equivalent. The FT-IR spectrum (Fig. S10) showed the presence of free hydroxyl group at 3396.36 cm^{-1}, a carbonyl of carboxylic acid at 1720.10 cm^{-1}, alkyl group at 2916.06/2847.45 cm^{-1} and a carbon-hydroxyl group at 1195.01cm^{-1}. The structure of compound **1** was deduced from NMR analysis (^1H, ^{13}C, DEPT 135, HMBC, HSQC and COSY) coupled with HR-ESI-MS data. ^1H NMR spectrum (Fig. S3) of **1** showed signals of two oxymethines protons at δ_H 4.47 (1H, qd, *J* = 6.5 and 3.0, H-2') and δ_H 4.33 (1H, br s, H-1'), two methyl groups at δ_H 0.90 (3H, t, *J* = 6.8, H-19) and δ_H 1.45 (3H, d, *J* = 6.4, H-3'). It also exhibited the presence of one aliphatic chain at δ_H 1.27 (25H, br s). ^{13}C NMR spectrum (Fig. S2) of **1** showed one signal at δ_C 177.8 (C-1) characteristic of a carboxylic acid. This spectrum coupled with DEPT 135 spectrum (Fig. S4) led us to identified in the positive phase two oxymethines carbon peaks at δ_C 71.2 (C-1') and δ_C 79.0 (C-2') and two methyl groups at δ_C 14.1 (C-19) and δ_C 13.7 (C-3'). On the same DEPT 135 spectrum, negative phase peaks were observed at δ_C 29.7–29.3 (C-6-16) characteristic of the aliphatic chain. All these information confirmed **1** as a dihydroxy fatty acid.

HMBC and COSY correlations as well as ^1H and ^{13}C NMR analysis (Table 2) led to the identification of the positions and the proximity of the oxymethine carbons at C-1' and C-2' as well as the positions of the two methyl carbons at C-19 and C-3'. Some important correlations were observed with the HMBC spectrum (Fig. S5) of **1**, such as the correlations between the methyl proton at δ_H 1.45 (3H, d, *J* = 6.4, H-3') with the carbons of the oxymethine at δ_C 71.2 (C-1') and δ_C 79.0 (C-2') and the carbons at δ_C 23.4 (C-3) and δ_C 47.5 (C-2). In $^2J_{C-H}$, a correlation of a proton at δ_H 4.33 (1H, br s, H-1') with a carbon of the carboxylic acid at δ_C 177.8 (C-1) was observed. In ^2J and ^3J the correlations of protons at δ_H 1.67 (1H, dtd, *J* = 14.7, 9.7 and 5.4, H-3) and δ_H 1.87–1.79 (2H, m, H-4) with a carbon of the carboxylic acid at δ_C 177.8 (C-1) were observed. Correlations were also observed between the methyl proton at δ_H 0.90 (3H, t, *J* = 6.8, H-19) with carbons at δ_C 22.7 (C-18) and δ_C 31.9 (C-17). Another correlation was observed between the proton at δ_H 1.27 (25H, br s,) with the carbons at δ_C 31.9 (C-17) and δ_C 14.1 (C-19).

Table 2

^{1}H ;^{13}C NMR; HMBC and COSY data for compound **1**

Position	Compound 1			
	δ ^{13}C	δ ^{1}H (nH, m, J in Hz)	HMBC	COSY
1	177.8	-	H-2 ; H-3 ; H-4 ; H-1'	
2	47.6	2.58 (1H, dt, 9.9 and 4.9)	H-3 ; H-4 ; H-3'	H-1' ; H-3 ; H-4
3	23.3	1.67 (1H, dtd, 14.7, 9.7 and 5.4) and 1.45 (1H, d, 6.4)	H-4 ; H-3'	H-2 ; H-4
4	22.7	1.87–1.79 (2H, m)	H-3	H-2 ; H-3
5	27.6	1.26 (2H, m)		
6–16	29.7–29.3	1.24–1.29 (25H, m)	H-3 ; H-4 ;	
17	31.9	1.33 (2H, d,13.8)	H-19 ; H-18	H-19
18	22.7	1.27 (2H, br s)	H-19	H-19
19	14.1	0.90 (3H, t, 6.8)	H-18	H-18 ; H-17
1'	71.2	4.33 (1H, br s,)	H-3 ; H-2' ; H-3'	H-2' ; H-2
2'	79.0	4.47 (1H, qd, 6.5 and 3.0)	H-2' ; H-1'	H-3' ; H-1'
3'	13.7	1.45 (3H, d, 6.4)	H-2'	H-2'

Its COSY spectrum (Fig. S7) displayed different scalar correlations between protons H-3' and H-2'; H-2' and H-1'; H-2 and H-1'; H-2 and H-3; H-3 and H-4; H-2 and H-4; H-19, H-18 and H-17. In the same spectrum (Fig. S7), proton at δ_H 1.45 (1H, d, J = 6.4 Hz, H-3') showed correlations with oxymethine at δ_H 4.47 (1H, qd, J = 6.5 and 3.0 Hz, H-2'), and oxymethine protons at δ_H 4.33 (1H, br s, H-1'). A correlation was also observed between the oxymethine proton at δ_H 4.47 (H-2') and the other oxymethine proton at δ_H 4.33 (H-1'). The HR-TOF-MS spectrum of **1** (Fig. S9) showed some important fragment ions such as the fragment ions at *m/z* 327.2320 $[M\text{-}C_2H_5O + Cl]^-$ and at *m/z* 119.9473 $[M\text{-}C_{18}H_{36} + Cl]^-$. Thus, the structure of compound **1** was elucidated as 2-(1,2-dihydroxypropyl) nonadecanoic acid, a new dihydroxy fatty acid that was given the trivial name gilgianic acid.

Compound **2** was obtained as a white powder in *n*-Hex/EtOAc (3:1, v/v). The analysis of HR-ESI-MS (Fig. S18) displayed a pseudo-molecular ion peak at *m/z* 385.2167 $[M + Cl]^-$ (calcd for $C_{21}H_{34}O_4Cl$, 385.2146) from which the molecular $C_{21}H_{34}O_4$ was deduced, containing five degrees of unsaturation. The analysis of ^{1}H and ^{13}C NMR spectra (Fig. S13 and Fig. S12) coupled with HSQC spectrum (Fig S16) revealed that compound **2** is closer to **1** with the only difference being the presence of a benzene ring attached to the alkyl chain of compound **2**. ^{1}H and ^{13}C NMR analysis (Table 2) of **2** indicated the presence of a monosubstituted aromatic ring with the presence of three aromatic protons peaks at δ_H 7.30 (2H, t, J = 7.5 Hz, H-3'), 7.21 (2H, d, J = 7.5 Hz, H-2'), 7.20 (1H, d, J = 7.6 Hz, H-4')) and four aromatic carbons peaks at δ_C 142.9 (C-1'), 128.4 (C-2'), 128.2 (C-3'

), 125.5 (C-4'). They also indicated the presence of two oxymethine protons at δ_H 4.53 (1H, q, *J* = 6.9, 6.8, 6.8Hz, H-2"), 4.19 (1H, d, *J* = 5.5 Hz, H-1") respectively carried by the carbons at δ_C 82.6 (C-2"), 73.8 (C-1"), a carbonyl group at δ_C 177.6 (C-1) characteristic of a carboxylic acid. In addition, ^{1}H-NMR also exhibited a peak of a methyl group at δ_H 1.36 (3H, d, *J* = 6.8 Hz, H-3"), carried by 18.1 (C-3"). We also observed on the ^{13}C-NMR spectrum (Fig. S12) and DEPT 135 (Fig. S14) peaks attributable to an aliphatic chain ranging from 27.7 to 31.5 ppm. The analysis of the HMBC (Fig. S15, Table 3) and COSY spectra (Fig. S17, Table 3) allowed us to observe some important correlations such as correlations between the protons at δ_H 2.64 (2H, m, H-12) with aromatic carbons at δ_C 142.9 (C-1'), 128.4 (C-2'), 128.2 (C-3') and with aliphatic carbons at δ_C 31.5 (C -11), 29.7 (C-10), 29.6 (C-9). In addition, correlations were also observed between a proton at δ_H 2.58 (1H, dt, 9.9 and 4.9 Hz, H-2) with the methylene carbons at δ_C 23.3 (C-3), 27.7 (C-4) and the carboxylic acid group at δ_C 177.6 (C-1). On the same spectrum, we observed correlations between diastereotopic protons at δ_H 1.87–1.79 (1H, m, H-3a) and 1.70 (1H, dt, *J* = 9.8, 5.0 Hz, H-3b) with carbons at δ_C 177.6 (C-1), 73.8 (C-1") and 43.8 (C-2), correlations between oxymethine protons at δ_H 4.19 (1H, d, J = 5.5 Hz, H-1") and 4.53 (1H, q, *J* = 6.9, 6.8, 6.8Hz, H-2"), respectively in $^{3}J_{C-H}$ and $^{4}J_{C-H}$ with the carboxylic group at δ_C 177.6 (C-1) and with the methyl group 18.1 (C-3") in $^{3}J_{C-H}$ and $^{2}J_{C-H}$ respectively. On its COSY spectrum, we observed a succession of scalar correlations between the protons H-3" and H-2"; H-2" and H-1"; H-1" and H-2; H-11 and H-12; H-12 and H-2'; H-2' and H-3'. All these correlations allowed us to attach the phenyl group with the aliphatic chain and the 1,2-dihydroxylpropyl group at position 2 of the skeleton. All this information from spectroscopic and spectrometric methods compared to compound **1** allowed us to elucidate compound **2** as 2-(1,2-dihydroxypropyl)-12-phenyldodecanoic acid, a new dihydroxyl fatty acid that was given the trivial name phenylgilgianic acid. Compounds **3**–**5** have been previously isolated from the genus *Trichilia*. Compounds **3** and **4** have been isolated from *T. casaretti* and *T. catigua* [20, 21], **5** has been isolated from *T. claussenii* [9], **6** was isolated for the first time from *Trichilia* genus but have been reported from other genera [22].

Table 3

^{1}H ;^{13}C NMR; HMBC and COSY data for compound **2**

Position	Compound 2			
	δ ^{13}C	δ ^{1}H (nH, m, J in Hz)	HMBC	COSY
1	177.6	-	H-2; H-1‟; H-2"; H-3a; H-3b	-
2	43.8	2.58 (1H, dt, 9.9 and 4.9)	H-3a; H-3b; H-2"	H-1"
3	23.4	1.87–1.79 ($1H_a$, m,) and 1.70 ($1H_b$, dt, J = 9.8, 5.0)	H-2	H-4
4	27.7	1.45 (2H, br d, *J* = 6.5)	H-2 ; H-3a ; H-3b	H-3
5–10	29.3–29.7	1.32 (12H, br d, *J* = 21.1)	H-3a; H-3b; H-4; H-11; H-12	-
11	31.5	1.67–1.62 (2H, m)	H-12	H-12
12	36.0	2.64 (2H, m)	H-3' ; H-2' ; H-11	H-2' ; H-11
1'	142.9	-	H-3' ; H-2' ; H-12 ; H-11	-
2'	128.4	7.21 (2H, d, J = 7.5)	H-12	H-12 ; H-3'
3'	128.2	7.30 (2H, t, *J* = 7.5)	H-12	H-2'
4'	125.5	7.20 (1H, d, J = 7.5)	H-3' ; H-2'	-
1"	73.8	4.19 (1H, d, J = 5.5)	H-3a; H-3b; H-2"	H-2 ; H-2"
2"	82.6	4.53 (1H, q, J = 6.9, 6.8, 6.8)	H-1"	H-3"; H-1"
3"	18.1	1.36 (3H, d, *J* = 6.8)	H-1"; H-2"	H-2"

Spectroscopic And Spectrometry Data Of The Isolated Compounds

Compound (**1**) gilgianic acid. White powder (8.1 mg). HRESIMS, *m/z* 407.2899 $[M + Cl]^-$ (calcd for $C_{22}H_{44}O_4Cl$, 407.2928). ^{1}H-NMR (500 MHz, Chloroform-*d*) δ_H 4.47 (1H, qd, *J* = 6.5 and 3.0 Hz, H-2'), 4.33 (1H, br s, H-1'), 2.58 (1H, dt, *J* = 9.9 and 4.9 Hz, H-2), 1.87–1.79 (2H, m, H-4), 1.67 (1H, dtd, *J* = 14.7, 9.7 and 5.4 Hz, H-3), 1.45 (4H, d, *J* = 6.4 Hz, H-3'), 1.27 (25H, br s,), 0.90 (3H, t, *J* = 6.8 Hz, H-19). ^{13}C-NMR (126 MHz, Chloroform-*d*) δ_C 177.8 (C-1), 47.6 (C-2), 23.3 (C-3), 22.7 (C-4), 27.6 (C-5), 29.7 (C-6), 29.7 (C-7), 29.65 (C-8), 29.63 (C-9), 29.6 (C-10), 29.5 (C-11), 29.4 (C-12), 29.3 (C-13), 29.7–29.3 (14–16), 31.9 (C-17), 14.1 (C-19), 71.2 (C-1'), 79.0 (C-2'), 13.7 (C-3') see Table 2.

Compound (**2**), phenylgilgianic acid. White powder (7.1 mg). HR-ESI-MS, *m/z* 385.2167 $[M + Cl]^-$ (calcd for $C_{21}H_{34}O_4Cl$, 385.2146). ^{1}H NMR (500 MHz, Chloroform-*d*) δ_H 7.30 (2H, t, *J* = 7.5 Hz, H-3'), 7.21 (2H, d, J = 7.5 Hz, H-2'), 7.20 (1H, d, *J* = 7.6 Hz, H-4'), 4.53 (1H, q, J = 6.9, 6.8, 6.8Hz, H-2"), 4.19 (1H, d, J = 5.5 Hz, H-1"), 2.64 (2H, m,

H-12), 2.58 (1H, dt, *J* = 9.9, 4.9 Hz, H-2), 1.87–1.79 (1H, m, H-3a),1.70 (1H, dt, J = 9.8, 5.0 Hz, H-3b), 1.67–1.62 (2H, m, H-11), 1.45 (2H, br d, *J* = 6.5 Hz, H-4), 1.36 (3H, d, *J* = 6.8 Hz, H-3"), 1.32 (12H, br d, *J* = 21.1Hz, H-5 to H-10). ^{13}C NMR (126 MHz, Chloroform-*d*) δ_C 177.6 (C-1), 142.9 (C-1'), 128.4 (C-2'), 128.2 (C-3'), 125.5 (C-4'), 82.6 (C-2"), 73.8 (C-1"), 43.8 (C-2), 36.0 (C-12), 31.5 (C-11), 29.7 (C-10), 29.6 (C-9), 29.57 (C-8), 29.5 (C-7), 29.4 (C-6), 29.3 (C-5), 27.7 (C-4), 23.3 (C-3), 18.(C-3") see Table 3.

Compound (**3**), *β*-sitosterol. White powder. 1H NMR (500 MHz, $CDCl_3$) δ_H 5.37 (1H, t, *J* = 5.0 Hz, H-6), 3.55 (1H, tt, *J* = 10.8, 4.7 Hz, H-3), 0.95 (3H, d, *J* = 6.5 Hz, H-21), 0.84 (3H, t, *J* = 7.2 Hz, H-29), 0.83 (3H, d, *J* = 6.5 Hz, H-27), 0.86 (3H, d, *J* = 6.5 Hz, H-28), 0.70 (3H, s, H-19), 1.08 (3H, s, H-18). ^{13}C NMR ($CDCl_3$, 126 MHz) δ_C 37.3(C-1), 36.2 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 31.7 (C-7), 31.9 (C-8), 50.2 (C-9), 36.5 (C-10), 21.1 (C-11), 39.9 (C-12), 42.2 (C-13), 56.1 (C-14), 23.3 (C-15), 28.3 (C-16), 56.1 (C-17), 11.9 (C-18), 18.8 (C-19), 40.7 (C-20), 19.9 (C-21), 34.0 (C-22), 26.2 (C-23), 45.9 (C-24), 29.2 (C-25), 19.1 (C-26), 19.4 (C-27), 24.3 (C-28), 12.0 (C-29). These data were in accordance with those reported by [23] and the compound was found to be *β*-sitosterol.

Compound (**4**), stigmasterol. White powder. ^{1}H NMR ($CDCl_3$, 500 MHz) δ_H 0.86 (3H, t, *J* = 7.2 Hz, H-28), 0.83 (3H, d, *J* = 6.7 Hz, H-27), 0.81 (3H, d, *J* = 6.7 Hz, H-26), 0.92 (d, *J* = 6.5 Hz, H-21), 0.74 (3H, s, H-19), 1.05 (3H,s, H-18); 3.53 (1H, tdd, *J* = 4.6, 4.5, 3.7 Hz, H-3), 5.34 (3H, t, *J* = 6.5 Hz, H-5), 4.98 (1H, m, H-22), 5.14 (1H, m, H-23). ^{13}C NMR ($CDCl_3$, 126 MHz) δ_C 37.5 (C-1), 32.3 (C-2), 72.4 (C-3), 42.5 (C-4), 141.4 (C-5), 121.7 (C-6), 31.6 (C-7), 31.9 (C-8), 50.6 (C-9), 36.3 (C-10), 21.7 (C-11), 40.0 (C-12), 42.2 (C-13), 56.5 (C-14), 24.6 (C-15), 29.4 (C-16), 56.1 (C-17), 12.4 (C-18), 18.7 (C-19), 40.7 (C-20), 21.8 (C-21), 138.9 (C-22), 129.8 (C-23), 46.5 (C-24), 29.7 (C-25), 19.7 (C-26), 20.6 (C-27), 25.7 (C-28), 12.8 (C-29). These data were in accordance with those reported by [24] and the compound was found to be stigmasterol.

Compound (**5**), sitosterol-3-O-*β*-D-glycoside. White amorphous solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ_H 2.97 (1H, m, H-3), 2.28 (1H, dt, *J* = 4.7, 8.12 Hz, H-4a), 1.97 (1H, ddd, *J* = 2.01, 12.91, 12.95 Hz, H-4b), 5.32 (1H, t, *J* = 3.7 Hz, H-6), 1.75 (1H, ddd, *J* = 2.7, 7.2, 15.9 Hz, H-7a), 1.93 (1H, ddd, *J* = 15.9, 2.7, 7.2 Hz, H-7b), 0.72 (3H, s, H-18), 0.92 (3H, s, H-19), 0.96 (3H, d, *J* = 6.7 Hz, H-21), 0.86 (3H, d, *J* = 7.2 Hz, H-26), 0.89 (3H, d, *J* = 7.2 Hz, H-27), 1.33 (2H, m, H-28), 0.99 3H, (t, *J* = 7.2 Hz, H-29), 4.24 (1H, d, *J* = 7.7 Hz, H-1'), 2.86 (1H, dt, *J* = 4.7, 8.1 Hz, H-2'), 3.29 (1H, dt, *J* = 4.7, 8.1 Hz, H-3') 3.10 (1H, dt, *J* = 4.7, 8.1 Hz, H-4'), 3.10 m (1H, dt, *J* = 4.7, 8.1 Hz, H-5'), 4.58 (1H, dd, *J* = 2.7, 11.8 Hz, H-6a'), 4.43 (1H, dd, *J* = 5.4, 11.8 Hz, H-6b'), 3.58 (1H, d, *J* = 4.8 Hz, OH-2'), 3.55 (1H, d, *J* = 4.7 Hz, OH-3'), 3.42 (1H, d, *J* = 4.7 Hz, OH 4'), 3.63 (1H, t, *J* = 4.7 Hz, OH-6'). ^{13}C NMR (DMSO-d_6, 126 MHz) δ_C 36.8 (C-1), 29.2 (C-2), 76.9 (C-3), 39.3 (C-4), 140.4 (C-5), 121.2 (C-6), 31.9 (C-7), 31.3 (C-8), 49.6 (C-9), 36.2 (C-10), 20.6 (C-11), 38.3 (C-12), 41.8 (C-13), 56.1 (C-14), 23.8 (C-15), 27.8 (C-16), 55.4 (C-17), 11.7 (C-18), 19.1 (C-19), 35.5 (C-20), 18.6 (C-21), 33.3 (C-22), 25.4 (C-23), 45.1 (C-24), 28.6 (C-25), 19.7 (C-26), 18.9 (C-27), 22.1 (C-28), 11.8 (C-29), 101.7 (C-1'), 73.5 (C-2'), 76.9 (C-3'), 70.2 (C-4'), 76.8 (C-5'), 62.9 (C-6'). These data were in accordance with those reported by [19] and the compound was found to be *β*-sitosterol-3-O- β -D-glucopyranoside.

Compound (**6**), lupeol. White powder. ^{1}H NMR ($CDCl_3$, 500 MHz): 4.72 (1H, s, H-29a), 4.56 (1H, s, H-29b), 3.1 (1H, m, H-3), 0.78 (3H, s, H-25), 0.80 (3H, s, H-24), 0.87 (3H, s, H-26), 0.95 (3H, s, H-27), 0.97 (3H, s, H-23), 1.68 (1H, s, H-30). ^{13}C NMR($CDCl_3$, 126 MHz): 38.7 (C-1), 27.8 (C-2), 79.6 (C-3), 38.5 (C-4), 55.1 (C-5), 18.3 (C-6), 33.5 (C-7), 41.8 (C-8), 50.5 (C-9), 37.2 (C-10), 20.3 (C-11), 23.5 (C-12), 32.7 (C-13), 42.4 (C-14), 27.3 (C-15), 40.6 (C-16), 48.4 (C-17), 53.5 (C-18), 47.6 (C-19), 148.3 (C-20), 27.8 (C-21), 44.6 C-22), 28.3 (C-23), 15.8 (C-24), 16.7 (C-

25), 16.2 (C-26), 15.4 (C-27), 16.7 (C-28), 109.4 (C-29), 19.3 (C-30). By comparison with literature data [18], this compound was found to be lup-20(29)-en-3β-ol or lupeol.

Discussion

Antibacterial activity

Several species from the *Trichilia* genus and their secondary metabolites have been reported to possess good and potent antimicrobial activities [21, 25–27]. Based on these previous reports, the crude extract and the major isolated compounds **1**–**5** were evaluated for their antibacterial activities against twelve bacterial strains: *Staphylococcus epidermidis* (*SE*), *Enterococcus faecalis* (*EF*), *Escherichia coli* (*EC*), *Mycobacterium smegmatis* (*MS*), *Staphylococcus aureus* (*SA*), *Enterobacter cloacae* (*ECL*), *Klebsiella oxytoca* (*KO*), *Proteus vulgaris* (*PV*), *Klebsiella pneumoniae* (*KP*), *Proteus mirabilis* (*PM*), *Klebsiella aerogenes* (*KA*) and *Bacillus subtilis* (*BS*). The crude extract and compounds exhibited activity against all the microorganisms with MIC values in a range of 2.3–73.5 μg/mL (Table 1, Figs. 2 and 3). Interestingly, the strongest activity was shown against *B. subtilis* and *K. aerogenes* for all tested compounds with MIC values of 2.3 μg/mL, which is even lower than that of ampicillin (MIC 3.3 µg/mL), used as positive control. In comparison to the other isolated compounds, the new fatty acids **1** and **2** showed the weakest activity against all the organisms tested. Compound **4** only differs from compound **3** by the presence of an extra double bond in its structure, however it is quite noticeable that the activity exhibited by compound **3** against all tested bacteria is higher than that of compound **4**, implying that the presence of a double bond on the aliphatic chain of compound **4** reduces the activity of this compound. However, the activity of compound **5** (which differs from **3** by the presence of a sugar) against *Proteus vulgaris* and *Proteus mirabilis* (MIC 4.6 µg/mL) was higher than that of compound **3**. Thus, it can be hypothesised that a slight modification of the chemistry of compound **3** could play an important role in the structure activity relationship (SAR) of this class of compounds.

Table 1
Minimum inhibitory concentrations of the isolated compounds and crude extract on different bacterial strains

Minimum inhibitory concentration (MIC, μg/mL)												
Compounds tested	Gram-positive					Gram-negative						
1	BS	MS	SA	SE	EF	KA	ECL	KO	PV	KP	EC	PM
2	2.3	18.3	36.7	73.5	36.7	2.3	18.3	36.7	18.3	36.7	18.3	36.7
3	2.3	18.3	36.7	73.5	36.7	2.3	18.3	36.7	18.3	36.7	18.3	36.7
4	2.3	18.3	18.3	18.3	2.3	2.3	18.3	18.3	18.3	9.2	18.3	18.3
	2.3	36.7	36.7	36.7	18.3	2.3	36.7	36.7	36.7	18.3	36.7	36.7
5	2.3	18.3	36.7	36.7	2.3	2.3	36.7	18.3	36.7	4.6	36.7	4.6
Crude extract	2.3	18.3	18.3	36.7	36.7	2.3	18.3	36.7	18.3	36.7	18.3	18.3
NLD	**2**	**64**	**8**	**8**	**>64**	**32**	**2**	**1**	**16**	**32**	**64**	**4**
STM	**2**	**0.5**	**32**	**1**	**16**	**2**	**64**	**2**	**16**	**2**	**8**	**16**
AMP	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**3.3**	**52**	**3.3**	**3.3**	**3.3**

STM, Streptomycin; NLD, Nalidixic acid; AMP, Ampicillin; *EC*, *Escherichia coli*; *ECL*, *Enterobacter cloacae*; *SE, Staphylococcus epidermidis*; *EF*, *Enterococcus faecalis*; *MS*, *Mycobacterium smegmatis*; *PV*, *Proteus vulgaris*; *KP*, *Klebsiella pneumoniae*; *SA*, *Staphylococcus aureus*; *BS*, *Bacillus subtilis; PM, Proteus mirabilis*; *KO*, *Klebsiella oxytoca*; *KA*, *Klebsiella aerogenes*

Conclusion

In summary, two novels fatty acids **1** and **2** together with four known compounds (**3**–**6**) were isolated from the stem bark of *Trichilia gilgiana* Harms (Miliaceae) and identified. To the best of our knowledge this is the first report of the isolation of a fatty acid from the genus *Trichilia*. Investigation of the antimicrobial activity of the isolates (**1**–**5**) revealed that they exhibited activity against all the microorganisms tested with MIC values ranging from 2.3–73.5 μg/mL. The most potent activity of all the tested compounds was shown against *B. substilis* and *K. aerogenes* with an MIC value of 2.3 μg/mL which was better than that of the positive control ampicillin (3.3 μg/mL). Although the new isolated fatty acid did not show an outstanding biological result in comparison to the other isolated compounds, this finding can allow us to explore the structural diversity of secondary metabolites from the genus *Trichilia* and offer new guidance to the discovery of new antibacterial agents.

Experimental

General experimental procedures and instrumentation

Reagents for extraction and isolation were used as supplied by Sigma-Aldrich South Africa without further purification. Column chromatography was carried out using Merck silica gel 60 (particle size 0.063-0.200 mm).

Thin-layer chromatography was carried out using pre-coated Merck silica gel F_{254} plates and viewed under UV light (Spectroline ENF – 260C/FE) (254/365 nm). High-resolution mass spectra were recorded using a Waters API Q-TOF Ultima spectrometer (University of Stellenbosch, South Africa). NMR spectra were recorded using Bruker 500 MHz spectrometer using signal frequency of 126 MHz for ^{13}C and DEPT 135 while all the other experiments used a signal of 500 MHz and were referenced using residual protonated solvent signals (δ_H: 7.260 ppm for $CDCl_3$ and 2.50 ppm for DMSO-d_6; δ_C: 77.000 ppm for $CDCl_3$ and 39.50 ppm for DMSO-d_6). Chemical shifts (δ) are expressed in ppm relative to tetramethylsilane (TMS) as internal standard and coupling constants (J) are given in Hz.

Plant material

The stem bark of *Trichilia gilgiana* Harms was collected on the 09th ofJune 2019 at Mount Kala, Yaounde, in the Centre Region of Cameroon. It was identified at the Cameroon National Herbarium, Yaounde by a botanist Victor Nana and a voucher specimen (No.: CNH/46221) was deposited. The harvested stem bark was chopped, air-dried and crushed, to afford 3200.92g of powder.

Extraction and isolation

A portion (2500.54 g) of the obtained powder was macerated in DCM/MeOH (1:1) at room temperature for 72 hours and evaporated using a rotary evaporator. Two successive macerations yielded 500.20 g of oily crude extract. 120.10 g of the obtained extract was mounted in a silica gel (0.63-0.200 mm) column chromatography and subjected to gradient elution with the solvent *n*-hexane-EtOAc (with increasing polarity from 100:0 to 00:100) and EtOAc-MeOH (from 100:0 to 60:40). 630 fractions of 200 mL were collected out of this fractionation procedure. Based on thin-layer chromatography (TLC) analysis, some fractions were combined and the precipitates obtained were filtered and recrystallized leading to compounds **1** (8.1 mg), **2** (7.1 mg), **3** (14.1 mg), **4** (11.8 mg) **5** (17.3 mg) and **6** (4.1 mg).

Antibacterial assay

For this study, microbial strains were purchased from Davies Diagnostic, South Africa, and were maintained in glycerol at –8 ºC prior use. It included Gram-positive strains namely: *Bacillus subtilis* (BS)(ATCC19659), *Enterococcus faecalis* (*EF*) (ATCC13047), *Staphylococcus epidermidis* (SE) (ATCC14990), *Staphylococcus aureous* (SA) (ATCC25923), *Mycobaterium smegmatis* (MS)(MC2155) and Gram-negative bacteria: *Enterobacter cloacae* (ECL)(ATCC13047), *Proteus vulgaris* (PV)(ATCC6380), *Klebsiella oxytoca* (KO) (ATCC8724), *Klebsiella pneumonia* (KP) (ATCC13882), *Proteus mirabilis* (PM)(ATCC7002), *Escherischia coli* (EC) (ATCC25922), *Klebsiella aerogenes* (KA) (ATCC13048).

The Microdilution method was used to evaluate the minimum inhibitory concentration (MIC) which is defined as the lowest concentration of the antimicrobial agent that inhibits the microbial growth after 24 h of incubation according to [28]. To achieve this, stock solution of substances were serially diluted in 100 µL of nutrient broth in a 96 microtiter well plate yielding concentrations of 588, 294, 147, 74, 37 and 18.5 μg/mL. Thereafter, 100 µL of an overnight bacterial culture for each of the bacterium to be tested was brought to 0.5 Mc Farland in nutrient broth, seeded in 96 microtiter plates and incubated at 37 °C for 24 h. Experiments were

carried out in duplicate. Streptomycin, ampicillin and nalidixic acid were used as positive control and negative control was prepared to contain 50 % nutrient broth in DMSO.

Declarations

CRediT authorship contribution statement

Michael Hermann Kengne Kamdem: Investigation, Writing - Original Draft. **Rostan Mangoua Talla:** Investigation. **Eric Morifi:** Resources, Investigation. **Blondelle Matio Kemkuignou:** Investigation. **Charlotte Mungho Tata:** Resources, Validation. **Gertrude Laura Foudjo Melacheu:** Project administration. **Marthe Carine Djuidje Fotsing:** Supervision. **Edwin Mpho Mmutlane:** Supervision. **Derek Tantoh Ndinteh:** Investigation, Writing - Review & Editing.

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Declaration of Competing Interest

No potential conflict of interest was reported by the authors.

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Figures

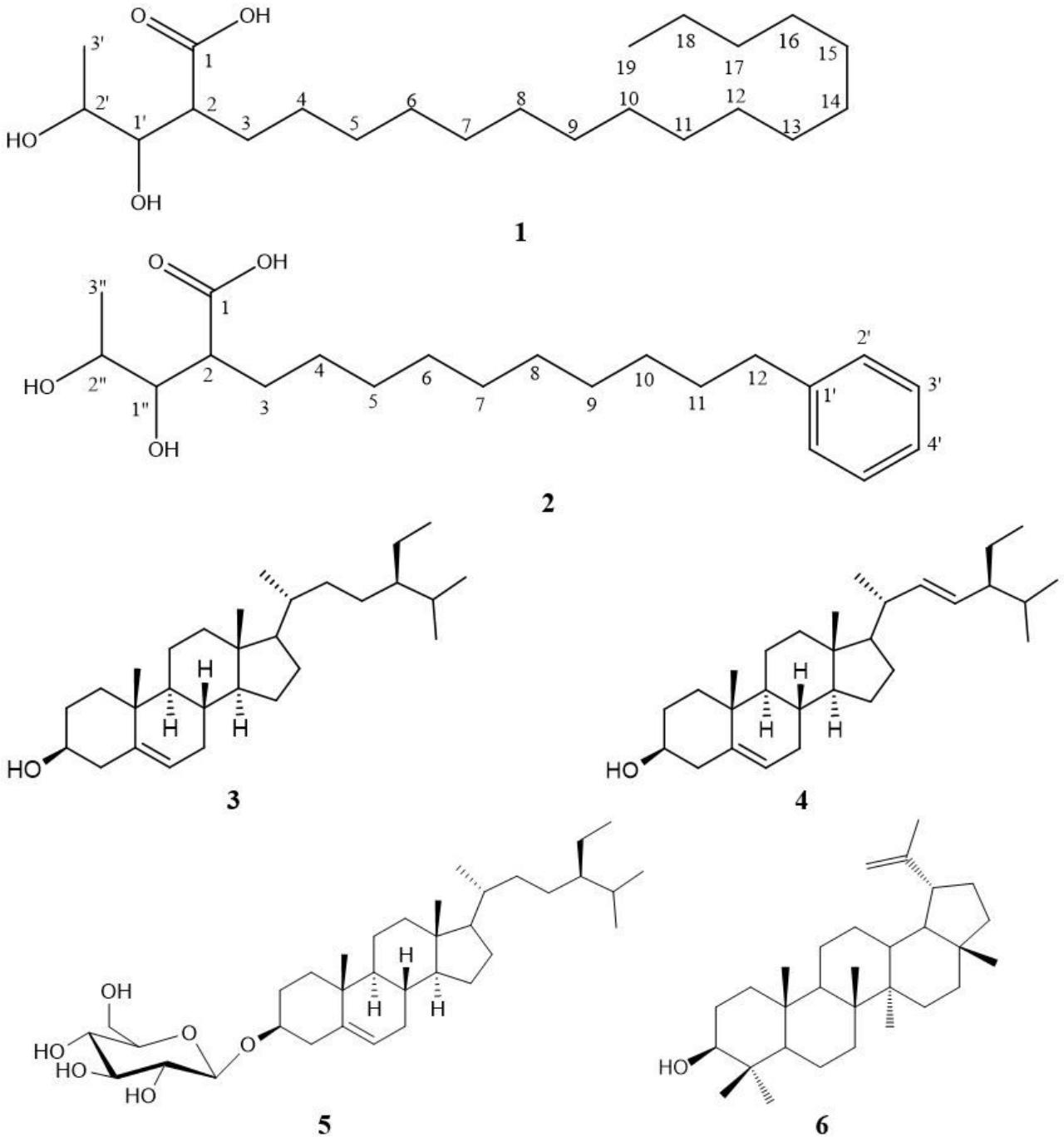

Figure 1

Chemical structures of compounds **1**–**6** isolated from the stem bark of *Trichilia gilgiana* Harms

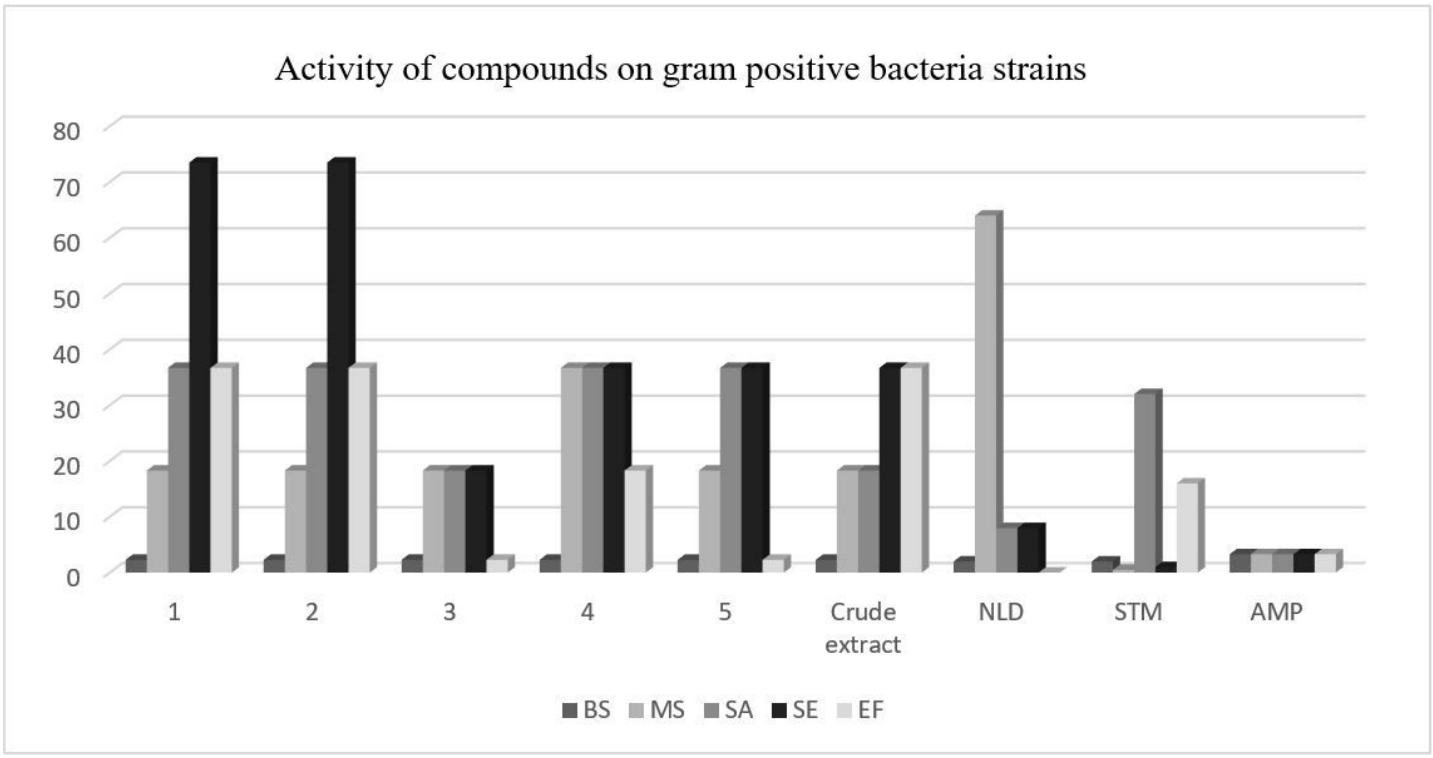

Figure 2

Activity of compounds on gram positive bacteria strains

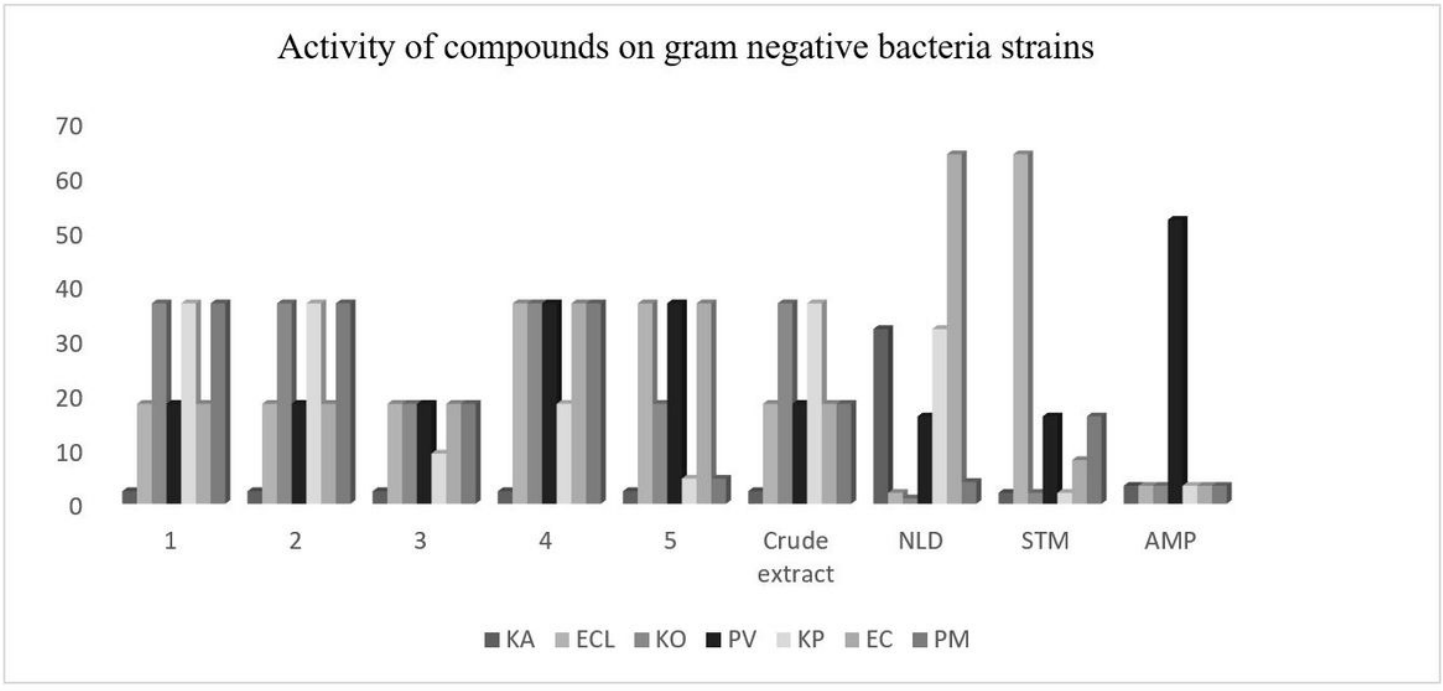

Figure 3

Activity of compounds on gram negative bacteria strains

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