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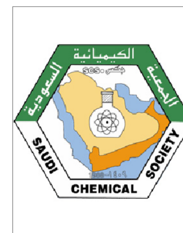

ORIGINAL ARTICLE

Pentacyclic Triterpenoids, Phytosteroids and Fatty Acid Isolated from the Stem-bark of *Cola lateritia* K. Schum. (Sterculiaceae) of Cameroon origin; Evaluation of Their Antibacterial Activity

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Abstract The phytochemical investigation on the chemical constituents of dichloromethane-methanol (1:1) stem-bark extract of *Cola lateritia* K. Schum. (Sterculiaceae) led to the isolation and characterization of five pentacyclic triterpenoids, one fatty acid and two phytosteroids. The compounds were identified as heptadecanoic acid (**1**), maslinic acid (**2**), betulinic acid (**3**), lupenone (**4**), lupeol (**5**), friedelin (**6**), β-stigmasterol (**7**) and *β*-sitosterol-3-*O*-ß-*D*-glucoside (**8**). Their structures were determined by NMR analysis (^{1}H, ^{13}C, DEPT-135, COSY, HMBC and HSQC), high-

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resolution mass spectrometry (HR-ESI-MS) and comparisons with published data in the literature. This work, to the best of our knowledge, is the first isolation and identification of these compounds in pure forms from *Cola lateritia*. Also, compounds **1**–**3** are reported for the first time from *Cola* genus. *In vitro* antibacterial activity of the isolated compounds (**1**–**8**) and the crude extract were evaluated against *Bacillus subtilis*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Mycobacterium smegmatis*, *Staphylococcus aureus*, *Enterobacter cloacae*, *Klebsiella oxytoca*, *Proteus vulgaris*, *Klebsiella pneumonia*, *Escherichia coli, Proteus mirabilis* and *Klebsiella aerogenes* with streptomycin, nalidixic acid and ampicillin as standard antibacterial drugs. Compound **2** was active against *E. faecalis* (MIC = 18.5 μg/mL), and it was 6.9 and 28 times lower and active than that of streptomycin (MIC 128 μg/mL) and nalidixic acid (MIC > 512 μg/mL) respectively. All the isolated compounds and crude extract showed significant activities against the tested bacterial strains.

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1. Introduction

Cola Schott & Endl. is an underutilized genus belonging to the family Sterculiaceae. The genus comprises about 125 species of medicinal plants, which are native to Western and Central Africa (Rätsch, 2005). *Cola* species are distributed mainly in the dry parts of the rainforest. However, some species, including *Cola millenii*, *Cola gigantea* and *Cola nitida* are commonly found in the dry and wet forest environments (N'Guessan, et al., 2018; Olorode, 1984). Traditionally, *Cola* species are used for treating many diseases and infections in different communities. *Cola millenii* leaves are ethnomedicinally used for treating ringworm, fever, opthalmia, dysentery and gonorrhoea (Odugbemi, 2006). The stems, flowers, twigs, leaves, and other parts of *Cola acuminate*, *Cola lateritia* and *Cola nitida* are used for the treatment of malaria, diarrhoea, dysentery, fever and coughs (Burkill, 1995; Irvine, 1961). Kola nuts, the mature fruits of the *Cola* species, are known for their characteristic bitter flavour and treatment of whooping cough, asthma, malaria and fever in many West African nations (Blades, 2000; Benjamin et al., 1991; Jayeola, 2001).

Available information from literature has shown that only few species of the *Cola* genus have been studied for their phytochemical constituents. Dongmo et al., 2019 identified unsaturated fatty acids and triterpenoids from the *Cola rostrata* K. Schum roots. Polyphenolic compounds were isolated from *Cola nitida* ssp. Alba (Atawodi et al., 2007). With regards to biological activities, some species from *Cola* genus have been studied for their various properties. The antibacterial activity of the aqueous extract of *Cola gigantea* leaves against *Escherichia coli, Bacillus subtilis*, and *Staphylococcus aureous* showed a very good activity (Sofowora et al., 2013; Kenneth et al., 2014). The antibacterial effects of hexane extracts of *Cola millenii* bark and leaves against *Escherichia coli* were greater than those of *Cola gigantean* (Nwidu et al., 2019). The decoction extract of *Cola acuminata* was reported to be toxic to the breast cancer cells (Abo et al., 2008). Currently, there is dearth of information on the chemical constituents as well as biological activities of *Cola lateritia*. Due to the numerous traditional uses of *Cola* species and our continuous attempt to search for novel antibacterial agents from plant sources, this study was aimed at investigating the phytochemical and antibacterial activity of *Cola lateritia*.

2. Material and methods

Materials: Reagents for extraction and isolation were used as supplied by Sigma-Aldrich. Thin layer chromatography was carried out using pre-coated Merck silica gel F_{254} plates. Merck silica gel 60 (0.040–0.063 mm) was used as stationary phase in carrying out column chromatography.

Instrumental: NMR spectra were recorded using Bruker 500 MHz and 600 MHz spectrometer using signal frequency of 125 MHz and 150.9 MHz for ^{13}C and DEPT 135 while all the other experiments used a signal of 500 MHz and 600 MHz and were referenced using residual protonated solvent signals (δH: 2.50 ppm for DMSO d_6, 3.31 ppm for CD_3-OD, 7.260 ppm for $CDCl_3$; δ_C: 77.000 ppm for $CDCl_3$, 39.50 ppm for DMSO d_6 and 49.00 ppm for CD_3OD). Chemical shifts (δ) are expressed relative to tetramethylsilane (TMS) in ppm as internal standard, and coupling constants are given in Hz. IR spectrum was recorded using a Perkin-Elmer FT-IR Spectrum 100 spectrometer operated in an attenuated total reflectance (ATR) mode. Mass spectra were recorded on a Waters API Q-TOF Ultima spectrometer (University of Stellenbosch, South Africa). Melting points were determined using a Reichert 281,313 hot-stage apparatus and were uncorrected.

2.1. General experimental procedures

2.1.1. Plant material

The stem-bark of *C. lateritia* was collected in September 2017 at Mount Kala, Yaounde, Cameroon. It was identified at the Cameroon National Herbarium, Cameroon where a voucher specimen (No.: CNH/56668) was deposited. Then, the harvested stem-bark were chopped, air-dried and crushed to afford 3200.92 g of powder.

2.1.2. Extraction and isolation

A portion (2500.54 g) of the obtained powder was macerated in CH_2Cl_2/MeOH (1:1) solvent mixture at room temperature for 72 h and evaporated using a rotary evaporator. Three successive macerations yielded 115.20 g of crude extract.

110.14 g of the crude extract obtained was reconstituted in CH_2Cl_2/MeOH and impregnated with silica (0.63–0.200 mm). After complete evaporation of the solvent, the powder

obtained was mounted in a silica gel column (0.63–0.200 mm) and subjected to gradient elution with the solvent mixture hexane, hexane/ethyl acetate, ethyl acetate, ethyl acetate / methanol and finally with methanol (100% hexane, fractions 1–29; 2% EtOAc in hexane, fractions 30–71; 5% EtOAc in hexane, fractions 72–90; 10% EtOAc in hexane, fractions 91–125; 15% EtOAc in hexane, fractions 126–147; 20% EtOAc in hexane, fractions 148–172; 25% EtOAc in hexane, fractions 173–201; 30% EtOAc in hexane, fractions 202–219; 35% EtOAc in hexane, fractions 220–232; 35% EtOAc in hexane, fractions 233–250; 40% EtOAc in hexane, fractions 251–272; 60% EtOAc in hexane, fractions 273–293; 80% EtOAc in hexane, fractions 294–301; 100% EtOAc, fractions 302–314; 10% MeOH in EtOAc, fractions 315–325; 100% MeOH 326–350). A total of 350 fractions of 200 mL each were collected and then grouped into 5 series indexed from A to E according to similar chromatographic profiles. A white precipitate obtained from fractions 35–40 was recrystallized with MeOH leading to compound **6** (20 mg). The precipitate obtained from fractions 75–81 was washed using MeOH and filtered, giving a white powder, compound **4** (31 mg). After 24 h, precipitates were formed in fractions 95–103 and were mixed based on TLC. The precipitate was then washed using MeOH and filtered, yielding compound **5** (40 mg) a white powder. Fractions 107–114 were combined on the basis of TLC and washed using MeOH leading to a white powder, compound **1** (34 mg). The precipitate obtained from fractions 117–123 were filtered several times using hexane leading to a white powder, compound **7** (37 mg). A precipitate obtained from fractions 175–181 were combined on the basis of TLC and washed using hexane and acetone leading to a white powder, compound **3** (25 mg). Fractions 260–267 were left at room temperature and after two days, a deposit of white powder was observed in them. The powder was washed with hexane and filtered to yield to a white powder, compound **2** (21 mg). Fractions 294–298 were mixed on the basis of TLC and washed with MeOH leading to a white precipitate, compound **8** (30 mg).

2.1.3. Antibacterial assay

In vitro antibacterial activity of the isolated compounds (**1**–**8**) and the crude extract were studied against twelve bacteria strains using broth microdilution technique. The bacteria tested comprised of Gram-positive and Gram-negative strains which were purchased from Davies Diagnostic, South Africa. These include *Staphylococcus aureus* (**SA**) (**ATCC25923**), *Bacillus subtilis* (**BS**) (**ATCC19659**), *Mycobacterium smegmatis* (**MS**) **(MC2155)**, *Enterococcus faecalis* (**EF**) (**ATCC13047**), *Staphylococcus epidermidis* (**SE**) **(ATCC14990)**, *Enterobacter cloacae* (**ECL**) (**ATCC13047**), *Escherischia coli* (**EC**) (**ATCC25922**), *Klebsiella aerogenes* (**Ka**) (**ATCC13048**), *Klebsiella oxytoca* (**KO**) (**ATCC8724**), *Proteus mirabilis* (**PM**) (**ATCC7002**), *Proteus vulgaris* (**PV**) (**ATCC33420**) and *Klebsiella pneumonia* (**KP**) (**ATCC13882**). Minimum inhibitory concentration (MIC) of the compounds and crude extract were carried out following the procedure described by Fonkui et al., 2018, with little modification. Briefly, stock solutions were prepared by adding 3.4 mL of DMSO to 4 mg of each isolated compound in a vial. Each of these solutions was then serially diluted (6 times) in 100 uL of nutrient broth in a 96 well plates to the desired concentrations (588, 294, 147, 74, 37 and 18.5 µg/mL). Thereafter, 100 µL (in duplicate) of each of these solutions was seeded with 100 µL of an overnight bacterial culture brought to 0.5 Mc Farland in nutrient broth. The positive controls consisted of ampicillin, streptomycin and nalidixic acid while the negative control was made up of 50% nutrient broth in DMSO. Viable bacteria were confirmed in the presence of resazurin dye after 4 h incubation, and the MIC was taken for each compound (Table 1). All equipment and culture media were sterilised before use.

2.1.4. Physical and spectroscopic data of the compounds 1–8

Compound 1, Heptadecanoic acid: white powder (34.0 mg); mp. 61.3 ^{0}C; HR-MS: $[M+Na]^{+}$ at *m/z*: 293.2447; ^{1}H NMR ($CDCl_3$): 2.22 (2H, t, *J* = 7.3 Hz, H-2); 1.28 [m, 26H,

Table 1 Minimum inhibitory concentration of isolated compounds and the crude extract compared with standard drugs.

Minimum inhibitory concentration (MIC, µg/mL)												
Test compound	Gram-positive						Gram-negative					
	BS	EF	SE	SA	MS	ECL	PV	KO	KP	PM	EC	KA
1	18.5	294	294	294	294	294	294	294	147	294	294	18.5
2	18.5	18.5	18.5	18.5	18.5	18.5	18.5	18.5	74.0	18.5	18.5	18.5
3	18.5	147	294	294	294	294	294	294	147	294	294	18.5
4	18.5	294	294	294	147	294	294	294	147	294	294	18.5
5	18.5	294	298	294	294	294	294	294	294	294	294	18.5
6	18.5	294	588	588	294	588	294	588	294	588	588	294
7	18.5	147	294	294	294	294	294	294	147	294	294	18.5
8	18.5	294	294	294	294	294	294	588	294	588	294	18.5
Crude Extract	18.5	74	294	147	74	147	294	147	74	294	294	18.5
AMP	26	26	26	26	26	26	416	26	26	26	26	26
STM	16	128	8	256	4	512	128	16	16	128	64	512
NLD	16	> 512	64	64	512	16	128	8	256	32	512	256

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

$(CH2)_{13}$]; 0.86 (t, 3H, *J* = 6.5 Hz, H-17), ^{13}C NMR ($CDCl_3$): 176.5 (C-1), 31.9 (C-2), 31.2 (C-3), 29.4 (C-4), 14.1 (C-17).

Compound 2, Maslinic acid: Colourless needles (21.0 mg); mp. 248–250 ^{0}C; HR-MS: $[M+Na]^+$ at *m*/*z* 495.4339; UV: 227 nm, IR (KBr) cm^{-1}: 3435, 2926, 2838, 2712, 1742, 1665, 1463, 1380, 1254, 1161, 1139, 1112, 1041, 1013, 975, 823, 794, 728, 580 cm^{-1}; ^{1}H NMR (DMSO d_6): 0.88 (s, 3H, Me-23), 0.96 (s, 3H, Me-24), 0.98 (s, 3H, Me-25), 0.99 (s, 3H, Me-26), 1.02 (s, 3H, Me-27), 1.22 (s, 3H, Me-28), 1.24 (s, 3H, Me-29 and Me-30), 3.25 (dd, 1H, *J* = 4.6 and 14.6 Hz), 3.36 (d, 1H, *J* = 9.1 Hz, H-3), 4.11 (ddd, 1H, *J* = 4.6, 9.1 and 11.1 Hz, H-2), 5.45 (s, H-12); ^{13}C NMR (CD_3OD, 150 MHz): 46.5 (t, C-1), 67.9 (d, C-2), 88.9 (d, C-3), 39.0 (s, C-4), 55.4 (d, C-5), 18.1 (t, C-6), 31.9 (t, C-7), 38.4 (s, C-8), 48.1 (d, C-9), 37.7 (s, C-10), 23.4 (t, C-11), 122.3 (d, C-12), 144.1 (s, C-13), 41.4 (s, C-14), 27.5 (t, C-15), 22.9 (t,C-16), 46.2 (s, C-17), 41.5 (d, C-18), 45.6 (t, C-19), 30.0 (s, C-20), 33.2 (t, C-21), 32.5 (t, C-22), 27.9 (q, C-23), 16.10 (q, C-24), 15.7 (q, C-25), 16.30 (q, C-26), 25.0 (q, C-27), 180.1 (s, C-28), 32.4 (q, C-29), 22.4 (q, C-30) These data are in agreement with those reported by Kyeong et al., (2014).

Compound 3, Betulinic acid: white needles powder (24.8 mg); mp. 277–279 °C; EI-MS (positive ion mode): $[M^+]$ at *m*/*z* 456; IR v max (CCl_4) cm^{-1}: CH, C = C, C = O, C-H and O-H bend at 1457, 1648, 1688, 2942/2870 and 3447 respectively; ^{1}H NMR ($CDCl_3$, 600 MHz): 0.73 (s, Me-25), 0.86 (s, Me-24), 0.95 (s, Me-26), 0.97 (s, Me-27), 0.99 (s, Me-23), 1.70 (s, Me-30), 4.73 (s, H-29a) and 4.56 (s, H-29b), 3.12 (dd, *J* = 4.6, 11.2 Hz, H-3), 2.34 (ddd, *J* = 2.6, 11.6 Hz, H-13) and 3.04 (ddd, *J* = 4.8, 11.1 Hz, H-19); ^{13}C NMR ($CDCl_3$, 150 MHz): 39.8 (C-1), 28.8 (C-2), 78.7 (C-3), 39.9 (C-4), 56.4 (C-5), 19.3 (C-6), 35.3 (C-7), 41.6 (C-8), 51.5 (C-9), 38.1 (C-10), 21.7 (C-11), 26.7 (C-12), 39.2 (C-13), 43.3 (C-14), 31.7 (C-15), 33.4 (C-16), 57.2 (C-17), 50.3 (C-18), 48.3 (C-19), 151.8 (C-20), 30.8 (C-21), 38.1 (C-22), 29.2 (C-23), 16.8 (C-24), 16.9 (C-25), 16.9 (C-26), 15.4 (C-27), 179.3 (C-28), 110.4 (C-29), 19.9 (C-30) These data are in agreement with those reported by Machado et al., (2018).

Compound 4, Lupenone: white needles powder (31.1 mg), mp. 168–170 °C; EI-MS: $[(M+H^+)]$ at *m*/*z* 425, 109, 119, 135, 142, 154, 168; IR v max (CCl_4) cm^{-1}:

3079.7 (-C = CH–), 2924.3–2853.8 (C-H), 1738.3 (C = O), 1645.5 and 1376.9 (C = C), 1461.5 (CH_3/CH_2); ^{1}H NMR ($CDCl_3$, 600 MHz): 1.21 (s, Me-23), 1.21 (s, Me-24), 0.71 (s, Me-25), 1.22 (s, Me-26), 1.26 (s, Me-27), 1.26 (s, Me-28), 1.26 (s, Me-29), 1.72 (s, Me-30), 4.45 (t, *J* = 1.64 Hz, H-29α), 4.72 (t, *J* = 1.65 Hz, H-29β), 2.54 (m, H-2a), 2.43 (m, H-2b); ^{13}C NMR ($CDCl_3$, 150 MHz): 39.7 (C-1), 34.4 (C-2), 218.5 (C-3), 47.2 (C-4), 54.7 (C-5), 19.9 (C-6), 32.8 (C-7), 41.8 (C-8), 49.9 (C-9), 36.7 (C-10), 21.6 (C-11), 21.4 (C-12), 32.6 (C-13), 42.5 (C-14), 23.7 (C-15), 40.3 (C-16), 48.7 (C-17), 53.6 (C-18), 47.7 (C-19), 148.1 (C-20), 27.6 (C-21), 44.4 (C-22), 26.8 (C-23), 20.8 (C-24), 15.6 (C-25), 16.7 (C-26), 16.7 (C-27), 15.4 (C-28), 109.2 (C-29), 19.6 (C-30). These data were in accordance with those reported by Tsai et al., (2012).

Compound 5, Lupeol: white needles powder (40.0 mg); mp. 212–214 °C; EI-MS: $[M^+]$ at *m*/*z* = 426; IR v max (CCl_4) cm^{-1}: 3057, 2930, 2315, 1595, 1437, 1260; ^{1}H NMR ($CDCl_3$, 600 MHz): 4.72 (s, H-29α), 4.56 (s, H-29β), 3.1 (m, H-3), 0.78 (s, Me-25), 0.79 (s, Me-24), 0.86 (s, Me-26), 0.95 (s, Me-27), 0.98 (s, Me-23), 1.68 (s, Me-30); ^{13}C NMR($CDCl_3$, 150 MHz): 38.6 (C-1), 27.6 (C-2), 79.2 (C-3), 38.6 (C-4), 55.2 (C-5), 18.2 (C-6), 33.2 (C-7), 41.9 (C-8), 50.2 (C-9), 37.4 (C-10), 20.6 (C-11), 23.7 (C-12), 32.5 (C-13), 42.5 (C-14), 27.5 (C-15), 40.4 (C-16), 48.6 (C-17), 53.8 (C-18), 47.8 (C-19), 148.1 (C-20), 27.6 (C-21), 44.5C-22), 28.2 (C-23), 15.7 (C-24), 16.8 (C-25), 16.1 (C-26), 15.3 (C-27), 16.8 (C-28), 109.3 (C-29), 19.4 (C-30). Based on these physical and spectroscopic data, compound **5** was assigned as Lup-20(29)-en-3*β*-ol or Lupeol which was in agreement with Tsai et al., (2012).

Compound 6, Friedelin: white powder (20.0 mg); m.p. 258–260 °C (lit. 260–262 °C, Subhadhirasakul and Pharkphoom, 2005); IR v max (CCl_4)cm^{-1}:, 1720, 2930, 1450; EIMS *m*/*z* (% intensity): 426 ($[M^+]$, 6.47), 411 ([M−CH3]), 302 (6.13), 273 (4.24), 55 (100.00); ^{1}H NMR ($CDCl_3$, 600 MHz): 1.17 (s, Me-28), 1.10 (s, Me-27), 1.05 (s, Me-26), 1.04 (s, Me-30), 0.97 (s, Me-29), 0.88 (s, Me-25) and 0.73 (s, Me-24), 0.87 (d, *J* = 6.6 Hz, Me-23), 2.24 (q, *J* = 6.7 Hz, H-4), 2.42 (ddd, *J* = 13.5, 5.0, 2.2 Hz, H-2) and 2.30 (ddd, *J* = 13.5, 7.2, 1.2 Hz, H-2) ; ^{13}C NMR ($CDCl_3$, 150 MHz): 21.9 (C-1), 40.8 (C-2), 212.8 (C-3), 58.0 (C-4), 41.8 (C-5), 41.1 (C-6), 18.0 (C-7), 53.5 (C-8), 37.7 (C-9), 59.6 (C-10), 35.7 (C-11), 30.5 (C-12), 39.3 (C-13), 38.7 (C-14), 32.5 (C-15), 36.1 (C-16), 29.7 (C-17), 42.6 (C-18), 34.9 (C-19), 28.5 (C-20), 32.6 (C-21), 39.4 (C-22), 6.9 (C-23), 14.8 (C-24), 18.2 (C-25), 20.4 (C-26), 18.8 (C-27), 32.3 (C-28), 35.4 (C-29), 31.5 (C-30). These data were in agreement with those reported by Almeida et al. (2011).

Compound 7, Stigmasterol: White powder (37.0 mg); mp: 175–177 °C; MS (*m*/*z*): 412 $[M^+]$, 394, 351, 314, 300, 271, 229, 213, 55**;** IR v max (CCl_4)cm^{-1}:, 3451, 2930, 1628, 1452; ^{1}H NMR ($CDCl_3$, 500 MHz): 0.86 (t, *J* = 7.2 Hz, Me-28), 0.83 (d, *J* = 6.7 Hz, Me-27), 0.81 (d, *J* = 6.7 Hz, Me-26), 0.92 (d, *J* = 6.5 Hz, Me-21), 0.74 (s, Me-19), 1.05 (s, Me-18); 3.53 (tdd, *J* = 4.6, 4.5, 3.7 Hz, H-3), 5.34 (t, *J* = 6.5 Hz, H-5), 4.98 (m, H-22), 5.14 (m, H-23), ^{13}CNMR ($CDCl_3$, 125 MHz): 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). The physical and spectral results were in agreement with the values reported by Jamal et al., (2009) and Subhadhirasakul and Pharkphoom (2005).

Compound 8, *β*-Sitosterol-3-*O*-*β*-*D*-glucoside: white amorphous solid (30.1 mg); mp: 279–281 °C; MS (*m*/*z*): 576.439 $[M^+]$; IR v max (CCl_4)cm^{-1}: 3410 (–OH), 2940/2870 (CH_3/CH_2), 1635 (C = C), 1458 (-C-H), 1370 (-C-H) , 1250 (C-O), 1165 (C-O); ^{1}H NMR (DMSO d_6, 500 MHz): 2.97 (m, H-3), 2.28 (dt, *J* = 4.7, 8.12 Hz, H-4a), 1.97 (ddd, *J* = 2.01, 12.91, 12.95 Hz, H-4b), 5.32 (t, *J* = 3.7 Hz, H-6), 1.75 (ddd, *J* = 2.7, 7.2, 15.9 Hz, H-7a), 1.93 (ddd, *J* = 15.9, 2.7, 7.2 Hz, H-7b), 0.72 (s, Me-18), 0.92 (s, Me-19), 0.96 (d, *J* = 6.7 Hz, Me-21), 0.86 (d, *J* = 7.2 Hz, Me-26), 0.89 (d, *J* = 7.2 Hz, Me-27), 1.33 (m, CH_2-28), 0.99 (t, *J* = 7.2 Hz, Me-29), 4.24 (d, *J* = 7.7 Hz, H-1′), 2.86 (dt, *J* = 4.7, 8.1 Hz, H-2′), 3.29 (dt, *J* = 4.7, 8.1 Hz, H-3′) 3.10 (dt, *J* = 4.7, 8.1 Hz, H-4′), 3.10 m (dt, *J* = 4.7, 8.1 Hz, H-5′),

4.58 (dd, J = 2.7, 11.8 Hz, H-6a'), 4.43 (dd, J = 5.4, 11.8 Hz, H-6b'), 3.58 (d, J = 4.8 Hz, OH-2′), 3.55 (d, J = 4.7 Hz, OH-3′), 3.42 (d, J = 4.7 Hz, OH 4′), 3.63 (t, J = 4.7 Hz, OH-6′), ; ^{13}C NMR (DMSO d_6, 125 MHz): 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 Arora et al., (2013)

3. Results and discussion

The phytochemical investigation of dichloromethane-methanol (1:1) extract of the stem-bark of *C. lateritia* led to the isolation and characterization of eight known compounds such as: heptadecanoic acid (**1**), maslinic acid (**2**), betulinic acid (**3**), lupenone (**4**), lupeol (**5**), friedelin (**6**), stigmasterol (**7**) and *ß*-sitosterol-3-*O*-*ß*-*D*-glucoside (**8**). All these compounds were isolated for the first time from *C. lateritia*. Spectroscopic data of compound **1** has not been reported in the literature. To the best of our knowledge, this is the first time it is isolated from a

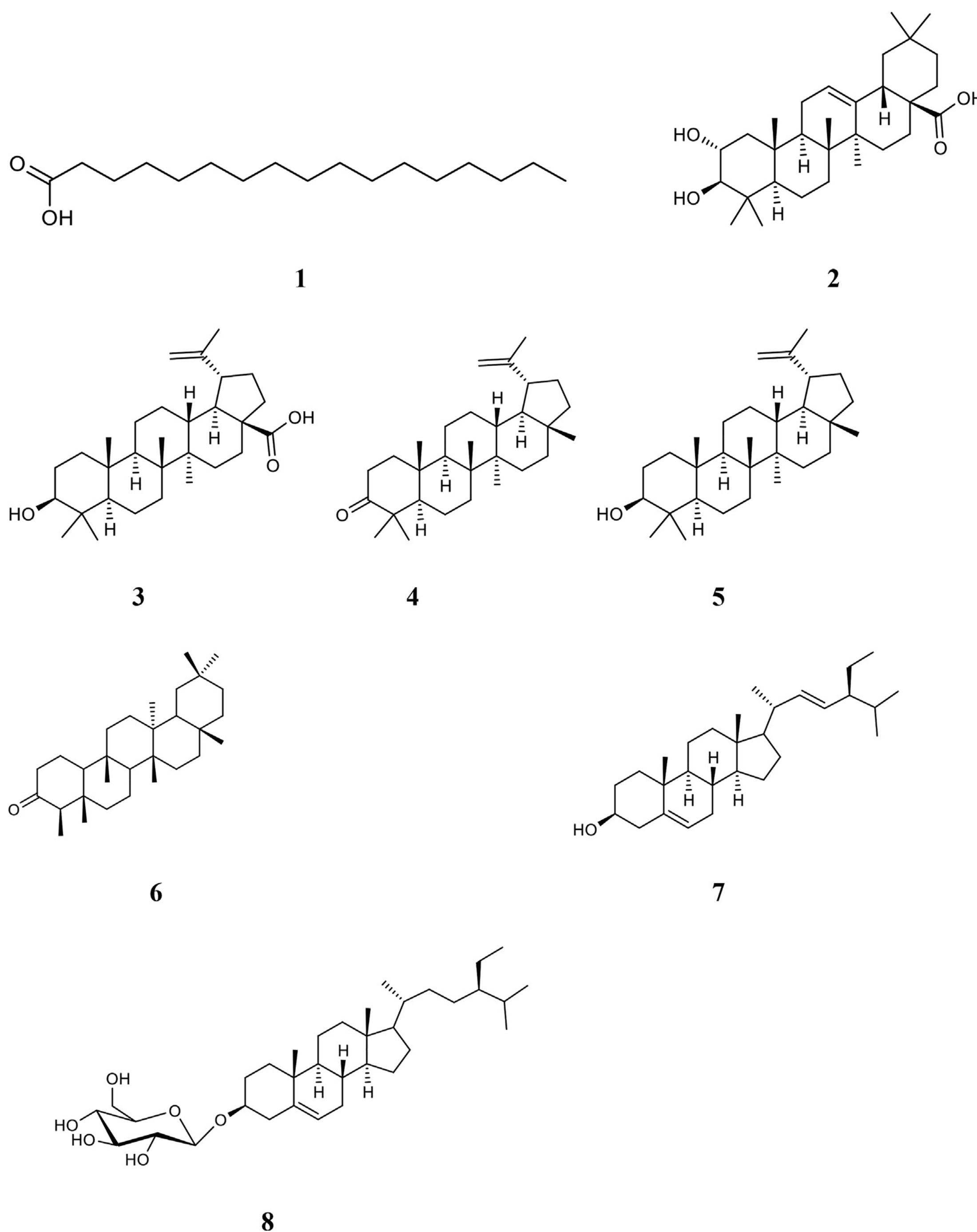

Fig. 1 Structures of compounds 1–8 isolated from the stem bark of Cola lateritia.

natural source in pure form, but it has been reported in the literature (Pfeuffer and Jaudszus, 2016). Fig. 1 depicts the chemical structures of all the isolated compounds.

The antibacterial activity of the eight compounds was tested against five Gram-positive and seven Gram-negative bacteria strains using microdilution method. Table 1 depicts the MICs of the eight compounds against twelve bacteria strains. Tested compounds exhibited broad spectrum of antibacterial activity and their efficacy was concentration-dependent. For instance, amongst the Gram-negative bacteria, *K. aerogenes* was the most susceptible strain to all the compounds, with an MIC of 18.5 μg/mL except compound **6** (MIC 294 μg/mL). Therefore, these compounds appear to be 27.7 times more potent than streptomycin (MIC 512 μg/mL), 13.8 times more potent than nalidixic acid (MIC 256 μg/mL) and 1.4 time more potent than ampicillin (MIC 26 μg/mL). Likewise, amongst the Gram-positive bacteria investigated, *B. subtilis* was the most susceptible strain. All the compounds exhibited MIC of 18.5 μg/mL and thus being 1.4 times more potent than ampicillin (MIC 26 μg/mL).

Furthermore, compound **2** displayed best activity among all the isolated compounds with an MIC value of 18.5 μg/mL on all the strains except *K. pneumonia* where it exhibited activity at 74 μg/mL. Compound **2** was very active against *E. faecalis* with an MIC value of 18.5 μg/mL, which was 6.9 times and > 28 times lower than that of streptomycin (MIC 128 μg/mL) and nalidixic acid (MIC > 512 μg/mL) respectively. Similar result was obtained with *P. vulgaris* with an MIC value of 18.5 μg/mL which is 7 times lower than that of streptomycin or nalidixic acid (MIC 128 μg/mL) and 22.5 times lower than that of ampicillin (416 μg/mL). These observations suggest that compound **2** may be a more potent antibacterial agent than standard drugs like streptomycin, nalidixic acid and ampicillin for some of the tested bacterial, including *E. faecalis*, *S. aureous*; *P. vulgaris, EC*, *Escherichia coli*, *K. aerogenes* and *P. mirabilis*. The significant antibacterial activities of compound **2** corroborates many studies which have shown that triterpenoids from the same carbon skeleton such as oleanolic acid, ursolic acid and their derivatives have been reported to have good antibacterial activity (Jesus et al., 2015; Chouaïb et al., 2015). Maslinic acid has also been reported to have various biological activities including antitumor activity (Li et al, 2010), Anti-inflammatory activity (Napetschnig and Wu, 2013), Antiviral activity (Xu et al., 1996) and Cardioprotective effect (Hussain et al., 2012). The MIC values of compound **6** for the different bacteria strains compared to the other compounds and the standard drugs revealed that compound **6** was the least active of all the isolated compounds with MIC values ranging from 18.5 to 588 μg/ml. The poor activity of compound **6** could be explained by considering structure–activity relationship and polarity; compound **6** is the least polar amongst all the isolated compounds. One important factor that controls the interaction of cell membrane with different molecules is their polarity. Structure-activity relationship (SAR) showed that polar substituents or polar compounds have greater activity than non-polar substituents/compounds. For instance, compound **2** has two hydroxyl (–OH) groups in positions 2 and 3 of the first ring of the compound and according to the MIC obtained, we noticed that, that compound has the best antimicrobial activity. On the other hand, we noticed that compound **6** had the lowest activities against the bacteria strains compared to the other compounds.

This study brings a considerable significance for the use of this species against bacterial infections and corroborates that of several authors who reported the antibacterial activities of others species of the *Cola* genus (Ekalu and James, 2020). For instance, *Cola acuminata* was reported to demonstrate MIC ranging between 14 and 50 μg/ml against bacterial strains (Obey and Swamy, 2014). In another study, however *Cola nitida* showed better microbial potential against *K. pneumonia*, *E. coli*, *S. aureus*, and *S. pneumonia,* with MIC values ranging between (6.25–200 mg/ml) (Oghenerobo and Falodun, 2013). On the other hand, the leaf and stem bark of *Cola gigantea* exhibited significant antibacterial activity against *S. aureus*, *E. coli*, *B. subtilis*, and *P. aeruginosa*, with MIC values ranging between 0.125 and 2.75 mg/mL (Austarheim et al., 2012). Despite many studies that reported antibacterial activities of crude extracts from *Cola* genus, no isolated compound has been linked to be responsible for the antibacterial activities of the genus. At present, triterpenoids, steroids, and fatty acid isolated in this study demonstrated antibacterial activities.

We also noticed some compounds were more active than the crude extract with some bacteria strains. These results could be explained by the synergetic and/or antagonistic effects of different metabolites in the crude extract. The crude extract contains many compounds; which have different efficacy against the different bacterial strains, thus an antagonistic effect of those compounds will reduce the toxicity of the crude extracts to the various strains. This could be the reason why some compounds exhibited good activity than the crude extract.

4. Conclusion

The phytochemical studies of dichloromethane-methanol stem-bark extract of *C. lateritia* led to the isolation of eight compounds: one fatty acid, five triterpenoids and two phytosteroids. The isolated compounds and the crude extract showed good antibacterial activity against the twelve strains used; with one of the isolated compounds (Maslinic acid) being more efficacious than some of the standard drugs. The results obtained from this study and those previously reported in the literature confirm that the genus *Cola* is very rich in triterpenoids, steroids and fatty acids. The multiple biological activities of the isolated compounds and the crude extract justify the multiples uses of plants from *Cola* genus in traditional pharmacopeia.

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Rostan M. Talla: Formal analysis. **Thierry Y. Fonkui:** Formal analysis. **Kevine K. Silihe:** Writing – review & editing. **Charlotte M. Tata:** Supervision, writing – review & editing. **Marthe C. D. Fotsing:** Project administration, supervision, writing – review & editing. **Edwin M. Mmutlane:** Supervision, writing – review & editing. **Derek T. Ndinteh:** Supervision, writing – review & editing.

Declaration of Competing Interest

We have no competing interests to declare.

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Appendix A. Supplementary data

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