A new antimicrobial nor-friedelane-type triterpenoid and other constituents from *Plectranthus glandulosus* Hook. f. (Lamiaceae)

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ABSTRACT

Chemical investigation of the ethanol extract from the whole plant of *Plectranthus glandulosus* led to the isolation of a new nor-triterpenoid (1) along with seventeen known compounds (2–18) including seven triterpenoids, nine flavonoids and one steroid. Their structures were established on the basis of 1D- and 2D-NMR, IR, and MS experiments, and by comparison of their spectroscopic data with those of similar compounds reported in the literature. The EtOH extract and some isolated triterpenoids (1–4 and 13) were subjected to in vitro antimicrobial assays against a panel of pathogenic microorganisms, including Gram-positive and Gram-negative bacteria, and fungi using broth microdilution method. The EtOH extract displayed moderate activity (MIC = 512 μg/mL) against *Staphylococcus aureus* MSSA1, *Shigella flexneri* SDINT and *Cryptococcus neoformans* H99. Compounds 1, 4 and 13 showed the most potent antimicrobial effect with MICs of 32–256 μg/mL.
1. Introduction

The genus *Plectranthus* represents a large and widespread group of plant species of the Lamiaceae family. It comprises approximately 300 species distributed in tropical and subtropical areas of Africa, Asia and Australia (Lukhoba et al. 2006). *Plectranthus glandulosus* Hook. f. is an evergreen perennial flowering, highly branched herb up to 3 meters tall, widely distributed in West, Central, and South Africa (Pele and Le Berre 1966; Amvam et al. 1998). It is used in Cameroon’s traditional medicine to treat dermatitis, bellyache, venereal diseases, internal inflammation, and nerve ache (Pele and Le Berre 1966; Danga et al. 2014). Known as Ava in ‘Ewondo’, a local tongue in Cameroon, the plant is used as a spice mainly in the southern part of the country (Pele and Le Berre 1966). The literature review on previous chemical investigations of plant species of the genus *Plectranthus* indicated some essential oil compositions (Padalia and Verma 2011) and the isolation of flavonoids, diterpenoids and triterpenoids as main constituents (Razdan et al. 1982; Abdel-Mogib et al. 2002; Gaspar-Marques et al. 2006; Stavri et al. 2009; Nzogong et al. 2018a; Tsopmejio et al. 2019). Previous biological studies on *P. glandulosus* reported antinociceptive and anti-inflammatory effects (Fongang et al. 2016), antioxidant and insecticidal activities (Goudoum et al. 2009; Danga et al. 2015) as well as antibacterial activity (Nanmeni et al. 2021) of this plant extract. In our continuous search for bioactive secondary metabolites from Cameroonian medicinal plants (Tene et al. 2009; Mpetga et al. 2014; Nzogong et al. 2018a; Sonfack et al. 2021; Nanmeni et al. 2021), we isolated and characterized a new antimicrobial nor-triterpenoid (1) along with seventeen known compounds (2–18) from the whole plant of *P. glandulosus*.

2. Results and discussion

Fractionation of the EtOH extract of the whole plant of *P. glandulosus* on silica gel column chromatography (CC), followed by recrystallization and filtration led to the isolation of eighteen compounds (Figure 1) including a new nor-triterpenoid (1) and the known friedelin (2) (Mahato and Kundu 1994), lupeol (3) (Tamokou et al. 2011), oleanolic acid (4) (Palu et al. 2019), pilloin (5) (Nunez-Alarcon 1971), chrysoeriol (6) (Park et al. 2007), luteolin 7-methyl ether (7) (Saewan et al. 2011), 5-hydroxy-7,4’-dimethoxy-flavone (8) (Mangoyi et al. 2015), 5,6-dihydroxy-7,3’,4’-trimethoxyflavone (9) (Miski et al. 1983), ladanein (10) (Tóth et al. 2007), maslinic acid (11) (Garcia-Granados et al. 1998), benthamic acid (12) (Mahato and Kundu 1994), hederagenin (13) (Kizu and Tomimori 1982), cylicodiscic acid (14) (Tchivounda et al. 1990), β-sitosterol 3-O-β-D-glucopyranoside (15) (Nualkaew et al. 2015), chrysoeriol 5-O-β-D-glucopyranoside (16) (Markham and Moore 1980), 7-O-methyl luteolin 5-O-β-D-glucopyranoside (17) (Ulubelen et al. 1982), and galuteolin (18) (Ji et al. 1986). The structure of the new compound was determined based on the analysis of its spectroscopic and spectrometric data and comparison of these data with those of similar compounds reported in the literature.

Compound 1 was obtained as a white powder in acetone; [α]$_D^{23}$ +37.2 (c 0.10, CHCl$_3$). Its molecular formula was determined as C$_{30}$H$_{48}$O$_4$ from its positive HR-ESI-MS which exhibited the pseudo-molecular ion peak at $m/z$ 495.3446 [M + Na]$^+$ (calcd for
The IR spectrum showed characteristic absorption bands at 3449 (OH), 3064 (Csp\(^2\)-H), 2945–2872 (Csp\(^3\)-H), 1720 (C=O), 1640 (C=C) and 1164 (C-O) cm\(^{-1}\) (Figure S1). The structure of 1 was fully assigned after careful analyses of\(^1\)H,\(^13\)C, DEPT, \(^1\)H \(^1\)H COSY, HSQC, HMBC and MS experiments (Figures S2–S10, Table S1). The \(^1\)H NMR spectrum had a set of signals at \(\delta_H\) 0.89 (3H, d, \(J = 7.4\) Hz), 0.92 (3H, s), 0.93 (3H, s), 0.96 (3H, s), 1.28 (3H, s), 3.70 (1H, br d, \(J = 2.0\) Hz), 4.17 (1H, br d, \(J = 4.1\) Hz), 4.77 (1H, br s) and 4.99 (1H, br s) (Table S1) assignable to one secondary methyl, four angular methyls, two oxygenated methine protons, and two exomethylene protons suggesting a triterpenoid nature for compound 1 (Figure 1). The \(^13\)C NMR spectrum of 1 displayed signals of 29 carbons that were partially sorted by DEPT and HSQC spectra into seven quaternary (\(\delta_C\) 36.9 (C-5), 37.5 (C-9), 52.4 (C-13), 41.3 (C-14), 31.2 (C-17), 147.0 (C-20) and 176.7 (C-27)), six methine (\(\delta_C\) 72.6 (C-3), 48.7 (C-4), 51.4 (C-8), 61.5 (C-10), 45.0 (C-18), and 71.6 (C-21)), eleven methylene (\(\delta_C\) 17.2 (C-1), 35.0 (C-2), 41.1 (C-6), 15.7 (C-7), 38.4 (C-11), 26.9 (C-12), 27.8 (C-15), 36.5 (C-16), 25.8 (C-19), 40.7 (C-22), and 113.5 (C-29)), and five methyl (\(\delta_C\) 11.5 (C-23), 16.3 (C-24), 17.5 (C-25), 19.7 (C-26), and 33.9 (C-28)) carbons (Table S1), all characteristic for a 30-nor-triterpenoid of the friedelane-type (Giner-Pons et al. 1992; Giner et al. 1993; Mpeta et al. 2014) bearing hydroxyl groups at positions C-3 and C-21, an exomethylene at position C-20/C-29 and a carbonyl function at position C-27. An additional signal for 1 at \(\delta_H\) 3.40/\(\delta_C\) 50.4 (Table S1) was attributable to a methoxyl group. This methoxyl was found to form a methyl ester group at C-27, as evidenced by the HMBC correlation between the methoxyl protons at \(\delta_H\) 3.40 and the carbonyl at \(\delta_C\) 176.7 (C-27). Correlations observed in the HMBC spectrum between H-3 (\(\delta_H\) 3.70) and C-1 and C-5,
between H-21 (δH 4.17) and C-17, C-19, C-20, C-22, and C-29, and between H-18 (δH 1.81) and C-12, C-13, C-14, C-17, C-20 and C-27, confirmed the position of the two hydroxyl groups, the exomethylene and the carboxymethyl functions (Table S1, Figures S7 and S11). The β-orientation of the hydroxyl group at position C-21 was confirmed by correlations observed in the NOESY spectrum (Figure S8) between H3-26 (δH 0.92) and H-18 (δH 1.81), H-18 and H3-28 (δH 1.28), H3-28 and H-22b (δH 1.17), and H-22a (δH 2.15) and H-21 (δH 4.17). Further correlations observed between H-3 (δH 3.70) and H-2b (δH 1.51) and H-4 (δH 1.24), H-4 and H-10 (δH 0.74), and, H-10 and H-8 (δH 1.40) also confirmed that the hydroxyl group at position C-3 was β-oriented (Figure S11). Therefore, the structure of the new compound, 1, was elucidated as 3β,21β-dihydroxy-30-nor-(D:A)-friedo-olean-20(29)-en-27-oic acid methyl ester, trivially named methyl plectranthusate.

The EtOH extract and some isolated compounds (1–4 and 13) were tested for their antimicrobial activity (Table S2) against nine microorganisms including four Gram-positive (*Staphylococcus aureus* ATCC25923, methicillin sensitive *S. aureus* MSSA1, methicillin resistant *S. aureus* MRSA3, methicillin resistant *S. aureus* MRSA4) and two Gram-negative (*Shigella flexneri* SDINT, *Pseudomonas aeruginosa* ATCC27853) bacteria and three fungal strains (*Candida albicans* ATCC10231, *Candida tropicalis* PK233 and *Cryptococcus neoformans* H99). The EtOH extract displayed antimicrobial activity towards 9/9 (100%) of tested bacterial and fungal strains with moderate activity (100 < MIC < 625 μg/mL) (Tamokou et al. 2017) against *S. aureus* MSSA1, *S. flexneri* SDINT and *C. neoformans* H99 (Table S2). Compounds 1 and 13 were the most active samples inhibiting the growth of all nine tested microbes with MICs ranging from 32–256 μg/mL and 64–256 μg/mL, respectively. They were followed in decreasing order by 4 (MIC = 64–256 μg/mL), 2 (MIC = 128–256 μg/mL) and 3 (MIC = 256–256 μg/mL). Compounds 1, 4 and 13 showed moderate activity (10 < MIC ≤ 100 μg/mL) (Tamokou et al. 2017) against 5/9 (55.55%), 2/9 (22.22%) and 4/9 (44.44%) tested microbial species, respectively. The best antifungal activity was observed with compound 1 against *C. neoformans* H99 (MIC = 32 μg/mL; MFC = 64 μg/mL) whereas the best antibacterial activity was recorded with compounds 1, 4 and 13 against *S. aureus* ATCC25923 and *S. flexneri* SDINT (MIC = 64 μg/mL; MBC = 128 μg/mL). As shown in Table S2, nystatin and ciprofloxacin used as standard drugs were more potent against fungi (yeasts), Gram-positive and Gram-negative bacteria than the isolates (1–4 and 13). Our data showed that the sensitivity of microbes to the test samples varied from one microorganism to the other. Compounds 1 and 2 are friedelane-type triterpenoids. The highly functionalized structure of compound 1 could justify the difference in their activity against the test microorganisms. Also, compounds 4 and 13 are oleanane-type triterpenoids. However, the activity of 13 (23-hydroxyoleanolic acid) was equal or higher than that of 4 (oleanolic acid). In the latter case (observed with *S. aureus* MSSA1, *S. aureus* MRSA4, *P. aeruginosa* ATCC27853, *C. albicans* ATCC10231 and *C. neoformans* H99), this can be attributed to the additional hydroxyl group at position 23 in 13. In most of the cases, a microbicidal (bactericidal or fungicidal) effect with MMC/MIC ratio ≤ 4 was noted for compounds 1, 4 and 13 suggesting their lethal effect. The known antimicrobial mechanisms associated to the group of chemicals to which the isolated compounds belong may explain the antimicrobial potency of the
EtOH extract. Membrane disruption has been suggested as one of the likely mechanisms of action (Cowan 1999; Shah et al. 2004). This might also explain the antimicrobial activities of isolated triterpenoids (Cowan 1999; Shah et al. 2004). Triterpenoids, a class of compounds widely distributed in nature have shown diverse biological activities, including antimicrobial properties (Jain et al. 2001; Chiozem et al. 2009; Tene et al. 2009; Tamokou et al. 2011; Nzogong et al. 2018b).

3. Experimental

3.1. General experimental procedures

Optical rotations were measured with a JASCO P-1020 digital polarimeter. The NMR spectra were recorded on Bruker AV-400 or DRX-500 NMR spectrometers. The chemical shifts ($\delta$) were reported in parts per million (ppm) with reference to TMS and coupling constants ($J$) given in Hz. Deuterated solvents, methanol (CD$_3$OD), dimethyl sulfoxide (DMSO-$d_6$), pyridine (C$_5$D$_5$N), and chloroform (CDCl$_3$) were used (as solvents) for the NMR experiments. ESIMS and HRESIMS were carried out on an API Qstar time-of-flight spectrometer. Column chromatography (CC) was performed with silica gel 60 F254 (70–230 mesh; Merck) and gel permeation with Sephadex LH-20 gel. TLC was carried out with precoated silica gel Kieselgel 60 F254 plates (0.25 mm thick), and spots were detected with UV light (254 and 366 nm) and further sprayed with 20% H$_2$SO$_4$ reagent followed by heating at 100 °C.

3.2. Plant material

The whole plant of *Plectranthus glandulosus* Hook. f. was collected in Dschang, West Region of Cameroon, in January 2017. Authentication was performed by Mr. Fulbert Tadjouteu, a botanist of the Cameroon National Herbarium in Yaounde, where our sample was deposited under the voucher number 49084/HNC.

3.3. Extraction and isolation

The dried and powdered plant material (3.5 kg) was macerated three times (72 h each time) in EtOH (15 L) at room temperature to afford 234 g (6.7% yield) of a crude extract, after filtration and removal of the solvent using a rotary evaporator. A portion (224.0 g) of this extract was subjected to silica gel column chromatography (CC) eluting with a mixture of petroleum ether/ethyl acetate (EtOAc) followed by EtOAc/MeOH in increasing polarity. 125 fractions of 400 mL each were collected and combined, based on their TLC profiles, into seven major fractions: A (27.0 g), B (46.0 g), C (22.0 g), D (18.0 g), E (14.5 g), F (11.5 g) and G (23.5 g).

Fraction A (27.0 g) was subjected to CC over silica gel eluted with petroleum ether/EtOAc in increasing polarity to afford friedelin (2; 11.0 mg), lupeol (3; 8.0 mg) and an inseparable mixture of fatty compounds.

Fraction D (18.0 g) was also subjected to silica gel CC eluting with a gradient of petroleum ether/EtOAc. 110 fractions of 75 mL each were collected and grouped based on their analytical TLC profiles into 5 subfractions (D1–D5). Subfraction D1 was filtered
and washed with EtOAc to give chrysoeriol (6, 18.0 mg). D2 was further subjected to silica gel CC using a gradient of petroleum ether/EtOAc to afford luteolin-7-methyl ether (7, 8.0 mg). D2 was further subjected to silica gel CC using a gradient of petroleum ether/EtOAc to afford luteolin-7-methyl ether (7, 8.0 mg). The purification of D3 over silica gel CC eluting with the isocratic system n-hexane/EtOAc (85:15) afforded 5-hydroxy-7,4’-dimethoxyflavone (8, 5.5 mg). D4 was subjected to silica gel CC eluted with a gradient of petroleum ether/EtOAc to give 5,6-dihydroxy-7,3’,4’-trimethoxyflavone (9, 26.0 mg) and ladanein (10, 31.0 mg). D5 was also subjected to silica gel CC eluting with the isocratic system petroleum ether/EtOAc (1:1) to afford 23 fractions of 50 mL each grouped based on their TLC profiles into 3 subfractions (D5-1 to D5-3). Repeated purifications (three times) of D5-1 over Sephadex LH-20 eluted with the isocratic system CH₂Cl₂-MeOH (1:1) afforded 1 (5.2 mg) and maslinic acid (11; 2.0 mg). Similarly, an attempt to purify D5-2 yielded a mixture of maslinic acid and benthamic acid (11 + 12, 6.0 mg).

Purifications of other fractions afforded respectively a mixture of sterols (30.0 mg) from fraction B, oleanolic acid (4; 3.5 mg) and pilloin (5, 5.0 mg) from fraction C, hederagenin (13, 5.5 mg) and cyclocidic acid (14, 4.5 mg) from fraction E, sitosterol 3-O-β-D-glucopyranoside (15, 7.5 mg) from fraction F, and, galuteolin (18, 20.5 mg) and a mixture of chrysoeriol 5-O-β-D-glucopyranoside and luteolin-7-O-methyl-5-O-β-D-glucopyranoside (16 + 17, 22.5 mg) from fraction G, as reported (Nanmeni et al. 2021).

3.4. Methyl plectranthate (1)
White powder; [α]D23 +37.2 (c 0.10, CHCl₃). IR ʋ max 3448, 3064, 2944, 2871, 1720, 1640, 1452, 1387, 1194, 1164 cm⁻¹; ¹H (CDCl₃, 400 MHz) δ 4.99 (1H, br s, H-29a), 4.77 (1H, br s, H-29b), 4.17 (1H, br d, J = 4.1 Hz, H-21), 3.70 (1H, br d, J = 2.0 Hz, H-3), 3.40 (3H, s, MeO-27), 2.75 (1H, dd, J = 7.6, 2.5 Hz, H-19a), 2.63 (1H, dd, J = 13.1, 5.7 Hz, H-15a), 2.55 (1H, m, H-19b), 2.23 (1H, dt, J = 14.0, 3.7 Hz, H-12a), 2.15 (1H, m, H-22a), 1.86 (1H, m, H-2a), 1.81 (1H, br d, J = 7.6 Hz, H-18), 1.70 (1H, m, H-16a), 1.68 (1H, m, H-6a), 1.62 (1H, m, H-11a), 1.52 (2H, m, H-1a, H-7a), 1.51 (1H, m, H-2b), 1.44 (1H, m, H-12b), 1.40 (1H, m, H-8), 1.37 (1H, m, H-16b), 1.36 (1H, m, H-7b), 1.30 (1H, m, H-1b), 1.28 (3H, s, H-28), 1.25 (1H, m, H-15b), 1.24 (1H, m, H-4), 1.17 (1H, m, H-22b), 0.98 (1H, m, H-6b), 0.96 (3H, s, H-26), 0.93 (1H, m, H-11b), 0.92 (3H, s, H-25), 0.91 (3H s, H-24), 0.89 (3H, d, J = 7.4 Hz, H-23), 0.74 (1H, dd, J = 11.2, 2.4 Hz, H-10); ¹³C (CDCl₃, 100 MHz) δ 176.7 (C-27), 147.0 (C-20), 113.5 (C-29), 72.6 (C-3), 71.6 (C-21), 61.5 (C-10), 52.4 (C-13), 51.4 (C-8), 50.4 (MeO-27), 48.7 (C-4), 45.0 (C-18), 41.3 (C-14), 41.1 (C-6), 40.7 (C-22), 38.4 (C-11), 37.5 (C-9), 36.9 (C-5), 36.5 (C-16), 35.0 (C-2), 33.9 (C-28), 31.2 (17), 27.8 (C-15), 26.9 (C-12), 25.8 (C-19), 19.7 (C-25), 17.5 (C-26), 17.2 (C-1), 16.3 (C-24), 15.7 (C-7), 11.5 (C-23); ESIMS m/z 495 [M + Na]+, 967 [2M + Na]+, HRESIMS m/z 495.3446 [M + Na]+ (calcd for C₃₀H₄₈O₄Na⁺, 495.3450).

3.5. Antimicrobial assay
3.5.1. Microorganisms and growth conditions
The studied microorganisms consisted of sensitive and multidrug resistant Gram-positive (Staphylococcus aureus ATCC25923, methicillin sensitive S. aureus MSSA1, methicillin resistant S. aureus MRSA3, methicillin resistant S. aureus MRSA4) and Gram-negative
(Shigella flexneri SDINT, Pseudomonas aeruginosa ATCC27853) bacteria, as well as three strains of yeasts (Candida tropicalis PK233, Candida albicans ATCC10231 and Cryptococcus neoformans H99) taken from our laboratory collection. The bacterial and fungal species were grown at 37°C and maintained on nutrient agar (NA, Conda, Madrid, Spain) and Sabouraud Dextrose Agar (SDA, Conda) slants, respectively.

3.5.2. Determination of minimum inhibitory concentration (MIC) and minimum microbicidal concentration (MMC)

INT colorimetric assay (Tamokou et al. 2011) was performed to assess the minimal inhibitory concentration (MIC) of compounds against a panel of yeasts, Gram negative and Gram-positive bacteria. Briefly, test samples were first dissolved in dimethylsulfoxide (DMSO). The solution obtained was then added to Mueller Hinton Broth (MHB) for bacteria or Sabouraud Dextrose Broth (SDB) for yeasts and serially diluted twofold (in a 96-well microplate). One hundred microlitres (100μL) of inoculum (1.5 × 10^6 CFU/mL for bacteria and 10^5 spores/ml for yeasts) prepared in MHB/SDB was added. The plates were covered and agitated to mix the contents of the wells using a plate shaker and incubated at 35°C for 24 h (for bacteria) or for 48 h (for yeasts). The final concentration of DMSO was 1% which does not affect the microbial growth. Wells containing MHB/SDB, 100μL of inoculum, and DMSO at a final concentration of 1% served as a negative control. Ciprofloxacin (Sigma-Aldrich, Steinheim, Germany) and nystatin (Merck, Darmstadt, Germany) were used as reference drugs for bacteria and yeasts, respectively. The MIC values of samples were determined by adding 40μL of a 0.2 mg/mL p-i odonitrotetrazolium violet solution followed by incubation at 35°C for 30 min. Viable microorganisms reduced the colourless dye to pink. The MIC was defined as the lowest sample concentrations that prevented this change and exhibited complete inhibition of microbial growth. All assays were performed in triplicate and repeated thrice.

For the determination of MMC values, a portion of liquid (5μL) from each well that showed no growth of microorganisms was plated on Mueller Hinton Agar or SDA and incubated at 35°C for 24 h (for bacteria) or 35°C for 48 h (for yeasts). The lowest concentrations that yielded no growth after this subculture were taken as the MMC values.

4. Conclusion

The phytochemical study of the whole plant of P. glandulosus afforded one undescribed nor-friedelane triterpenoid together with seventeen known compounds, including seven triterpenoids, nine flavonoids and one steroid. The EtOH extract displayed moderate antimicrobial activity against Staphylococcus aureus MSSA1, Shigella flexneri SDINT and Cryptococcus neoformans H99. Our results on the antimicrobial activity, and particularly the antifungal activity of the new compound, methyl plectranthusate (1), against C. neoformans H99 could be valuable in further investigations for the search of secondary metabolites with similar structures that may have good activity and would be useful as antifungal agents against this yeast. The obtained results partially justified the use of P. glandulosus in traditional medicine in the treatment of infectious diseases caused by the tested pathogenic microorganisms.
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Disclosure statement

The authors declare no competing interest.

Authors’ contribution

ATT and J-D-DT carried out the study; JDSM and RTN contributed to structural determination; ICK helped in analysis of biological data; ATT, J-D-DT, JDSM and MT wrote the manuscript; MT and X-JH provided access to instrumentation; J-D-DT provided the microbial strains and facilities for the antimicrobial testing; J-D-DT and MT designed and supervised the work; all authors read and approved the final manuscript.

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