

Preliminary *in vitro* antimicrobial screening of chemical constituents isolated from the root of *Leplaea mayombensis* (Meliaceae)

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ABSTRACT

The Phytochemical study of Methanol/dichloromethane extract of root of *Leplaea mayombensis* led to the isolation and identification four limonoids: (8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-6,11-dihydroxy-14,15-epoxy-4,4,8,10,13-pentamethyl-17-(2-oxo-2,5-dihydrofuran-3-yl)-11,12,13,15,16,17-hexahydro-4*H*-cyclopent[α] phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (1), (8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-6,11-dihydroxy-14,15-epoxy-4,4,8,10,13-pentamethyl-17-(23-methoxy-2-oxo-2,5-dihydrofuran-3-yl)-11,12,13,15,16,17-hexahydro-4*H*-cyclopent [α] phenanthrene 3,7(8*H*,9*H*,10*H*,14*H*)-dione (2), (8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-6,11-dihydroxy-14,15-epoxy-17- ((*s*)-2-hydroxy-5-oxo-2,5-dihydrofuran-3-yl)-4,4,8,10,13-pentamethyl-10,11,12,13,15,16,17-octahydro-4*H*-cyclopent [α] phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (3), (8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-1,6,11-trihydroxy-14,15-epoxy-17-(((2*s*)-2-hydroxy-5-oxo-2,5-dihydrofuran-3-yl))-4,4,8,10,13-pentamethyl-1,10,11,12,13,15,16,17-octahydro-4*H*-cyclopent[α]phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (4), one cycloartane : 9,10-cyclopropyl-(3*S*,5*R*,8*R*,13*R*,14*S*,17*R*)-4,4,13,14,17-pentamethyl-17-((2*R*,5*S*,6*R*)-5,6,7-trihydroxy-6-methylheptan-2-yl)-hexadecahydro-1*H*-cyclopentane[α]phenanthren-3-ol (5) and three steroids: β -sitosterol (6), stigmaterol (7) and stigmaterol 3-O- β -D-glucopyranoside (8). Their structures were elucidated on the basis of spectroscopic analysis and by comparison of their spectral data with those reported in the literature. To the best of our knowledge, all these compounds were isolated for the first time from the *Leplaea mayombensis*. The antimicrobial studies showed that isolated compounds exhibit antimicrobial activity with inhibition zone diameters varying from 0.00 \pm 0.00 to 29.00 \pm 0.00 mm on both bacteria and fungi. 9,10-cyclopropyl-(3*S*,5*R*,8*R*,13*R*,14*S*,17*R*)-4,4,13,14,17-pentamethyl-17-((2*R*,5*S*,6*R*)-5,6,7-trihydroxy-6-methylheptan-2-yl)-hexadecahydro-1*H*-cyclopentane[α]phenanthren-3-ol (5) was the most active against *Escherichia coli* (28.00 \pm 0.00 mm), *Pseudomonas sp* (27.00 \pm 0.00 mm), *Lactobacillus acidophilus* (29.00 \pm 0.00 mm), *Streptococcus pneumonia* (23.00 \pm 0.00 mm) and *Serratia entomophili* (29.00 \pm 0.00 mm), *C. albicans* (12 \pm 0.00 mm), and *T. viridae* (13 \pm 0.00 mm) The results from this study support the conclusion that *L. mayombensis* contain many classes of antimicrobial compounds and therefore justify their traditional usage in the treatment of infectious diseases.

INTRODUCTION

Natural products are the most consistently successful source of drug leads. Natural products continue to provide greater structural diversity than standard combinatorial chemistry and so they offer major opportunities for finding novel low

molecular weight lead structures that are active against a wide range of assay targets (Harvey, 2000). Plants supply most of the active ingredients of traditional medicinal products, and plant extracts have long been used in screening programs in pharmaceutical companies and university institutes. It might be thought that most of the plant kingdom has been thoroughly examined in the search for biologically active molecules but only 10% of plants species existing around the world have been tested

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for some type of biological activity (Verpoorte, 1998). The genus *Leplaea* (meliaceae) has 8 species mainly widespread in tropical Africa. In Cameroon, 5 *Leplaea* species have been identified (Kouma *et al.*, 1998). Significant chemical constituents of meliaceae family are triterpenoids and limonoids (Amit and Shailendra, 2006). Plants of meliaceae family are rich source of limonoids, which proves to be a class of bioactive natural products with highly oxygenated and modified triterpene skeleton (Li-Li *et al.*, 2014). Limonoids are derivatives of terpenoids. meliaceae limonoids have attracted interest due to their diverse structures and biological activities, such as insect antifeedant, antimalarial, cytotoxic, and 11 β -HSD1 inhibitory effect (Tsamo *et al.*, 2013; Nugroho *et al.*, 2013; Rao *et al.*, 2012; Tan *et al.*, 2011).

In continuation of investigations of medicinal plants with aim to search for potent antimicrobial agents, the present study was designed to isolate compounds from the root of *Leplaea mayombensis*, and study their antibacterial and antifungal activities.

MATERIALS AND METHODS

General experimental technique

All organic solvents used were of analytical grade and Fractions were monitored by TLC and performed on precoated silica gel 60 F254 plates (Merck, Dramstadt, Germany). The spots were revealed using both ultra-violet light (254 nm and 366 nm) and 10 % H₂SO₄ spray reagent. The structures of isolated compounds were elucidated by means of spectroscopic experiments mainly 1D and 2D NMR performed, on a 500 and 150 MHz Bruker Avance III-600 spectrometer equipped with a 5mm BBFO+ probe at 300K and ESIMS / HRESIMS analyses recorded on a SYNAPT G2 HDMS (Waters) mass spectrometer and by comparison with literature data.

A Shimadzu HPLC, model LC-6AD, equipped with a Shimadzu SPD-6AV UV detector (detection UV λ 217 and 254) and a Shodex Asahipak GS-310 2Ga column (460 x 25 mm, 10 μ m particle size) was used for the analysis. For the column chromatography, Silica gel 60 (Acros Organics) and Sephadex LH 20 (Amersham Pharmacia Biotech AB) were used.

Sample collection

L. mayombensis root was harvested in Yaoundé-Cameroon, (October, 2014) and identified by Mr. Victor Nana (Plant taxonomist) of the Cameroon National Herbarium (HNC), where a voucher specimens are deposited (46220/HNC). Then, root were collected, cut into small pieces, dried at room temperature and powdered.

Extraction and isolation

5 kilograms powdered roots were extracted by maceration in MeOH: DCM (1:1 v/v) at room temperature. The filtrates were concentrated in rotavapor under reduce pressure to yield 450 g of extract (R). 300 g of this crude extract were subjected to fractionation by flash chromatography using *n*-

hexane, *n*-hexane: AcOEt (3:1), *n*-hexane: AcOEt (1:1), AcOEt, and MeOH to yield five fractions: R1 (81.9 g), R2 (53.5 g), R3 (40 g), R4 (25.4 g) and R5 (71.9 g). Base on their TLC profile, fractions R1 and R2 were mixed together to yield fraction (R') and fractions R3 and R4 were mixed to obtain (R''). The three fractions R' (135.4 g), R'' (65.4 g) and R5 (71.9 g) were used to follow the fractionation process. Fraction R' (50 g) was further subjected to column chromatography (CC) over silica gel (4x150 cm, 250 g, 70 - 230 mesh) and eluted with *n*-hexane: AcOEt mixture with increasing polarity from *n*-hexane: AcOEt (9:1) to *n*-hexane: AcOEt (1:3).

Fractions obtained were subjected to other column chromatography with *n*-hexane-CH₂Cl₂ gradient mixtures. Eight compounds were obtained including; β -sitosterol (**6**, 12 mg), stigmasterol (**7**, 26 mg) and Stigmasterol 3-O- β -D-glucopyranoside (**8**, 27 mg), 9,10-cyclopropyl-(3*S*,5*R*,8*R*,13*R*,14*S*,17*R*)-4,4,13,14,17-pentamethyl-17-((2*R*,5*S*,6*R*)-5,6,7-trihydroxy-6-methylheptan-2-yl)-hexadecahydro-1*H*-cyclopentane[α]phenanthren-3-ol (**5**, 7 mg), (8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-6,11-dihydroxy-14,15-epoxy-4,4,8,10,13-pentamethyl-17-(2-oxo-2,5-dihydrofuran-3-yl)-11,12,13,15,16,17-hexahydro-4*H*-cyclopent[α]phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (**1**, 7 mg), (8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-6,11-dihydroxy-14,15-epoxy-4,4,8,10,13-pentamethyl-17-(23-methoxy-2-oxo-2,5-dihydrofuran-3-yl)-11,12,13,15,16,17-hexahydro-4*H*-cyclopent[α]phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (**2**, 5 mg), (8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-6,11-dihydroxy-14,15-epoxy-17-((*s*)-2-hydroxy-5-oxo-2,5-dihydrofuran-3-yl)-4,4,8,10,13-pentamethyl-1,10,11,12,13,15,16,17-octahydro-4*H*-cyclopent[α]phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (**3**, 7 mg) and (8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-1,6,11-trihydroxy-14,15-epoxy-17-(((*s*)-2-hydroxy-5-oxo-2,5-dihydrofuran-3-yl))-4,4,8,10,13-pentamethyl-1,10,11,12,13,15,16,17-octahydro-4*H*-cyclopent[α]phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (**4**, 6 mg). All of the compounds were isolated for the first time from this plant.

(8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-6,11-dihydroxy-14,15-epoxy-4,4,8,10,13-pentamethyl-17-(2-oxo-2,5-dihydrofuran-3-yl)-11,12,13,15,16,17-hexahydro-4*H*-cyclopent[α]phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (1**)**

¹H NMR (500 MHz, CD₅CN): δ 7.25 (1H, d, 10.0 Hz, H-1), 7.10 (1H, s, H-22), 6.24 (1H, d, 10.0 Hz, H-2), 5.03 (1H, d, 6.3 Hz, H-11), 4.71 (2H, m, H-23), 4.08 (1H, s, H-15), 3.02 (1H, m, H-17), 2.95 (1H, d, 15.1 Hz, H-12b), 2.85 (1H, s, H-9), 2.26 (1H, dd, 15.1, 6.3 Hz, H-12a), 2.14 (1H, dd, 13.5, 6.5 Hz, H-16b), 2.04 (1H, m, H-16a), 1.88 (3H, s, H-28/H-29), 1.81 (3H, s, H-19), 1.78 (3H, s, H-30), 0.92 (3H, s, H-18) and ¹³C NMR (150 MHz, CD₅CN): 203.9 (C-3), 199.00 (C-7), 174.4 (C-21), 154.1 (C-1), 148.9 (C-22), 143.9 (C-6), 134.1 (C-5), 133.1 (C-20), 127.3 (C-2), 71.1 (C-23), 69.4 (C-14), 66.2 (C-11), 56.5 (C-15), 49.3 (C-4), 47.3 (C-8), 47.1 (C-12), 45.9 (C-9), 43.1 (C-17), 41.6 (C-13), 41.5 (C-10), 31.5 (C-16), 27.2 (C-28), 26.6 (C-19), 23.4 (C-30), 22.6 (C-18), 21.9 (C-29).

(8S,9R,10R,11S,13R,17R)-6,11-dihydroxy -14,15-epoxy -4,4,8,10, 13-pentamethyl- 17-(23-methoxy-2-oxo-2,5-dihydrofuran-3-yl)- -11,12,13,15,16,17- hexahydro-4H-cyclopent[α]phenanthrene 3,7 (8H,9H,10H,14H)-dione (2)

^1H NMR (500 MHz, CD₅CN) δ 6.39 (2H, s, H-21), 6.08 (1H, s, H-22), 4.87 (1H, m, H-11), 4.02 (1H, m, H-15), 3.22 (1H, m, H-17), 2.81 (1H, dd, 18.8, 9.2 Hz, H-2a), 2.71 (1H, m, H-12b), 2.69 (1H, dd, 18.8, 9.6 Hz, H-2b), 2.51 (1H, s, H-9), 2.35 (1H, dd, 13.1, 9.5 Hz, H-1b), 2.21 (1H, m, H-12a), 2.11 (1H, m, H-16b), 2.06 (1H, m, H-16a), 1.86 (1H, m, H-1a), 1.83 (3H, s, H-29), 1.75 (3H, s, H-28), 1.71 (3H, s, H-30), 1.60 (3H, s, H-19), 1.01 (3H, s, H-18) and ^{13}C NMR (150 MHz, CD₅CN): 214.8 (C-3), 199.3 (C-7), 171.4 (C-23), 170.9 (C-20), 142.7 (C-6), 140.0 (C-5), 119.9 (C-22), 100.0 (C-21), 68.9 (C-13), 65.9 (C-11), 69.5 (C-13), 56.3 (C-14), 48.6 (C-9), 46.9 (C-8), 46.6 (C-12), 45.2 (C-16), 42.0 (C-12), 40.0 (C-10), 36.1 (C-1), 33.4 (C-2), 31.1 (C-15), 25.0 (C-28), 24.0 (C-30), 23.1 (C-18), 21.4 (C-29).

(8S,9R,10R,11S,13R,17R)-6,11-dihydroxy-14,15-epoxy-17-((s)-2-hydroxy-5-oxo-2,5-dihydrofurano-3-yl) -4,4,8,10,13 – pentamethyl -1,10,11,12,13,15,16,17- octahydro- 4H-cyclopent[α]phenanthrene -3,7(8H,9H,10H,14H)-dione(3)

^1H NMR (500 MHz, CD₅CN) δ 6.44 (1H, s, H-21), 6.10 (1H, s, H-22), 4.92 (1H, m, H-11), 4.09 (1H, s, H-15), 3.25 (1H, m, H-17), 2.85 (1H, dd, 18.8, 9.2 Hz, H-2a), 2.69 (1H, dd, 18.8, 9.6 Hz, H-2b), 2.75 (1H, m, H-12b) 2.56 (1H, s, H-9), 2.35 (1H, dd, 13.2, 9.6 Hz, H-1b), 2.23 (1H, m, H-12a), 2.16 (1H, m, H-16b), 2.07 (1H, m, H-16a), 1.89 (1H, m, H-1a), 1.85 (3H, s, H-29), 1.77 (3H, s, H-29), 1.74 (3H, s, H-30), 1.61 (3H, s, H-19), 1.02 (3H, s, H-18) and ^{13}C NMR (150 MHz, CD₅CN): 214.9 (C-3), 199.5 (C-7), 171.6 (C-23), 170.8 (C-20), 142.9 (C-6), 140.0 (C-5), 120.0 (C-22), 100.4 (C-21), 69.7 (C-13), 66.1 (C-11), 69.7 (C-13), 56.5 (C-14), 48.8 (C-9), 47.0 (C-8), 46.7 (C-12), 45.4 (C-16), 42.1 (C-12), 40.1 (C-10), 36.2 (C-1), 33.6 (C-2), 31.5 (C-15), 25.0 (C-28), 24.0 (C-30), 21.5 (C-29), 17.9 (C-18).

8S,9R,10R,11S,13R,17R)-1,6,11-trihydroxy-14,15 -epoxy-17-[(2s)-2-hydroxy-5-oxo-2,5-dihydrofurano-3-yl] -4,4,8,10,13-pentamethyl-1,10,11,12,13,15,16,17-octahydro -4H- cyclopent [α]phenanthrene -3,7(8H,9H,10H,14H)-dione(4)

^1H NMR (500 MHz, CD₅CN) δ 6.27 (1H, s, H-21), 6.03 (1H, s, H-22), 5.05 (1H, m, H-11), 4.68 (1H, m, H-1b), 4.13 (1H, m, H-15), 3.74 (1H, m, H-9), 3.25 (1H, m, H-17), 3.21 (1H, m, H-2b), 3.14 (1H, m, H-2a), 2.70 (1H, m, H-12b), 2.20 (1H, m, H-12a), 2.15 (1H, m, H-16b), 2.11 (3H, s, H-28), 2.06 (1H, m, H-16a), 1.95 (3H, s, H-29), 1.82 (3H, s, H-30), 1.67 (3H, s, H-19), 1.07 (3H, s, H-18) and ^{13}C NMR (150 MHz, CD₅CN): 214.9 (C-3), 199.6 (C-7), 171.6 (C-23), 170.8 (C-20), 145.3 (C-6), 138.4 (C-5), 119.9 (C-22), 100.3 (C-21), 71.1 (C-1), 70.0 (C-14), 66.6 (C-11), 56.6 (C-15), 49.0 (C-4), 46.8 (C-8), 46.5 (C-12), 45.5 (C-10), 45.4 (C-17), 45.0 (C-2), 42.2 (C-13), 40.4 (C-9), 25.0 (C-28), 24.1 (C-30), 22.9 (C-18), 22.1 (C-29), 18.1 (C-19).

9,10-cyclopropyl-(3S,5R,8R,13R,14S,17R)) -4,4,13,14,17-pentamethyl-17-((2R,5S,6R)-5,6,7-trihydroxy-6-methylheptan-2-yl)-hexadecahydro-1H-cyclopentane[α]phenanthren-3-ol (5).

^1H NMR (500 MHz, CD₅CN) δ 4.31 (1H, d, 10.6 Hz, H-26a), 4.17 (1H, m, H-24), 4.11 (1H, d, 10.6 Hz, H-26b), 3.53 (1H, dd, 4.5, 11.5 Hz, H-3), 2.13 (1H, m, H-15b), 2.01 (1H, m, H-11a), 2.00 (1H, m, H-2a), 1.98 (1H, m, H-23a), 1.97 (1H, m, H-16a), 1.87 (1H, m, H-23b), 1.80 (1H, m, H-22a), 1.71 (1H, m, H-22b), 1.66 (1H, m, H-16b), 1.97 (1H, m, H-23a), 1.64 (3H, m, H-27), 1.61 (2H, m, H-12), 1.57 (1H, m, H-6a), 1.55 (1H, m, H-20), 1.52 (1H, m, H-1a), 1.48 (1H, dd, 4.6, 12.3, H-8), 1.34 (1H, m, H-2b), 1.32 (1H, m, H-5), 1.31 (1H, m, H-7b), 1.27 (1H, m, H-15a), 1.25 (1H, m, H-1b), 1.24 (3H, m, H-28), 1.14 (1H, m, H-11b), 1.11 (3H, m, H-29), 1.02 (3H, m, H-21), 1.00 (1H, m, H-7a), 1.00 (1H, m, H-1b), 1.00 (1H, s, H-17), 1.00 (3H, m, H-18), 0.90 (3H, s, H-30), 0.78 (1H, m, H-6b), 0.53 (1H, m, H-19a), 0.30 (1H, m, H-19b) and ^{13}C NMR (150 MHz, CD₅CN): 78.9 (C-3), 76.6 (C-24), 75.1 (C-25), 69.5 (C-26), 53.3(C-17), 49.5 (C-14), 48.5 (C-8), 47.7 (C-5), 45.7 (C-13), 41.3 (C-4), 36.5 (C-21), 36.2 (C-15), 34.5 (C-22), 33.5 (C-12), 32.7 (C-1), 31.5 (C-23), 30.5 (C-19), 28.7 (C-16/C-2), 27.0 (C-11), 26.8 (C-10), 26.5 (C-7), 26.3 (C-28/69.5 (C-26), 21.6 (C-6), 20.5 (C-27), 20.3 (C-9), 19.7 (C-30), 18.7 (C-18), 15.5 (C-29).

β -Sitosterol (6)

^{13}C NMR (150 MHz, CDCl₃): δ 37.24 (C-1), 31.66 (C-2), 71.81 (C-3), 42.31 (C-4), 140.75 (C-5), 121.72 (C-6), 31.90, 31.89 (C-7, C-8), 50.12 (C-9), 36.14 (C-10), 21.07 (C-11), 39.76 (C-12), 42.31 (C-13), 56.75 (C-14), 24.30 (C-15), 28.24 (C-16), 56.04 (C-17), 11.85 (C-18), 19.39 (C-19), 36.49 (C-20), 19.02 (C-21), 33.93 (C-22), 29.13 (C-23), 45.82 (C-24), 26.04 (C-25), 18.77 (C-26), 19.81 (C-27), 23.05 (C-28), 11.97 (C-29).

Stigmasterol (7)

^{13}C NMR (150 MHz, CDCl₃): δ 37.24 (C-1), 31.66 (C-2), 71.81 (C-3), 42.30 (C-4), 140.75 (C-5), 121.72 (C-6), 31.89, 31.90 (C-7, C-8), 50.12 (C-9), 36.49 (C-10), 21.07 (C-11), 39.67 (C-12), 42.20 (C-13), 56.75 (C-14), 24.35 (C-15), 28.91 (C-16), 55.94 (C-17), 12.04 (C-18), 19.39 (C-19), 40.49 (C-20), 21.07 (C-21), 138.31(C-22), 129.26 (C-23), 51.23 (C-24), 31.89 (C-25), 21.20 (C-26), 18.97 (C-27), 25.40 (C-28), 12.25 (C-29).

Stigmasterol 3-O- β -D-glucopyranoside (8)

^{13}C NMR (150 MHz, CD₅D₅N): δ C 141.1 (C-5), 139.0 (C-22), 129.6 (C-23), 122.1 (C-6), 102.8 (C-1'), 78.8 (C-3'), 78.7 (C-5'), 78.3 (C-3), 75.5 (C-2'), 71.9 (C-4'), 63.0 (C-6'), 57.0 (C-14), 56.4 (C-17), 51.6 (C-24), 50.5 (C-9), 42.6 (C-13), 41.0 (C-20), 40.1 (C-12), 39.5 (C-4), 37.7 (C-1), 37.1 (C-10), 32.4 (C-7), 32.2 (C-8, C-25), 30.4 (C-2), 29.6 (C-16), 25.9 (C-28), 24.7 (C-15), 21.7 (C-21), 21.5 (C-11, C-26), 19.6 (C-19), 19.4 (C-27), 12.7 (C-29), 12.3 (C-18).

Phytochemical screening of plant extract and fractions

Crude extract and fractions were subjected to phytochemical screening to detect the presence of alkaloids, tannins, saponins, triterpenoids, steroids, flavonoids, phenol, coumarins and anthraquinones using protocols described by Harbone, 1973.

Test microorganisms

The test microorganisms includes: Gram-positive bacteria (*Streptococcus pneumoniae*, *Enterococcus faecalis* and *Lactobacillus acidophilus*), Gram-negative bacteria (*Escherichia coli*, *Serratia entomophila* and *Pseudomonas sp.*) and fungal strains (*Aspergillus flavus*, *Trichophyton viridae* and *Candida albicans*) obtained from the Sri Paramakalyani Centre of Excellence in Environmental Sciences Manonmaniam Sundaranar University, Alwarkurichi -627412, Tamil Nadu, India.

Stock solutions and disc preparation

For the antimicrobial activity, stock solutions of compounds were prepared at 0.02 mg/mL in DMSO 10 %. Ampicillin, Chloramphenicol, Tetracyclin, Fluconazole and Nystatin were prepared in the same conditions. For disc preparation, 10 µL of each stock solution was dropped onto sterilized paper disks (6 mm diameter) and dried at room temperature for a final concentration of 0.02 mg/disc.

Antimicrobial activity

In vitro antimicrobial activity was assessed by disc diffusion method using Mueller Hinton (Bauer *et al.*, 1966) Agar (MHA) and Sabouraud dextrose Agar (SDA) obtained from Mast Group Ltd. The Agar plates were prepared by pouring 15 mL of molten media into sterile plates (90 mm). The plates were allowed to solidify for 5 min and 0.1 mL of inoculum suspension was swabbed uniformly and the inoculum allowed drying for 5 minutes.

The different compounds and reference drugs loaded at 0.02 mg /discs were placed on the surface of the medium and allowed to diffuse for 5 min. The plates were incubated at 35 °C for 24 hours for bacteria and for 48 hours for fungi. Negative control was prepared using 10 % DMSO. At the end of incubation, inhibition zones formed around the disc were measured with a Vernier Calliper in millimeter. Each experiment was performed in triplicate.

RESULTS AND DISCUSSION

Phytochemical content of crude extract and fractions

The preliminary phytochemical study (Table 1) showed that extract from root of *L. mayombensis* contain phenols, steroids, terpenoids, flavonoids, coumarins, alkaloids, saponins and tannins. The same chemical composition was found with methanol fraction. Only alkaloids were absent in ethyl acetate fraction of this extract. Whereas, hexane fraction contains only terpenoids, steroids and coumarins.

Table 1: Phytochemical composition of extracts and fractions of *L. mayombensis*

Chemical class	Crude extract		Fractions	
	M-C	H-F	AE-F	M-F
Alkaloids	+	-	-	++
Anthraquinones	+	-	++	++
Flavonoids	++	-	++	++
Phenols	++	-	++	++
Saponins	++	-	+	++
Sterols	++	++	++	++
Tanins	++	-	+	++
Terpenoids	++	++	++	++
Coumarins	++	+	++	++

+: Slight presence, ++: Heavy presence, -: Absence; M-C: MeOH-CH₂Cl₂ (1:1, v/v) crude extract; H-F: Hexane fraction, AE-F: ethyl acetate fraction, M-F: Methanol fraction.

Fractionation and isolation of compounds

Fractionation of MeOH/DCM root extract of *L. mayombensis* by column chromatography lead to isolation and purification of eight compounds (1-8). The structures of the isolated compounds were determined by spectroscopic analysis, especially, ¹H, and ¹³C NMR spectra in conjunction with 2D experiments, COSY, HSQC, HMBC and direct comparison with reference data from available literature (Figure 1).

They are: (8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-6,11-dihydroxy-14,15-epoxy-4,4,8,10,13-pentamethyl-17-(2-oxo-2,5-dihydrofuran-3-yl)-11,12,13,15,16,17-hexahydro-4*H*-cyclopent[α]phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (1) (Luo *et al.*, 2000, Vieira *et al.*, 2013, Kai-Long *et al.*, 2014), (8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-6,11-dihydroxy-14,15-epoxy-4,4,8,10,13-pentamethyl-17-(23-methoxy-2-oxo-2,5-dihydrofuran-3-yl)-11,12,13,15,16,17-hexahydro-4*H*-cyclopent[α]phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (2) (Luo *et al.*, 2000, Vieira *et al.*, 2013, Kai-Long *et al.*, 2014), (8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-6,11-dihydroxy-14,15-epoxy-17-((*s*)-2-hydroxy-5-oxo-2,5-dihydrofuran-3-yl)-4,4,8,10,13-pentamethyl-1,10,11,12,13,15,16,17-octahydro-4*H*-cyclopent[α]phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (3) (Luo *et al.*, 2000, Vieira *et al.*, 2013, Kai-Long *et al.*, 2014), 8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-1,6,11-trihydroxy-14,15-epoxy-17-(((2*s*)-2-hydroxy-5-oxo-2,5-dihydrofuran-3-yl))-4,4,8,10,13-pentamethyl-1,10,11,12,13,15,16,17-octahydro-4*H*-cyclopent[α]phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (4) (Luo *et al.*, 2000, Vieira *et al.*, 2013, Kai-Long *et al.*, 2014); 9,10-cyclopropyl-(3*S*,5*R*,8*R*,13*R*,14*S*,17*R*)-4,4,13,14,17-pentamethyl-17-(((2*R*,5*S*,6*R*))-5,6,7-trihydroxy-6-methylheptan-2-yl)-hexadecahydro-1*H*-cyclopentane[α]phenanthren-3-ol (5) (Luo *et al.*, 2000, Vieira *et al.*, 2013, Kai-Long *et al.*, 2014), steroids yields β -sitosterol (6) (Tagatsing *et al.*, 2011, Rao *et al.*, 2012, Khalijah *et al.*, 2012, Tchouya *et al.*, 2013), stigmasterol (7) (Tagatsing *et al.*, 2011, Rao *et al.*, 2012, Khalijah *et al.*, 2012, Tchouya *et al.*, 2013) and stigmasterol 3-O- β -D-glucopyranoside (8) (Tagatsing *et al.*, 2011, Rao *et al.*, 2012, Khalijah *et al.*, 2012, Tchouya *et al.*, 2013). Our results represent the first report of isolation of constituents 1-8 from *L. mayombensis*. Moreover, these compounds have been found in others plants species such as *Walsura yunnanensis*, *Walsura trifoliata* (Luo *et al.*, 2000, Vieira *et al.*, 2013, Kai-Long *et al.*, 2014).

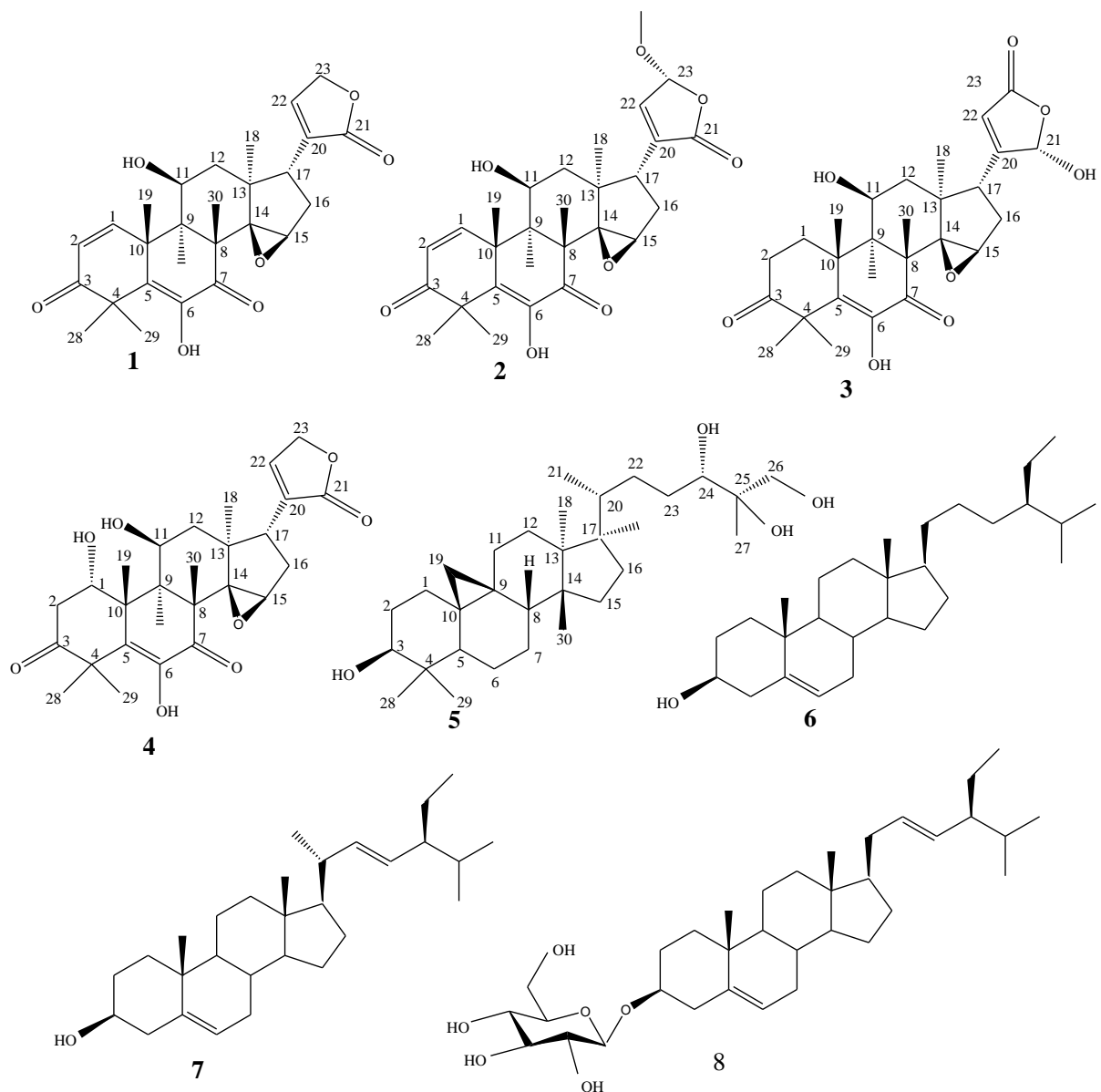


Fig. 1: Compounds isolated from roots of *L. mayombensis*.

Antimicrobial activities

The bacterial inhibition zone diameters of crude extracts, fractions and compounds are summarized in table 2. The results below indicate that the inhibition zone diameters vary from 0.00 ± 0.00 to 29.00 ± 0.00 mm on both bacteria and fungi. This inhibition was found to depend to compounds and microorganism tested. (8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-6,11-dihydroxy-14,15-epoxy-4,4,8,10,13-pentamethyl-17-(2-oxo-2,5-dihydrofuran-3-yl)-11,12,13,15,16,17-hexahydro-4*H*-cyclopent[α]phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (**1**) was found to be ineffective against bacteria strains but showed moderate inhibition (11-12 mm) against fungal strains. β -sitosterol (**6**) were active only against two gram negative (9 mm) bacteria (*S. entomophila* and *Pseudomonas sp*) and filamentous fungi (10 mm) (*A. flavus* and *T viridae*). Stigmasterol (**7**) was active against one gram negative and one filamentous

fungus (9 mm). Compounds 8 inhibit the growth (9-10mm) of two gram negative, one gram positive and one filamentous fungus. The good activity was observed with broad spectrum antimicrobial 9,10-cyclopropyl-(3*S*,5*R*,8*R*,13*R*,14*S*,17*R*)-4,4,13,14,17-pentamethyl-17-((2*R*,5*S*,6*R*)-5,6,7-trihydroxy-6-methylheptan-2-yl)-hexadecahydro-1*H*-cyclopentane [α]phenanthren-3-ol (9,10-cyclopropyl-(3*S*,5*R*,8*R*,13*R*,14*S*,17*R*)-4,4,13,14,17-pentamethyl-17-((2*R*,5*S*,6*R*)-5,6,7-trihydroxy-6-methylheptan-2-yl)-hexadecahydro-1*H*-cyclopentane [α]phenanthren-3-ol (**5**) which were the most active against both bacteria, yeast and filamentous fungi with inhibition diameter ranging from 12 to 29 mm.

All the compounds tested have showed antimicrobial activities on at least one microorganism. This activity was better than those exhibits by referents antibiotics.

Table 2: Inhibition zone diameter of compounds **1, 5, 6-8** isolated from root of *L.mayombensis*.

Microorganisms	Inhibition zone diameter (mm± SD)									
	Compounds (0.02mg/dic)					Reference(Positive control)				
	1	5	6	7	8	Amp	Chl	Tet	Flu	Nys
<i>S.entomophila</i>	0.00±0.00	19.00±0.00	9.00±0.00	0.00±0.00	10.00±0.00	15.00±0.00	38.00±0.00	31.00±0.00	nd	nd
<i>E.caccae</i>	0.00±0.00	13.00±0.00	0.00±0.00	0.00±0.00	10.00±0.00	13.00±0.00	40.00±0.00	6.00±0.00	nd	nd
<i>P. sp</i>	0.00±0.00	27.00±0.00	9.00±0.00	9.00±0.00	9.00±0.00	28.00±0.00	28.00±0.00	28.00±0.00	nd	nd
<i>E. coli</i>	0.00±0.00	28.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	17.00±0.00	12.00±0.00	nd	nd
<i>L.acidophilus</i>	0.00±0.00	29.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	25.00±0.00	27.00±0.00	nd	nd
<i>S.pneumonia</i>	0.00±0.00	23.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	6.00±0.00	0.00±0.00	37.00±0.00	nd	nd
<i>A.flavus</i>	12.00±0.00	0.00±0.00	10.00±0.00	0.00±0.00	0.00±0.00	nd	nd	nd	28.00±0.00	0.00±0.00
<i>C. albicans</i>	11.00±0.00	12.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	nd	nd	nd	33.00±0.00	0.00±0.00
<i>T.viridae</i>	0.00±0.00	13.00±0.00	10.00±0.00	9.00±0.00	10.00±0.00	nd	nd	nd	39.00±0.00	35.00±0.00

Each experiment was performed three times, and the data were averaged (n = 3). Values are means of three replication ± SD. Microorganisms: *S. entomophila* = *Serratia entomophila*, *E. caccae* = *Enterococcus caccae*, *P.sp* = *Pseudomonas sp*, *E.coli* = *Escherichia coli*, *L. acidophilus* = *Lactobacillus acidophilus*, *S. pneumonia* = *Streptococcus pneumonia*, *A. flavus* = *Aspergillusflavus*, *C. albicans* = *Candida albicans*, *T. viridae* = *Trichophytonviridae*. References (Positive control): Amp = Ampicillin, Chl = Chloramphenicol, Tet = Tetracycline, Flu = Fluconazole, Nys = Nystatin. nd: not determine

The preliminary phytochemical screening revealed the presence of phenols, steroids, terpenoids, flavonoids, coumarins, alkaloids, saponins, tannins and the difference of secondary metabolites composition of fractions compared to crude extracts. This result can be explained by the fact that during fractionation, compounds are separated according to their affinity and solubility with extraction solvent (Cowan *et al.*, 1999). However, the increasement of quantity of alkaloids and anthraquinones in methanol fraction compared to the loss of some classes of secondary metabolites in hexane and ethyl acetate fractions can be explain by the fact that fractionation could concentrated some classes of secondary metabolites and reduce others (Harbone, 1984; Bolou *et al.*, 2011). The presence of all these classes of secondary metabolites in extract of root of *L. mayombensis* is an indication of its pharmacological importance. In fact, these classes of metabolites are reported to possess many importance biological activities including, antimicrobial, anticancer, antioxydant (Shariff, 2001; Ghoghari and Rajani, 2006; Hadacek, 2002; Panda and Kar, 2007). The fractionation of root extracts was lead to isolation and identification of 8 compounds and their antimicrobial activity was determined by disc diffusion method. The results showed that activity is closely related to structure of compounds tested. β -sitosterol and stigmaterol, respectively, two compounds of the same classes of steroids were found to target the same group of microorganisms. The antimicrobial activities of stigmaterol were found to be moderate as compared the reference drugs. This observation is in good agreement with previous reports that showed low antimicrobial (or antibacterial) activities of stigmaterol against *Acetobacter sp.*, *E. coli*, *S. aureus*, and *Streptococcus sp*, *P. aeruginosa* (Ahamed *et al.*, 2002; Tamokou *et al.*, 2011). On the other hand, antibacterial activity of β -sitosterol was also found to be consistent with literature reports that discuss low/moderate antibacterial activity of β -sitosterol against several bacterial species that include *S. aureus*, *E. coli*, and *P. aeruginosa* (Beltrame *et al.*, 2002). : (8*S*,9*R*,10*R*,11*S*,13*R*,17*R*)-6,11-dihydroxy-14,15-epoxy-4,4,8,10,13-pentamethyl-17-(2-oxo-2,5-dihydrofuran-3-yl)-11,12,13,15,16,17-hexahydro-4*H*-cyclopent[α]phenanthrene-3,7(8*H*,9*H*,10*H*,14*H*)-dione (**1**) was the less active compounds studied. This can be explain the steric

congestion around the hydroxyl group limiting his reactivity. The inhibitory activity of stigmaterol 3-O- β -D-glucopyranoside (**8**) was better than compounds **1, 6** and **7**. This activity was due to the substitution of the single and free hydroxyl on the carbon skeleton by the glucopyranoside group which enhance the activity of this stigmaterol. In fact, this glucopyranoside group is known as responsible for antimicrobial activity due to their ability to complex with bacteria cell wall, (Cowan, 1999) and therefore, inhibiting the microbial growth. The best and high spectrum antimicrobial activity was observed with 9,10-cyclopropyl-((3*S*,5*R*,8*R*,13*R*,14*S*,17*R*)-4,4,13,14,17-pentamethyl-17-((2*R*,5*S*,6*R*)-5,6,7-trihydroxy-6-methylheptan-2-yl)-hexadecahydro-1*H*-cyclopentane[α]phenanthren-3-ol) (**5**) which is a cycloartane triterpenoids. In fact, terpenoids are involve in membrane disruption by the lipophilic compounds which lead to the death of microorganisms (Mendoza *et al.*, 1997). The antimicrobial activities varied with the bacterial and fungal species. These variations may be due to genetic differences between the microorganisms. The findings of the present study showed that there were differences between the antimicrobial activities of isolated compounds. This suggests that the root extract of *L. mayombensis* contains several antifungal and antibacterial principles with different polarities as shown by the phytochemical study. Therefore, it is clear that limoinds and cycloartane are the active constituents of meliaceae family plant.

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