International Journal of Innovative Studies in Sciences and Engineering Technology (IJISSET)

ISSN 2455-4863 (Online) **www.ijisset.org** *Volume: 5 Issue: 6 | 2019*

Chemical Constituents of Root from *Turraeanthus africanus* (Meliaceae) and *in Vitro* Antimicrobial Activity

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Abstract: *Turraeanthus africanus* a plant native to Africa belonging to the Meliaceae family is used in traditional medicine by indigenes to treat various ailments such as: fever, headache, epilepsy, cough, gastric ulcer, asthma, intestinal worms, high blood pressure, skin infections and oral infections. The purpose of this study was to evaluate the antimicrobial activities of compounds isolated from the roots of *Turraeanthus africanus* (Meliaceae). *In vitro* studies conducted by many researchers have shown that *Turraeanthus africanus*has antimicrobial properties. The chemical study of the CH_3OH/CH_2Cl_2 extract of the roots of *Turraeanthusafricanus* led to the isolation and identification eight compounds: threelimonoids: Dregeanin**(1)** , Methyl Angolensate**(2)**, Rohituca-3 **(3)**; two triterpenoids : Ursolicacid**(4)** ,3β-Lup-20(29)-en-3-ol or Lupeol(**5**) and three steroids:*β*-sitosterol (**6**), stigmasterol (**7**) andstigmasterol 3-O-*β*-D-glucopyranoside (**8**), isolated for the first time from the roots of *Turraeanthus africanus*. 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 *Turraeanthus africanus* **.**The antimicrobial studies showed that isolated compounds exhibit antimicrobial activity with inhibition zone diameters varying from 0.00 ± 0.00to 30.00 ± 0.00mm on both bacteria and fungi.3β-Lup-20(29)-en-3-ol or Lupeol(**5**) was the most active against *Echerhichia coli* (29.00±0.00 mm), *Pseudomonas sp*(28.00±0.00 mm), *Lactobasillus acidophilus* (30.00±0.00 mm)*, Streptococcus pneumonia* (24.00±0.00 mm) and *Serratiaentomophili*(30.00±0.00 mm), *Candida albicans* (13±0.00 mm), and *Trichophytonviridae*(14±0.00 mm).The results from this study support the conclusion that *Turraeanthus africanus* contain many classes of antimicrobial compounds and therefore justify their traditional usage in the treatment of infectious diseases.

***Keywords*:** Antimicrobial Activity, Limonoids, Meliaceae, *Turraeanthus africanus,* steroids, Triterpenoids.

1. INTRODUCTION

Plants play a vital role in the daily life of men. Since time immemorial, they are used as firewood, raw materials in the real estate, decoration and in the treatment of the diseases [1]. Today, these are a hive of drugs because they are fully integrated into African customs and play a pivotal role in traditional pharmacopoeia in the fight against many diseases[1]. 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[2]. 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 for some type of biological activity [3].*Turreanthus africanus* is a softwood and fine-grained forest tree that grows in wetlands near swamps and sandy soils. It can reach 35 to 45m long with a diameter of between 100 and 120cm, fluted or provided with short buttresses at the base sometimes spreading in great superficial roots, and deprived of branches on a height of 15 to 30m [4]. Bark surface is smooth, sometimes superficially fissured with vertical rows of pale gray or brown lenticels. The inner bark ranges from pale yellow to pale brown with a cedar odor. The heartwood is cream-white or pale yellow and turns yellow when exposed to light[5]. Significant chemical constituents of Meliaceae family are triterpenoids and limonoids [6]. Previous chemical studies on *Turreanthus africanus* have led to the isolation and characterization of a few secondary metabolites, including steroids, terpenoids, limonoids, and diterpenoids [7-11].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 [12]. Limonoids are derivatives of terpenoids. Meliaceous limonoids have attracted interest due to their diverse structures and biological activities, such as insect antifeedant, antimalarial, cytotoxic, and 11*β*-HSD1 inhibitory effects[13-15]. 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 *Turreanthus africanus*, and study their antibacterial activities.

2. MATERIALS AND METHODS

2.1. General experimental technique

After drying, crushing of the roots of *Turreanthus africanus* was carried out using a crushing machine. The maceration of the powder in CH_3OH/CH_2Cl_2 was done in a tightly sealed 25 L can.

An electronic scale of the HANGING SCALE Electronic type was used to measure the powdered mass of the crushed leaves;

An electronic scale of the LUTRON GM-300P type was used to weigh the mass of the raw extract of the sub-fractions and the products obtained;

A Buchi brand Heidolph WB 200 rotary evaporator wasused for the evaporation and the condensation of the crude extract and the various fractions obtained;

The flash chromatography was carried out using a VELP Scientifica vacuum cleaner, a micropore Buchner and a vacuum flask;

The column chromatography was carried out in a column 2 cm in diameter and 40 cm in length and another 3 cm in diameter and 23 cm in length. KIESELGEL silica type 60 (0.04-0.063 mm) was used as stationary phase;

Thin-layer analytical and preparative chromatographies were carried out on aluminum support plastic plates of dimension 20 x 20 cm^2, 0.2 mm thick and covered with a 60F254 silica layer Merck;

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 % H_2SO_4 spray reagent. The structures of isolated compounds were elucidated by means of spectroscopic experiments mainly 1D and 2D NMR performed, on a 600 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 λ217 and 254) and a ShodexAsahipak GS-310 2Ga column (460 x 25 mm, 10 µm 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.

2.2. Plant material

Turreanthus africanus root was harvested in Mount Kalla, Yaounde-Cameroon, (January, 2018) and identified by Mr. Victor Nana (Plant taxonomist) of the Cameroon National Herbarium (HNC), where a voucher specimens are deposited (56024/HNC). Then, root were collected, cut into small pecies, dried at room temperature and powdered.

Fig 1: *Roots of Turreanthus africanus*

2.3. Extraction and Isolation

3 kilograms powdered roots were extracted by maceration in CH_3OH: DCM (1:1 *v/v*) at room temperature. The filtrates were concentrated in rotavapor under reduce pressure to yield 250 g of extract (TA). 200 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 CH_3OH to yield five fractions: TA1 (75 g), TA2 (35 g), TA3 (38 g), TA4 (26 g) and TA5 (75 g). Base on their TLC profile, fractions TA1 and TA2 were mixed together to yield fraction (TA') and fractions TA3 and TA4 were mixed to obtain (TA''). The three fractions TA' (130 g), TA'' (60 g) and TA5 (75 g) were used to follow the fractionation process. Fraction TA' (40 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-CH_2Cl_2 gradient mixtures. Eight compounds were obtained including; *β*-sitosterol (**6)**(10 mg), stigmasterol (**7)**(20 mg) and Stigmasterol 3-O-*β*-*D*-glucopyranoside(**8)**(25 mg), 3β-Lup-20(29)-en-3-ol or Lupeol**(5)** (6 mg), Dregeanin**(1)**(6 mg), methylangolensate(**2)**(4 mg), Rohituca-3 (**3) (** 6 mg) and Ursolic acid(**4)** (6 mg). All of the compounds were isolated for the first time from this plant.

2.4. Physical and spectral data of compounds (1- 8)

Dregeanin (**1**): ^{1}H NMR (500 MHz, $CDCl_3$): *δ* 7.32 (1H, s, H-23), 7.09 (1H, s, H-21), 6.10 (1H, s, H-22), 5.55 (1H, d, 9.4 Hz, H-12), 5.42 (1H, s, H-30a), 5.35 (1H, s, H-30b), 4.22(1H, d, 11.0 Hz, H-11), 4.21 (1H, d, 11.3 Hz, H-29a), 4.11 (1H, dd, 5.4, 11.0 Hz, H-1), 3.97 (1H,d, 11.3 Hz, H-29b), 3.91 (1H, s, H-15), 3.78 (1H, s, H-2'), 3.20 (1H, d, 8.9 Hz, H-9), 3.01 (1H, m,H-2a), 2.96 (1H, m, H-17), 2.81 (1H, m, H-2), 2.80 (1H, m, H-6a), 2.71 (1H, m, H-6b), 2.42 (1H,dd, 9.3, 9.3 Hz, H-5), 2.26 (1H, dd, 7.1, 14.1 Hz, H-16a), 1.84 (1H, dd, 11.0, 14.1 Hz, H-16b),1.67 (1H, m, H-3'), 1.63 (3H, s, H-28), 1.35 (1H, m, H-4'a), 1.24 (3H, s, H-19), 1.18 (1H, m, H-4'b), 0.92 (3H, d, 6.9 Hz, H-6'), 0.82 (3H, t, 7.5 Hz, H-5'), 0.79 (3H, s, H-18)

^{13}C NMR (125MHz, CDCl3): 175.3 (C-1'), 171.3 (C-7). 167.7 (C-3), 142.9 (C-23), 140.3 (C-21), 137.2 (C-8),122.5 (C-20), 121.2 (C-30), 111.4 (C-22), 79.9 (C-11), 79.8 (C-1), 79.2 (C-4), 75.1 (C-2'), 74.7(C-12), 72.1 (C-29), 71.5 (C-14), 59.3 (C-15), 55.6 (C-9), 51.1 (C-10), 44.7 (C-5), 44.5 (C-13),39.5 (C-3'), 38.0 (C-1'), 37.7 (C-2), 33.4 (C-16), 31.2 (C-6), 26.7 (C-28), 24.0 (C-4'), 17.5 (C-19),15.0 (C-6'), 13.5 (C-5'), 12.0 (C-12).

Methyl angolensate (**2**): Calculated mass of $C_{27}H_{34}O_7$; m/z = 470.2304 ; **PF :** 110-111°C

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.48 – 7.38 (m, 25H), 7.28 (s, 4H), 7.13 (d, *J* = 10.2 Hz, 13H), 6.37 (s, 13H), 5.87 (d, *J* = 10.2 Hz, 13H), 5.62 (s, 13H), 4.21 – 4.00 (m, 3H), 3.93 (s, 13H), 3.60 (s, 14H), 3.51 (s, 3H), 2.61 – 2.42 (m, 27H), 2.09 – 1.61 (m, 82H), 1.61 – 1.44 (m, 48H), 1.40 (d, *J* = 15.1 Hz, 2H), 1.33 – 1.18 (m, 80H), 1.18 – 0.04 (m, 131H), 0.92 – 0.04 (m, 10H).

^{13}C NMR (126 MHz, $CDCl_3$) δ 204.65 (s), 168.35 (s), 157.83 (s), 142.97 (s), 141.16 (s), 125.75 (s), 120.60 (s), 109.96 (s), 78.47 (s), 77.18 (d, *J* = 23.4 Hz), 77.02 (s), 76.77 (s), 70.03 (s), 69.69 (s), 57.81 (s), 44.53 (s), 44.17 (s), 43.63 (s), 40.18 (s), 38.32 (s), 37.91 (s), 27.30 (d, *J* = 6.5 Hz), 26.34 (s), 21.51 (s), 19.96 (s), 18.69 (s), 17.76 (s), 15.01 (s).

Rohituca-3(3): Calculated mass of $C_{32}H_{40}O_{11}$; m/z = 600 g/mol, m/z: 600.2571 (100.0 %).

^{1}H NMR (600 MHz, $CDCl_3$) δ (ppm): 0.79 (3H, s); 0.83 (3H, t, 7.5); 0.93 (3H, d, 6.5); 1.17 (1H, m); 1.25 (3H, s); 1.33 (1H, m); 1.64 (3H, s); 1.66 (1H, m); 2.40 (1H, m); 2.44 (1H, t; 2.0); 2.72 (1H, m); 2.79 (1H, m); 2.80 (1H, m); 2.81 (1H, m); 2.98 (1H, t; 9.0); 3.22 (1H, d; 10.0); 3.78 (1H, d, 3.5); 3.99 (1H, d, 11.0); 3.99 (1H, t; 3.0); 3.01 (1H, m); 4.22 (1H, t; 10.0); 4.24 (1H, d, 11.0); 5.35 (1H, s); 5.42 (1H, s); 5.56 (1H, d; 10.0); 6.10 (1H, s); 7.07 (1H, s); 7.32 (1H, s).

^{13}C NMR (150 MHz,$CDCl_3$) δ (ppm): 12.1 (q); 13.1 (q); 15.1(q); 16.9 (q); 24.2 (t); 24.2 (t); 26.9 (q), 31.4 (t); 31.4 (t); 35.1 (d); 37.3 (t); 37.3 (t); 39.4 (d); 42.1 (t); 42.1 (t); 43.9 (d); 48.2 (s); 51.8 (s); 54.8 (d); 72.0 (t); 72.0 (t); 74.5 (d); 75.2 (d); 78.7 (d); 79.3 (s); 79.7(s); 79.8 (d); 110.6 (d); 121.5 (s); 122.7 (d); 122.7 (d); 138.9 (s); 140.0 (d); 143.3 (d); 167.7(s); 171.3 (s); 175.4 (s); 209.1 (s).

Ursolic acid: Calculated mass of **$C_{30}H_{48}O_3$**: m/z =456 ; **PF :**283-287°C

^{1}H NMR (600 MHz; DMSO-d6) δ 1.54 (1H,m,Ha-1), 0.94(1H,m,Hb-1), 4.31 (1H, d, J=4.8Hz, Ha-2), 1.45 (1H, m, Hb-2), 3.00 (1H, m , H-3),0.66 (1H, m, Hb-5), 1.45 (1H, m, Ha-6), 1.45(1H, m, Hb-6), 1.45 (1H, m, Ha-7), 1.28 (1H, m, Hb-7), 1.45 (1H, m, H-9), 1.84 (1H, m, Ha-11), 0.86 (1H, m, Hb-11), 5.13 (1H, t, J = 3.2Hz, Ha-12), 1.79 (1H, m, Ha-15), 1.00 (1H, m, Hb-15), 1.92 (1H, m, Ha-16), 1.54 (1H, m, Hb-16), 2.10 (1H, d, J=11.3Hz, H-18), 1.28 (3H, m, H-19), 0.89 (1H, m, H-20), 1.45 (1H, m, Ha-21), 1.28 (1H, m, Hb-21), 1.31 (2H, m, H-22), 0.89 (3H, s, H-23), 0.67(3H, s, H-24), 0.87 (3H, s, H-25), 0.75 (3H, s, H-26), 1.04 (3H, s, H-27), 0.90 (3H, d, *J* = 6.1 Hz, H-29), 0.81 (3H, d , *J* = 6.1 Hz, H-30).

^{13}C NMR (150 MHz ; DMSO-d_6) δ38.4 (C-1), 27.0 (C-2), 76.8 (C-3), 36.3 (C-4), 54.8 (C- 5), 18.0 (C-6), 32.7 (C-7), 38.2 (C-8), 47.0 (C-9), 39.3 (C-10), 22.8 (C-11), 124.6 (C-12), 138.2(C-13), 41.6 (C-14), 27.5 (C-15), 23.8 (C-16), 46.8 (C-17), 52.4 (C-18), 38.5 (C-19), 38.4 (C-20), 30.2 (C-21), 36.5 (C-22), 28.3 (C-23), 16.0 (C-24), 15.2 (C-25), 16.9 (C- 26), 23.3 (C-27), 178.3 (C-28), 21.0 (C-29), 17 (C-30).

(3β)-lup-20(29)-en-3-ol or **Lupeol (5):**Calculated mass of $C_{30}H_{50}O$; m/z = 426 g/molm/z: 426.3862 (100.0 %), 427.3895 (32.4 %), 428.3929 (5.1 %)

^{1}H NMR (600 MHz, $CDCl_3$)δ(ppm) 0.76; 0.79; 0.83; 0.94; 0.97; 1.03; 1.68; 3.18; 4.56; 4.685.

^{13}C NMR (150 MHz, $CDCl_3$) δ (ppm) 14.5; 16.2; 16.5; 17.2; 18.7; 18.7; 19.6; 21.3; 25.5; 27.7; 27.8; 28.3; 29.8; 34.6; 36.0; 37.5; 38.5; 39.1; 39.2; 40.4; 41.4; 43.0; 43.2; 48.4; 48.7; 50.8; 55.7; 79.4; 109.7; 151.0.

***β*-Sitosterol** (**6**): ^{13}C NMR (150 MHz, CDCl3): δ37.24 (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, C_5D_5N): δC 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).

2.5. Phytochemical screening of plant extract and fractions

Crude extract and fractions were subjected to phytochemical screening to detect the presence of alkaloids, Limonoids, saponins, triterpenoids, steroids, flavonoids, phenol, coumarins and antraquinones using protocols described by Harbone, 1973**[16].**

2.6. Antimicrobial activity

2.6.1. Test microorganisms

The test microorganisms includes: Gram-positive bacteria (*Streptococcus pneumonia*, *Entrecoccus coaccae* and *Lactobasillus acidophilus*), Gram-negative bacteria (*Escherichia coli*, *Serratia entomophilia* and *Pseudomonas sp*.) and fungal strains (*Aspergillus flavus*, *Trichophyton viridae*and *Candida albicans*) obtained from the fro CHU (University Hospital Center).

2.6.2. Stock solutions and disc preparation

For the antimicrobial activity, stock solutions of compounds were prepared at 0.02 mg/mL in DMSO 10 %. Amplicillin, 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.

- ***Principe***

In vitro antimicrobial activity was assess by disc diffusion method using Mueller Hinton [17] 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.

- ***Method***

The different compounds and references 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.

2.6.3. Preparation of extract solutions, compounds and reference antibiotics.

500 mg of the various extracts and compounds were weighed and dissolved in 1 mL of 100 % DMSO to obtain a stock solution of 500 mg/mL extract concentration. Antibiotic solutions (ciprofloxacin and fluconazole) were prepared under the same conditions by dissolving respectively in 2 mg in 1 mL of distilled water.

3. RESULTS AND DISCUSSION

3.1. Phytochemical content of crude extract and fractions

The preliminary phytochemical study (Table 1) showed that extract from root of *Turreanthus africanus* contain phenols, steroids, terpenoids, limonoids, coumarins 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 *Turreanthus africanus*

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

+: Slight presence, ++: Heavy presence, -: Absence; M-C: MeOH-CH_2Cl_2 (1:1, v/v) crude extract; H-F: Hexane fraction, AE-F: ethyl acetate fraction, M-F: Methanol fraction.

3.2. Fractionation and isolation of compounds

Fractionation of CH_3OH/CH_2Cl_2root extract of *Turreanthus africanus* 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 ^{13}C NMR spectra in conjunction with 2D experiments, COSY, HSQC, HMBC and direct comparison with reference data from available literature (Fig 2).

They are: Dregeanin (**1**)[18]; Methyl angolensate(**2**)[19-20]; Rohituca-3 (**3**) **[21]**; Ursolic acid (**4**) [22]; (3β)-lup-20(29)-en-3-ol or Lupeol(**5**)[23] ; steroids yields *β*-sitosterol (**6**)[24-27]; stigmasterol (**7**)[24-27] and stigmasterol 3-O-*β*-D-glucopyranoside (**8**)[24-27] . Our results represent the first report of isolation of constituents **1-8** from*Turreanthus africanus*. Moreover, these compounds have been found in others plants species such as *Walsurayunnanensis*, *Walsuratrifoliata*[28-30].

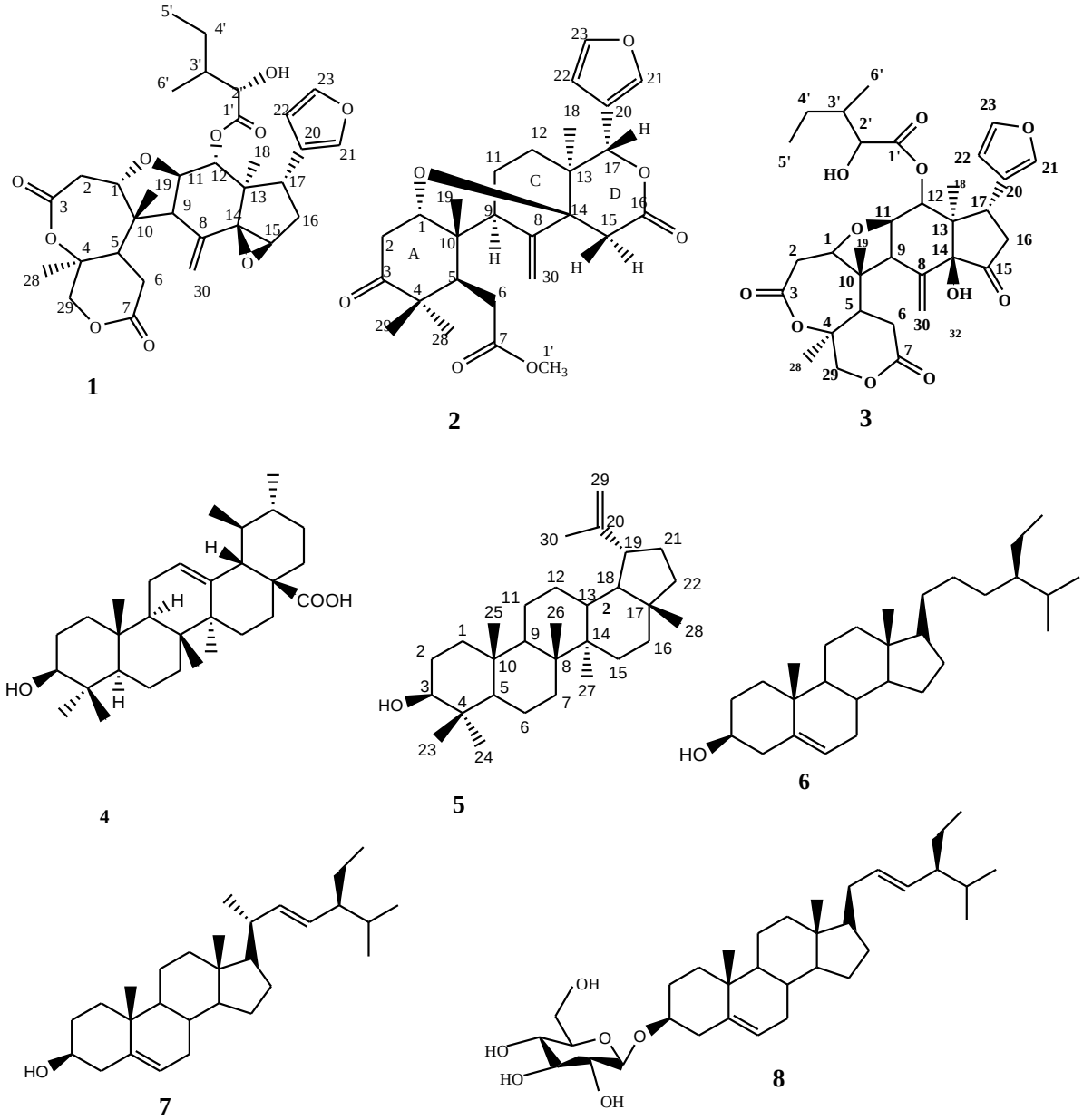

Fig 2: Structure of compounds 1 to 8 isolated from *Turreanthus africanus*

3.3. Antimicrobial results

Previous pharmacological studies on *Turreanthus africanus* have shown several activities including amongst others: antimicrobial and antifungal. It is in this context that antimicrobial tests on the crude methanol/dichloromethane extract and compounds isolated from theroots of *Turreanthus africanus* were carried out. This is to confirm their use in traditional medicine. The active extracts and/or the isolated products could thus justify the use of this plant in traditional medicine and also permit starting from the present work, to open other avenues of research.The antimicrobial tests carried out show that the target targets for these different antimicrobial tests are: bacteria, which contain a class of living organisms called microbes. Therefore, it is necessary to carry out the antimicrobial tests of the methanolic extract and compounds isolated from *Turreanthus africanus*. According to Aligiannis *et al.*[31].

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 ± 0.00to 30.00 ± 0.00mm on both bacteria and fungi. This inhibition was found to depend to compounds and microorganism tested.

Table 2:*Inhibition zone diameter of compounds **1**, **5**, **6-8** isolated from root of Turreanthus africanus*

Microoganisms	Inhibition zone diameter (mm± SD)									
	Compounds (0.02mg/dic)					Reference(Positive control)				
	1	**5**	**6**	**7**	**8**	Amp	Chl	Tet	Flu	Nys
S.entomophilia	0.00±0.00	20.00±0.00	10.00±0.00	0.00±0.00	11.00±0.00	16.00±0.00	39.00±0.00	32.00±0.00	nd	nd
E.caccae	0.00±0.00	14.00±0.00	0.00±0.00	0.00±0.00	11.00±0.00	14.00±0.00	41.00±0.00	7.00±0.00	nd	nd
P. sp	0.00±0.00	28.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00	29.00±0.00	29.00±0.00	29.00±0.00	nd	nd
E. coli	0.00±0.00	29.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	18.00±0.00	13.00±0.00	nd	nd
L.acidophilus	0.00±0.00	30.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	26.00±0.00	28.00±0.00	nd	nd
S.pneumonia	0.00±0.00	24.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	7.00±0.00	0.00±0.00	38.00±0.00	nd	nd
A .flavus	13.00±0.00	0.00±0.00	11.00±0.00	0.00±0.00	0.00±0.00	nd	nd	nd	29.00±0.00	0.00±0.00
C. albicans	12.00±0.00	13.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	nd	nd	nd	34.00±0.00	0.00±0.00
T.viridae	0.00±0.00	14.00±0.00	11.00±0.00	10.00±0.00	11.00±0.00	nd	nd	nd	40.00±0.00	36.00±0.00

Each experiment was performed three times, and the data were averaged (n = 3). Values are means of three replication ± SD.

Microorganisms: S. entomophilia = Serratiaentomophilia, E. caccae= Entrecoccuscaccae, P.sp= Pseudomonas sp,E.coli= Echerhichia coli,

L. acidophilus =Lactobasillus acidophilus, S. pnemonia= Streptococcus pnemonia, A. flavus= Aspergillusflavus, C. albicans= Candida albicans, T. viridae=Trichophytonviridae. References (Positive control): Amp = Amplicillin, Chl = Chloramphenicol, Tet = Tetracycline, Flu = Fluconazole, Nys = Nystatin. nd: not determine

Dregeanin (**1**) was found to be ineffective against bacteria strains but showed moderate inhibition (12-13 mm) against fungal strains. *β*-sitosterol (**6**)were active only against two gram negative (10 mm) bacteria (*Serratia entomophilia entomophilia* and *Pseudomonas sp*) and filamentous fungi (11 mm) (*Aspergillus flavus and Trichophyton viridae*). Stigmasterol (**7**) was active against one gram negative and one filamentous fungus (10 mm). Compounds 8 inhibit the growth (10-11mm) of two gram negative, one gram positive and one filamentous fungus. The good activity was observed with broad spectrum antimicrobial lupeol (**5**) which were the most active against both bacteria, yeast and filamentous fungi with inhibition diameter ranging from 13 to 30 mm. All the compounds tested have showed antimicrobial activities on at least one microorganism. This activity was better than those exhibits by referents antibiotics.

4.DISCUSSION

Antibiotics are chemical substances produced by microorganisms or by chemical synthesis of molecules derived from natural compounds. They prevent the growth of other microorganisms and in some cases, can destroy them [32].

The preliminary phytochemical screening revealed the presence of steroids, terpenoids, flavonoids, coumarins,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 [30]. 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 [33-34]. The presence of all these classes of secondary metabolites in extract of root of *Turreanthus africanus* is an indication of his pharmacological importance. In fact, these classes of metabolites are reported to possess many importance biological activities including, antimicrobial, anticancer, antioxydant [35-38]. 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 stigmasterol, respectively, two compounds of the same classes of steroids were found to target the same group of microorganisms. The antimicrobial activities of stigmasterol 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 stigmasterol against *Acetobacter sp*, *Echerhichia coli*, *Staphylococcus aureus*, and *Streptococcus sp*, *P. aeruginosa*[39-40].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*[41] .Dregeanin(**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 stigmasterol 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 enhances the activity of this stigmasterol. In fact, this glucopyranoside group is known as responsible for antimicrobial activity due to their ability to complex with bacteria cell wall[30] and therefore, inhibiting the microbial growth. The best and high spectrum antimicrobial activity was observed with 3β-Lup-20(29)-en-3-ol or Lupeol**(5)** which is a cycloartanetriterpenoïds. In fact, terpenoïds are involve in membrane disruption by the lipophilic compounds which lead to the death of microorganisms [42]. The antimicrobial activities varied with the bacterial and fungal species. These variations may be due to genetic differences between the microorganisms.

5. CONCLUSION

The objective of this work was to evaluate the antimicrobial activities of the compounds isolated from the roots of *Turreanthus africanus*, a plant of the Meliaceae family. Found in a large part of Africa, this plant is of great importance both in terms of medicine and socio-economically for indigenous peoples. The chemical study of the roots led to the isolation ofeigth compounds, Dregeanin**(1)** , Methyl Angolensate**(2)** , Rohituca-3 **(3)**; two triterpenoids : ursolic acid **(4)** ,3β-Lup-20(29)-en-3-ol or Lupeol(**5**) andthreesteroids:*β*-sitosterol (**6**), stigmasterol (**7**) andstigmasterol 3-O-*β*-D-glucopyranoside (**8**),**)**. Antimicrobial tests of compounds isolated from *Turreanthus africanus* are strongly related to microorganisms and the composition of extracts. Indeed, the wide range of antimicrobial properties can be explained by the presence of various groups of potentially active secondary metabolites. 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 *Turreanthus africanus* contains several antifungal and antibacterial principles with different polarities as shown by the phytochemical study. Therefore, it is clear that limonoids and cycloartane is makers of Meliaceae family plant.

Acknowledgement

The authors thank, Mr. NaNa Victor for the collection of *Turreanthus africanus* and Mr. Simo Nemg Fredy Brice, of the Laboratory of Phytobiochemistry and Medicinal Plant, Department of Biochemistry, Faculty of Sciences, University of Yaounde I for antimicrobial tests.

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