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Antibacterial Effects of Crude Methanolic Extract, Ethyl Acetate Extract and Isolated Compounds from the Fruits of *Combretum molle* (*Combretaceae*)

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Abstract: *Combretum molle* species are extensively used in traditional medicine in the Northern regions of Cameroon against inflammation, infections, diabetes, malaria, bleeding, diarrhea, digestive disorders and others as a diuretic as back pain. This study was conducted to evaluate the antibacterial effects of crude methanolic extract, ethyl acetate extract and isolated compounds from the fruits of *Combretum molle* using the Muller-Hinton solid medium disc method. Isolation of secondary metabolites from the fruits of *Combretum molle* was done by the means of usual chromatographic techniques and their structures were confirmed on the basis of spectroscopic data and those available in literature. The first phytochemical exploration of the fruits of *Combretum molle* afforded sitosterol-3-O-β-D-glucoside (1), oleanolic acid (2), mollic acid (3), mollic acid-3-O-β-D-glucoside (4), 3, 5-dihydroxy-4’,7-dimethoxyflavone (5), and 5,4’-dihydroxy-7-methoxyflavone (6). To the best of our knowledge, compounds (5) and (6) are isolated and reported for the first time from this plant. At the concentration of 25mg/ml methanolic extract, ethyl acetate extract and compounds 3, 4 and 5 presented good antibacterial potential on *Salmonella typhimurium* (ATCC13311) strain with diameters of the inhibition zone of 18.2±0.1mm, 15.3±0.2mm, 14.2± 0.3mm, 20.2±0.1mm and 15.1± 0.2mm respectively. Also compound 4 and 6 were sensitive on *Pseudomonas aeruginosa* (ATCC27853) with diameters of inhibition of 18.2±0.2 mm and 14.1± 0.4mm respectively. The results were compared to TE30 (tetracyclin), CIP5 (ciprofloxacin), C30 (chloremphinincol) and NA30 (nalidicic acid) used as references. These results are in agreement with the enthnobotanical uses of the plant and some previous reports on pharmacological activities such as anti-inflammatory, antioxidant, anticancer effects of some isolates and their related previously isolated and identified from several plants.

Keywords: *Combretum molle*, Fruit, Triterpenes, Flavonoid, Antibacterial Effect

1. Introduction

The genus *Combretum* belongs to *Combretaceae* family which includes 20 genera and about 600 species of plants distributed especially in the tropical and subtropical regions [1]. *Combretum molle* is used in African traditional medicine for the treatment of fever, abdominal pains, convulsion and worm infections [2]. It is a tree with large, straighter pole than most species of *Combretum*, distinguished by its rough bark and dense crown. It is commonly called Kinkeliba velouté (French) and Soft-leaved *combretum,* Velvet leaf willow (English) and occurs throughout tropical Africa in areas where woodlands and wooded grasslands predominate [3, 4]. *Combretum molle* has been found to possess antidiabetic [5]

and Antiasthmatical effects [6], which are in accordance with the use of this plant for treatment of infectious diseases and wounds in African traditional medicine. Moreover, the methanolic extracts of the roots and leaves of *Combretum molle* show cytotoxic effects against the T-24 bladder cancerous cell line [7]. Methanolic extract of the leaves of this plant has antimalarial activity against *Plasmodium falciparum* and molluscidal effect of the aqueous extract against *Biomphalaria pfeifferi* is shown [8]. The antimicrobial activity of acetone fraction extract of the stem bark of *Combretum molle* was evaluated by Mirriam [9]. Thus, the objective of this study is to isolate, characterize and evaluate the antibacterial activity of crude methanolic extract, acetate extract and secondary metabolites isolated from the fruits of *Combretum molle.*

2. Experimental

2.1. Plant Materials

The fruits of *Combretum molle* R. Br Ex G. Don were collected in March 2022 in Maroua, Far-North Region of Cameroon. The identification of the plant was performed by Mr. Victor Nana of the National Herbarium of Cameroon, Yaoundé, where a voucher specimen (Ref: 6518/SRF/CAM) has been deposited.

2.2. Extraction and Isolation

The fruits were dried, crush (1960g) and extracted with MeOH (3 × 10 L) at room temperature. After removing the solvent, the residue (215 g) was suspended in H_2O and extracted with AcOEt. The AcOEt-soluble fraction (80 g) was subjected to column chromatography (CC) eluting with the mixture of n-hexane/AcOEt and AcOE/MeOH in increasing order of polarity, to give six different fractions (fractions 1–6). Fraction 3 (3.5g) from n-hexane/AcOEt [90:10] was combined and subjected to CC on a silica gel column (3 cm × 45 cm, 50 g), eluting with n-hexane/AcOEt gradient mixtures to afford compounds 2 (75mg), 6 (45.4mg) and 5 (37mg). Fraction 4 (2g) from n-hexane/AcOEt [80: 20] was combined and subjected to CC on a silica gel column (2 cm × 45 cm, 35 g), eluting with n-hexane/AcOEt gradient mixtures to afford compounds 1 (85mg) and 3 (69mg). Fraction 5 (1.6g) from EtOAc/MeOH [95: 5] was subjected to column chromatography (CC) eluting with DCM/MeOH gradient mixture to give compound 4 (67mg).

2.3. Antibacterial Activity

2.3.1. Bacterial Strains

The microorganisms used in this study are Gram negative bacteria (*Pseudomonas aeruginosa* and *Salmonella typhimurium*) and Gram positif bacteria (*Staphylococcus aureus*). These are clinical isolates provided by the "Centre Pasteur", Yaounde, Cameroon. The biochemical and serological tests were used for confirmation of three bacterial strains.

2.3.2. Study of Antibacterial Activity

First, the discs are prepared and then the different concentrations of compounds (25mg/ml, 12.5mg/ml and 6.25mg/ml). These compounds were dissolved in methanol before preparing the concentrations. These solutions are coated and sterilized in an autoclave (121°C for 15mn). Blotting paper discs of 6 mm diameter are impregnated with these different concentrations and even methanol (negative control disc). All the discs prepared are dried in an oven at 37°C. Some classes of antibiotics are used to search for the best reference antibiotics [10].

2.3.3. Agar Diffusion Method

The antibacterial activity of the different compounds was evaluated by the agar diffusion method as described by Bauer and collaborators [11] and taken up by Barry and collaborators [12]. From 18 to 24-hour young colonies, a bacterial suspension is made in sterile distilled water for each strain. The turbidity of this suspension was adjusted to 0.5Mc Farland and then diluted to 1/100. An estimated inoculum of 10^6 colony-forming units per milliliter (cfu/ml) was then obtained. This inoculum was inoculated by flooding on petri dishes containing Muller-Hinton agar [13]. The discs impregnated with the different concentrations of compounds, extracts, methanol and antibiotics were then delicately deposited on the surface of the agar. The petri dishes were first left for 1 hour at room temperature for pre-diffusion of the substances, before being incubated at 37°C in an oven for 24 hours [14]. Antibacterial activity was determined by measuring the diameter of the inhibition zone around each disc [15].

3. Results and Discussion

3.1. Identification of Compounds

Compound 1 crystallizes in the n-Hexane/AcOEt [2/8] solvent system to form a white powder. It is soluble in pyridine. Libermann-Buchard test of compound 1 is positive. This information confirms that the compound belong to the triterpene family. The ^{1}H NMR spectrum (Pyridine-d5, 500 MHz), presents between 0.64 – 0.97 ppm the signals of the six (06) methyl groups. Two singlets at δ 0.65 (3H, s, Me-18) and 0.84 (3H, s, Me-19). Three doublets at δ 0.86 (3H, d, J=3.5Hz, Me-26); 0.88 (3H, d, J=3.5Hz, Me-27) and 0.97 (3H, d, J = 8.0 Hz, Me-21) and one triplet at δ 0.91 (3H, t, J = 6.0 Hz, Me-29).

The signal at δ 1.08 (2H, dq, J = 7.5; 3.0 Hz, H-28) characteristic of a methylene in alpha of methyl and methine. Two protons signals each at δ 2.41 (1H, m, H-4b) and 2.78 (1H, m, H-4a). A signal at δ 4.29 (1H, m) attributable to the H-3 and the presence of an anomeric proton at δ 5.06 (d, J=9.5 Hz), suggest that compound 1 has a sugar. A triplet signal of proton olefinic at δ 5.33 (1H, t, J = 3.0 Hz) attributable to the H-6 proton of β-sitosterol. The signals at δ 4.26 (2H, d, J = 7.5 Hz) and 5.03 (1H, d, J = 7.5 Hz) attributable respectively to the protons of the –CH2OH group of glucose. DEPT 135 Spectrum combine with the ^{13}C NMR Spectrum show clearly the presence of 35 carbon atoms including; Six methyls which appear at δ 12.0 (Me-18); 12.2

(Me-29); 19.0 (Me21); 19.2 (Me-27); 19.4 (Me-19) and 20.0 (Me-26). Twelve methylenes at δ 21.3 (C-11); 23.4 (C-28); 24.5 (C-15); 26.4 (C23); 28.5 (C-16); 30.2 (C-2); 32.0 (C-7); 34.2 (C-22); 37.5 (C-1); 39.3 (C-4); 39.9 (C-12) and 62.8 (C-6'). Fourteen methines at δ 29.5 (C-25); 32.2 (C-8); 36.4 (C-20); 46.0 (C-24); 50.3 (C-9); 56.2 (C-17); 56.8 (C-14); 71.7 (C-4'); 75.3 (C-2'); 78.1 (C5'); 78.5 (C-3'); 78.6 (C-3); 102.6 (C-1') and 121.9 (C-6). Three quaternary carbons at δ 36.9 (C-10); 42.3 (C-13) and 140.9 (C-5). The spectral data are shown in table 1. All the data compared to the literature confirms that compound 1 is a sitosterol-3-O-β-D-glucoside isolated from *Ravenala madagascariensis* and *Ipomoea digitata* [16, 17].

Compound 2 crystallizes in the n-Hexane/AcOEt [1/9] solvent system. It is soluble in DCM and responds positively to the Liebermann-Buchard test, characteristic of triterpenes. The ^{1}H NMR (CD3OD, 500MHz) and ^{13}C NMR spectra of compound 2 shows the signals characteristic of a compound of the olean-12-ene class. We observe 7 singlets at δ 0.75 (3H, s, Me-24); 0.81 (3H, s, Me-26); 0.90 (3H, s, Me-30); 0.93 (3H, s, Me-25); 0.94 (3H, s, Me-29); 0.95 (3H, s, Me-23) and 1.15 (3H, s, Me-27) confirming the presence of seven methyls of a pentacyclic triterpene skeleton. We also observe the presence of the signal at δ 5.23 (1H, t, J = 6.5 Hz) attributable to the proton of a methine group H-12. The signal at δ 3.14 (1H, dd, J = 12; 5 Hz) characteristic a proton of carbone oxymethine C-3 position. The pic at δ 2.85 (1H, dd, J = 11; 4 Hz) was assigned to methine at position C-18. The ^{13}C NMR spectrum shows 30 signals that confirm the triterpene skeleton with seven methyls. These methyls are carried by the quaternary carbons C-4 (Me-23, δ 28.7 and Me-24, δ 16.3); C-10 (Me-25, δ 15.9); C- 8 (Me-26, δ 17.7); C-13 (Me-27, δ 26.4) and C-20 (Me-29, δ 33.6 and Me-30, δ 23, 9). The double bond is confirmed by the presence of signals at δ 145.3 and 123.6. The oxymethyne group is confirmed by the chemical shift at δ 79.7. The resonant signal at δ 56.8 indicates the identity of the C-5 carbon. The spectral data are shown in table 1. All these informations combined with the literature permitted to identify compound 2 as oleanolic acid [18].

Compound 3 crystallizes in the n-Hexane/AcOEt [3/15]. The compound is white powder soluble in MeOH. It responds positively to the Liebermann-Buchard test. The ^{1}H NMR spectrum (Pyridine-d_5, 600 MHz) presents two signals at δ 0.82 (1H, d, J = 2.5 Hz) and 0.54 (1H, d, J = 2.5 Hz) are attached to methylene C-19 of a cycloartane type skeleton [19]. Six signals methyl groups including five appear in singular form at δ 1.72 (Me-29); 1.68 (Me-27); 1.59 (Me-26); 1.04 (Me-18); 0.98 (Me-30) and one doublet at δ 0.94 (3H, d, J = 6 Hz, Me- 21). Two signals at δ 2.49 (1H, d, J = 12 Hz) and 2.29 (1H, t, J = 12Hz) attributable to the H-2. Others signals at δ 3.42 (1H, d, J = 12 Hz, H-5); δ 3.91 (1H, s, H-1); δ 5.23 (1H, t, J = 6 Hz, H-24) and δ 5.56 (1H, dl, J = 12 Hz, H-3). The DEPT 135 Spectrum combine with the ^{13}C NMR Spectrum detect the presence of 30 signals including: Six primary carbons signals at δ 25.6 (Me-27); 19.2 (Me-30); 18.3 (Me-18); 18.1 (Me-21); 17.5 (Me-26); 9.6 (Me-29); Ten secondary carbons signals at δ 23.2 (C-6); 25.1 (C-23); 25.7 (C-7); 26.0 (C-11); 28.2 (C-16); 29.6 (C-19); 33.1 (C-12); 35.6 (C-15); 36.4 (C-22) and 38.6 (C-2); Seven tertiary carbons signals at δ 125.6 (C-24); 72.3 (C-1); 70.5 (C-3); 52.3 (C-17); 47.9 (C-8); 37.5 (C-5); 35.9 (C-20) and Seven quaternary carbons signals at δ 179.8 (C-28); 130.6 (C-25); 55.5 (C-4); 48.9 (C-14); 45.5 (C-13); 30.1 (C-10); 20.6 (C-9). The spectral data are shown in table 2. The analyses of all these spectral data as well as their comparison with the data of the literature permitted to identify compound 3 as mollic acid [19].

Compound 4 crystallizes in the AcOEt/MeOH [1/19] solvent system. It is white powder soluble in MeOH. It responds positively to the Liebermann-Buchard test. The ^{1}H NMR spectrum (MeOD, 500 MHz) presents two doublets at δ 0.73 (1H, d, J = 5 Hz) and 0.53 (1H, d, J = 5 Hz) are attached to methylene C-19 of a cycloartane type skeleton. The signals of six methyl groups between 0.93 and 1.69 ppm therefore five appear to singular form at δ 1.69 (Me-27); 1.62 (Me-26); 1.01 (Me-30); 1.16 (Me-29); 1.01 (Me-18), one doublet at δ 0.93 (3H, d, J = 10 Hz, Me- 21). Two signals at δ 2.21 (1H, d, J = 15 Hz) and 1.92 (1H, m,) attributable to the H-2 protons carried by the methylene at δ 35.6 (C-2). Others protons at δ 2.60 (1H, dd, J = 10; 5 Hz) attributable to proton H-5; δ 3.55 (1H, s) attributable to proton H-1; δ 5.12 (1H, t, J = 5 Hz) attributable to proton olefinic H-24 and δ 4.61 (1H, dd, J = 10; 5 Hz), attributable to the hydroxy group proton H-3. We observe the proton peaks in the sugar zone at δ 4.36; 3.13; 3.33; 3.24; 3.90; 3.32 and 3.65 attributable respectively to protons H-1'; H-2'; H-3'; H-4'; H-5' H-6'a and H-6'b. The DEPT 135 Spectrum combine with the ^{13}C NMR Spectrum detect the presence of 36 carbon atoms signals at δ 24.4 (Me-27); 18.4 (Me-30); 18.4 (Me-18); 17.4 (Me-21); 16.3 (Me-26); 8.4 (Me-29); 35.6 (C-2); 22.4 (C-6); 25.1 (C-7); 25.2 (C-11); 32.6 (C-12); 36.0 (C-15); 27.9 (C-16); 29.6 (C-19); 36.6 (C-22); 25.6 (C-23), 61.6 (C6'); 124.8 (C-24); 72.3 (C-1); 80.0 (C-3); 52.2 (C-17); 48.7 (C-8); 37.2 (C-5); 35.7 (C-20); 104.1 (C-1'); 73.9 (C-2'); 76.4 (C-3'); 70.3 (C-4'); 76.6 (C-5'); 180.0 (C-28); 131.0 (C-25); 55.5 (C-4); 51.0 (C-14); 46.0 (C-13); 30.9 (C-10); 20.9 (C-9). The COSY spectrum shows the different correlations between the protons: The proton H-24 correlates with the protons H-23 and H-22; H-3 correlates with the protons H-2 and H-3'; H-5 correlates with the proton H-6; H-7 correlates with the proton H-6; H-5 correlates with the proton H-30 and H-19a correlates with the proton H-19b. The HMBC spectrum show the following correlations: δ 4.61 correlates with the signals of carbons C-28, C-1' and C-29: δ 4.36 correlates with the signal of carbon C-3; δ 3.65 correlates with the signal of carbon C-5'; δ 3.56 correlates with the signal of carbon C-3; δ 3.33 correlates with the signals of carbons C-2' and C-4'; δ 3.24 correlates with the signal of carbon C-5'; δ 3.13 correlates with the signals of carbons C-1' and C-5'; δ 1.17 correlates with the signals of carbons C-3 and C-5; δ 0.93 correlates with the signals of carbons C-17 and C-15; δ 1.01 correlates with the signal of carbon C1-7; δ 0.73 correlates with the signals of carbons C-1, C-5, C-8 and C-11 and δ 0.53 correlates with the signals of carbons C-1, C-5, C-8 and C-11. The spectral data are shown in table 2. All the data compared

to the literature confirms that compound 4 is a mollic acid -3-O-β-D-glucoside [19].

Compound 5 is a yellow powder; it is soluble in MeOH. It was purified in an n-Hexane/AcOEt [1/9] solvent system. It is UV fluorescent at 254 nm and 365 nm. The ^{1}H-NMR (MeOD, 500 MHz) spectrum indicates the presence of signals at δ 8.11 (1H, dd, J = 10: 5 Hz, H-2' and H-6'); δ 6.90 ppm (1H, dd, J = 10: 5 Hz, H-3' and H-5'); δ 6.42 (1H, d, J = 5 Hz, H-8) and δ 6.20 (1H, d, J = 5 Hz, H-6. A singlet at δ 3.55 (6H, s) corresponding to methoxyl groups (7-OCH3 and 4'-OCH3). HSQC spectrum shows the following correlations at: δ 8.11 correlates with the signal at δ 128.5; δ 6.90 correlates with the signal at δ 113.5; δ 6.42 correlates with the signal at δ 96.9; δ 6.20 correlates with the signal at δ 92.5 and δ 3.33 correlates with the signal at δ 54.6 ppm. The signal at δ 96.9 ppm is a value of the characteristic chemical shift of the C-8 of a flavone. The ^{13}C DEPT 90 NMR spectrum shows the presence of 6 carbon atoms at δ 128.5 (C-2' and C-6'), 113.5 (C-3' and C-5'), 96.9 (C-8) and 92.5 (C-6). The HMBC NMR spectrum shows the different correlations: δ 8.11 correlates with the signals at δ 145.4 (C-2), δ 174.7 (C-4) and δ 128.5 (C-2', C-6'); δ 6.90 correlates with the signals at δ 121.3 (C-1'), δ 145.4 (C-2) and 174.7 (C-4); δ 6.42 correlates with the signals at δ 102.7 (C-10), δ 162.9 (C-7) and δ 155.6 (C-9); δ 6.20 correlates with the signals at δ 102.7 (C-10), δ 162.9 (C-7), δ 92.5 (C-6) and δ 159.8 (C-5). The ^{13}C NMR, DEPT and HSQC spectrum makes it possible to detect the presence of 17 signals corresponding to 17 carbon atoms including 9 quaternary carbons at (δ 102.7, 121.3, 134.5, 145.4, 155.6, 159.8, 157.9, 174.7, 162.9 ppm), 6 tertiary carbons at (δ 92.5, 96.9, 113.4, 113.5, 128.4, 128.5 ppm) and 2 primary carbons at (δ 54.6ppm). The spectral data are shown in table 3. All the data compared to the literature confirms that compound 5 is a 3, 5-dihydroxy-4', 7-dimethoxyflavone [20].

Compound 6 is a yellow powder; it is soluble in MeOH. It was purified in the n-Hexane/AcOEt [1/9] solvent system. It is UV fluorescent at 254 nm and 365 nm. The ^{1}H-NMR (DMSO, 500 MHz) spectrum shows the signals at δ 7.95 (1H, dd, J = 10; 5 Hz, H-2' and H-6'), δ 6.95 (1H, dd, J = 10; 5 Hz, H-3' and H-5'), δ 6.77 (1H, d, J = 5 Hz, H-8), δ 6.38 (1H, d, J =5 Hz, H-6), δ 6.83 (1H, s, H-3) and a singlet at δ 3.68 corresponding to methoxy group (7-OCH3), δ 12.97 (1H, s) characteristic of proton OH group at C-5 of a flavonoid. HSQC spectrum shows the following correlations: δ 7.95 correlates with the signal at δ 129.0; δ 6.95 correlates with the signal at δ 116.4; δ 6.83 correlates with the signal at δ 103.5; δ 6.77 correlates with the signal at δ 93.2; δ 6.38 correlates with the signal at δ 98.4 and 3.68 correlates with the signal at δ 56.5. ^{13}C NMR spectrum and DEPT allow us to detect the presence of 13 signals of carbon indicated the presence of 16 carbon atoms including 8 quaternary carbons at (δ 182.4; 165.6; 164.5; 161.8, 161.7; 157.7; 121.6; 105.2 ppm), 7 tertiary carbons at (δ 129.0; 129.1; 116.4; 116.5; 103.5; 98.4; 93.2 ppm) et one primary carbons at (δ 56.5ppm). The spectral data are shown in table 3. All these informations spectroscopic combined with the literature permitted to identify compound 6 as 5, 4'-dihydroxy-7-methoxyflavone [21]. See figure 1 to the chemical structure of compounds 1-6.

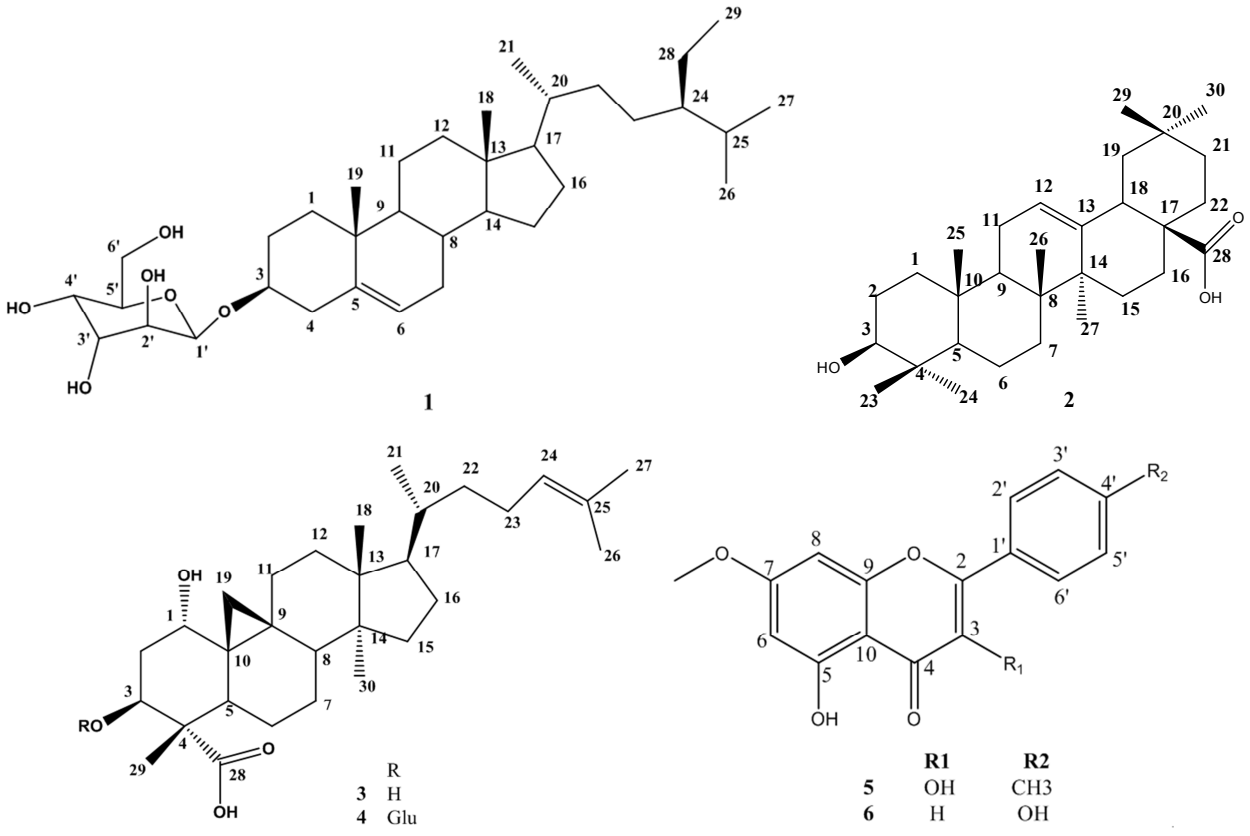

Figure 1. *Chemical structure of compound 1-6.*

Table 1. *^{13}C. ^{1}H NMR Spectrum of compounds 1 and 2.*

Position	1		2	
	δ_C	δ_H	δ_C	δ_H
1	37,5	1,71 (2H, m)	39,9	0,98; 1,64
2	30,2	1,69 (2H, m)	27,8	1,55; 1,61
3	78,5	4,29 (1H, m)	79,7	3,14 (1H, *dd*, *J* = 12; 5 Hz)
4	39,3	2,41 (1H, m) 2,78 (1H, m)	38,2	-
5	140,9	/	56,8	0,74
6	121,9	5,32 (1H, d, *J* = 3,0 Hz)	19,5	1,41; 1,57
7	32,0	1,69 (2H, m)	34,0	1,32; 1,53
8	32,2	1,41 (1H, m)	40,6	-
9	50,3	0,98 (1H, m)	49,2	1,59
10	36,9	/	33,8	-
11	21,3	1,41 (2H, m)	24,1	1,59; 1,91
12	39,9	1,09 (2H, m)	123,6	5,23 (1H, t, *J* = 6,5 Hz)
13	42,3	/	145,3	-
14	56,8	0,92 (1H, m)	42,9	-
15	24,5	1,56-1,03; m	27,8	1,55; 1,63
16	28,5	1,83-1,90, m	24,5	1,90; 2,00
17	56,2	1,11 (2H, m)	47,7	-
18	12,0	0,64 (3H, s)	42,8	2,85 (1H, *dd*, *J* = 11; 4 Hz)
19	19,4	0,84 (3H, s)	47,3	1,14; 1,69
20	36,4	1,34-1,42 (1H, m)	31,6	-
21	19,0	0,97 (3H, d, *J* = 8,0 Hz)	28,9	1,09; 1,79
22	34,2	1,38-1,08 (2H, m)	34,9	1,20; 1,40
23	26,4	1,23 (2H, m)	28,7	0,95 (3H, s)
24	46,0	0,99 (1H, m)	16,3	0,75 (3H, s)
25	29,5	1,64-1,68 (1H, m)	15,9	0,93 (3H, s)
26	20,0	0,86 (3H, d, *J* = 3,5 Hz)	17,7	0,81 (3H, s)
27	19,2	0,88 (3H, d, *J* = 3,5 Hz)	26,4	1,15 (3H, s)
28	23,4	1,08 (2H, dq, J = 7,5; 3,0 Hz)	180,4	-
29	12,2	0,91 (3H, t, *J* = 6,0 Hz)	33,6	0,94 (3H, s)
30	/	/	23,9	0,90 (3H, s)
1’	102,6	5,06 (1H, d, *J* = 9,5 Hz)	/	/
2’	78,1	4,05 (1H, m)	/	/
3’	78,6	4,45 (1H, m)	/	/
4’	71,7	4,30 (1H, m)	/	/
5’	78,5	3,80 (1H, m)	/	/
6’	62,8	4,26 (1H, d, *J* = 7,5 Hz) 5,03 (1H, d, *J* = 7,5 Hz)	/	/

Table 2. *^{13}C, ^{1}H NMR Spectrum of compounds 3 and 4.*

Position	3		4	
	δ_C	δ_H	δ_C	δ_H
1	72,3	3,91 (1H, s)	72.3	3.55 (1H, s)
2	38,6	2,49 (1H, d, *J* = 13 Hz) 2,29 (1H, t, *J* = 12 Hz)	35.6	2.21 (1H, d, *J* = 15 Hz) 1.92 (1H, t, *J* = 15 Hz)
3	70,5	5,56 (1H, dl, *J* = 12 Hz)	80.0	4.61 (1H, dd, *J* = 10; 5 Hz)
4	55,5	-	55.5	-
5	37,5	3,42 (1H, dl, *J* = 12 Hz)	37.2	2.60 (1H, dd, *J* = 10; 5 Hz)
6	23,2	1,71; 1,23	22.4	1.32; 1.13
7	25,7	2,12; 1,58	25.1	2.43; 1.72
8	47,9	1,60	48.7	1.53
9	20,6	-	20.8	-
10	30,1	-	30.9	-
11	26,0	2,74; 1,23	25.2	2.43; 1.72
12	33,1	1,68	32.6	1.65
13	45,5	-	46.0	-
14	48,9	-	51.0	-
15	35,6		36.0	1.30
16	28,2	-	27.9	1.93; 1.25
17	52,3	1,60	52.2	1.65
18	18,3	1,04 (3H, s)	18.4	1.01 (3H, s)

Position	3		4	
	δ_C	δ_H	δ_C	δ_H
19	29,6	0,82 (1H, d, *J* = 2,5 Hz) 0,54 (1H, d, *J* = 2,5 Hz)	29.6	0.73 (1H, d, *J* = 5 Hz) 0.53 (1H, d, *J* = 5 Hz)
20	35,9	1,28	35.7	1.30
21	18,1	0,94 (3H, d, *J* = 6 Hz)	17.4	0.93 (3H, d, *J* = 10 Hz)
22	36,4	1,71	36.6	1.65
23	25,1	2,00; 1,80	25.6	2.03; 1.90
24	125,6	5,23 (1H, t, *J* = 6 Hz)	124,8	5.12 (1H, t, *J* = 5 Hz)
25	130,6	-	131,0	-
26	17,5	1,59 (3H, s)	16.3	1.62 (3H, s)
27	25,6	1,68 (3H, s)	24.4	1.69 (3H, s)
28	180,0	-	180.0	-
29	9,6	1,72 (3H, s)	8.4	1.17 (3H, s)
30	19,2	0.98 (3H, s)	18.4	1.01 (3H, s)
1’	-	-	104.1	4.36
2’	-	-	73.9	3.13
3’	-	-	76.4	3.33
4’	-	-	70.3	3.24
5’	-	-	76.6	3.32
6’	-	-	61.6	3.90 3.65

Table 3. *The ^{13}C, ^{1}H NMR, HMBC Spectrum of compound 5 and 6.*

Position	5			6		
	δ_C	δ_H	HMBC	δ_C	δ_H	HMBC
2	145.4	-		164.5	-	
3	134.5	-		103.5	6.83 (1H, s)	C1’, C2, C4, C10
4	174.7	-		182.4	-	
5	159.8	-		161.8	-	
6	92.5	6.20 (1H, d, J= 5Hz)	C6, C7, C10	98.4	6.38 (1H, d, J=)	C2, C7, C8, C10
7	162.9	-		165.6	-	
8	96.9	6.42 (1H, d, J=5Hz)	C7, C8, C9, C10	93.2	6.77 (1H, d, J=)	C6, C7, C9, C10
9	155.6	-		157.7	-	
10	102.7	-		105.2	-	
1’	121.3	-		121.6	-	
2’	128.4	8.11 (1H, dd, J = 10; 5 Hz)	C6’, C2, C4’	129.0	7.95 (1H, dd, J = 10 Hz)	C6’, C2, C3’
3’	113.4	6.90 (1H, dd, J = 10; 5 Hz)	C4’, C5’	116.4	6.95 (1H, dd, J = 10 Hz)	C5’, C1’, C2,
4’	157.9	-		161.7	-	
5’	113.5	6.90 (1H, dd, J = 10; 5Hz)	C4’, C3’	116.5	6.95 (1H, dd, J = 10 Hz)	C3’, C1’, C2
6’	128.5	8.11 (1H, dd, J = 10; 5Hz)	C2’, C2, C4’	129.1	7.95 (1H, dd, J = 10 Hz)	C2’, C2, C5’
7-OCH_3	54.6	3.33 (3H, s)		56.5	3.68 (3H, s)	
4’-OCH_3	54.6	3.33 (3H, s)		/	/	

3.2. Results and Discussion on Antibacterial Activity

The evaluation of the antibacterial activity of compounds and extract was carried out by the Muller-Hinton agar diffusion method, as proposed in the recommendations of the Antibiogram committee of the French Society of Microbiology [22]. The antibacterial ability was classified according to the DIZ (diameters of the inhibition zone) as follows: not sensitive (DIZ < 8.0mm) moderately sensitive (8.0 < DIZ <14.0mm); sensitive (14.0 < DIZ < 20.0), and extremely sensitive (DIZ > 20.0mm) [23, 24]. The results obtained on the antibacterial activity of the isolated compounds and extracts varied significantly depending on the concentration. For the compound 4, the highest concentration (25mg/ml), the diameter of the inhibition zone was 20.2±0.1mm (Table 4), at this dose it was extremely sensitive because DIZ > 20.0mm. We can say that, at this concentration, compound 4 has the similar effects as the antibiotic CIP5 (ciprofloxacin, 32.2±0.3mm), on *Salmonella typhimurium* (ATCC13311) strain. At the same concentration methanolic extract, ethyl acetate extract and compounds 3 and 5 were sensitive on *Salmonella typhimurium* (ATCC13311) strain with diameters of the inhibition zone of 18.2±0.1mm, 15.3±0.2mm, 14.2± 0.3mm and 15.1± 0.2mm respectively (Table 4), because the DIZ are between (14.0<DIZ<20.0mm), at this concentration they had the larger effects on *Salmonella typhimurium* than the reference antibiotic C30 (chloremphinincol, 11.5±0.2mm) (Table 7). Methanolic extract and compound 4 and 6 showed the sensitive effect at a concentration of 25mg/ml, their diameters inhibitions zone were 15.2±0.2mm, 18.2±0.2 and 14.1± 0.4 respectively (Table 5) *on Pseudomonas aeruginosa*. At the same concentration compounds 2, 3, 4, 5 and 6 on *Staphylococcus aureus* were moderately sensitive with diameters of the inhibition 13.2± 0.1mm, 12.1± 0.3mm, 13.5±0.1mm, 11.8±0.2mm and 10.7±0.2mm respectively (Table 6), we can say that, at this concentration, compounds 2, 3, 4, 5 and 6 had the same effects as the antibiotic NA30 (nalidicic acid, 11.4±0.2mm). At the concentration 12.5mg/mL methanolic extract and compound 4 were sensitive on *Salmonella typhimurium*, with the diameters of the inhibition 15.1±0.2mm and 16.1±0.3mm respectively. Compounds 3, 5 and 6 were also moderately sensitive with the diameters of the inhibition are 11.2± 0.3mm, 11.3±0.1mm and 10.0± 0.2mm respectively on *Salmonella typhimurium*, at this

concentration they had same effects as antibiotic C30 (chloremphinincol, 11.5±0.2mm). Our results are similar to that of Xiao and his collaborators, who tested *dihydromyricetin* on *Salmonella typhimurium*, at a dose of 11.34mg/ml and the diameter of the inhibition zone obtained was 13.1±0.5mm [24].

Table 4. *Effect of Compounds 1-6* and the Extracts on *Salmonella Typhimurium.*

Concentrations	*Diameters of inhibition zone (mm)*								
(mg/mL)	MeOH	MeOH Extract	AcOEt Extract	1	2	3	4	5	6
6.25	ND	11.2±0.1	10.3±0.2	ND	ND	10.0± 0.1	10.7±0.1	ND	ND
12.5	ND	15.1±0.2	12.1±0.1	ND	9.3±0.2	11.2± 0.3	16.1±0.3	11.3±0.1	10.0± 0.2
25	ND	18.2±0.1	15.3±0.2	9.1± 0.3	11.1± 0.3	14.2± 0.3	20.2±0.1	15.1± 0.2	11.8± 0.1

ND: not detected, N= 3, E. AcOEt: Acetate Extract, E. MeOH: Methanolic Extract.

Table 5. *Effect of Compounds 1-6* and the Extracts on *Pseudomonas Aeruginosa.*

Concentrations	*Diameters of inhibition zone (mm)*								
(mg/mL)	MeOH	MeOH Extract	AcOEt Extract	1	2	3	4	5	6
6.25	ND	9.7±0.1	8.8± 0.3	ND	8.7±0.1	ND	9.2±0.1	ND	9.5± 0.1
12.5	ND	11.2±0.2	9.4±0.2	ND	10.1±0.2	8.4± 0.1	12.2±0.2	ND	11.9± 0.1
25	ND	15.2±0.2	11.4±0.3	9.4± 0.2	12.2±0.1	10.1± 0.2	18.2±0.2	08.1±0.3	14.1± 0.4

ND: not detected, N= 3, E. AcOEt: Acetate Extract, E. MeOH: Methanolic Extract.

Table 6. *Effect of Compounds 1-6* and the Extracts on *Staphylococus Aureus.*

Concentrations	*Diameters of inhibition zone (mm)*								
(mg/mL)	MeOH	MeOH Extract	AcOEt Extract	1	2	3	4	5	6
6.25	ND	9.2±0.1	8.6± 0.1	ND	9.1± 0.1	ND	9.8±0.1	8.4± 0.2	ND
12.5	ND	11.1±0.2	10.1±0.1	ND	11.3± 0.2	10.1± 0.3	11.1±0.2	9.1±0.1	8.3±0.1
25	ND	14.2±0.1	13.9±0.2	ND	13.2± 0.1	12.1± 0.3	13.5±0.1	11.8±0.2	10.7±0.2

ND: not detected, N= 3, E. AcOEt: Acetate Extract, E. MeOH: Methanolic Extract.

Table 7. *Effect of Antibiotics on Salmonella Typhimurium, Pseudomonas Aeruginosa and Staphylococcus Aureus.*

Antibiotics / Bacteria	*Diameter of inhibition zone (mm)*					
	TE30	CIP5	AMC30	PB50	C30	NA30
Salmonella typhimurium	ND	32.2±0.3	9.4±0.1	ND	11.5±0.2	ND
Staphylococcus aureus	15.2±0.1	30.1±0.2	31.4±0.1	ND	21.4±0.2	11.4±0.2
Pseudomonas aeruginosa	9.1± 0.1	34.5±0.3	ND	ND	ND	8.1±0.2

ND: not detected, TE30 = tetracyclin, CIP5 = ciprofloxacin, AMC30 = amoxicillin/flavonic acid, PB50 = polymexin B, C30 = chloremphinincol, NA30 = nalidicic acid. Values represent means of 3 independent replicates ± SD.

4. Conclusion

In this study of fruits of *Combretum molle*, we isolated and identified six compounds confirmed by 1D and 2D NMR spectra and literature data. To our knowledge, this is the first study carried out on the fruits of this plant and it is the first time that compounds 5 and 6 have been isolated from this specie. We found that at the different concentrations (25mg/ml, 12.5mg/ml and 6.25mg/ml), ethyl acetate extract and methanolic extract and compounds 3, 4 and 5 had greater effects on *Salmonella typhimurium* than on *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Therefore, all activities confirm the rationale behind the use of this plant in traditional medicine.

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