Advances in Biological Chemistry, 2025, 15(1), 18-30
https://www.scirp.org/journal/abc
ISSN Online: 2162-2191
ISSN Print: 2162-2183

Chemical Constituents from Ethyl Acetate Extract of *Graptophyllum glandulosum* Turrill and New Semi-Synthetic Derivative with Antimicrobial Activities

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How to cite this paper: Tagousop, C.N., Tsamo, D.L.F., Dongmo, A.J.N., Harakat, D., Voutquenne-Nazabadioko, L., Tamokou, J.-de-D. and Ngnokam, D. (2025) Chemical Constituents from Ethyl Acetate Extract of *Graptophyllum glandulosum* Turrill and New Semi-Synthetic Derivative with Antimicrobial Activities. *Advances in Biological Chemistry*, **15**, 18-30.
https://doi.org/10.4236/abc.2025.151002

Received: January 17, 2025
Accepted: February 15, 2025
Published: February 18, 2025

Abstract

From the ethyl acetate extract of the medicinal plant *Graptophyllum glandulosum* Turrill, five known compounds: Lupeol (**1**), Oleanolic acid (**2**), Chrysoeriol (**3**), *N*-methyl-isonicotinamide (**4**) and *β*-sitosterol 3-*O*-*β*-*D*-glucopyranoside (**5**) were isolated. In addition, oxidation reactions carried out on lupeol (**1**) yielded two semi-synthetic compounds, including a previously unreported: (20*R*)-formyloxy-29-*nor*-lupan-3-one (**1b**) and one other well-known Lupenone (**1a**). The structures of natural and semi-synthetic compounds were determined by analysis of 1D-(^{1}H, ^{13}C), 2D-(COSY, HSQC and HMBC) NMR data in conjunction with mass spectrometry (TOFESIMS and HR-TOFESIMS) and by comparison with the reported data. The evaluation of antimicrobial activities of substrate (**1**) as well as semi-synthetic derivatives (**1a** and **1b**) using broth microdilution method showed that compound **1b** was the most active (16 ≤ MIC ≤ 32 µg/mL) against *Escherichia coli*, *Staphylococcus aureus* and *Candida albicans* compared to the starting material **1** (16 ≤ MIC ≤ 64 µg/mL) and derivative **1a** (32 ≤ MIC ≤ 64 µg/mL).

Keywords

Graptophyllum glandulosum Turrill, Lupeol, Oxidation Reaction, Antimicrobial Activities

1. Introduction

Graptophyllum glandulosum Turrill (Acanthaceae) is a shrub with 4 angled, almost glabrous branches, normal green leaves and reddish-purple flowers. It is one of several shrubs and trees of the *Graptophyllum* genus, which grows mainly in West and Central Africa, but also in the Pacific regions [1]. Leaves, roots and other parts of *G. glandulosum* are used in folk medicine in Cameroon to treat wounds, abscesses, skin diseases, respiratory infections and diarrhoea. In our previous studies, we reported the characterization and evaluation of the antimicrobial activities of flavonoid glycosides from the *n*-BuOH extract of *G. glandulosum* and their mechanism of action using lysis, leakage and osmotic stress assays [2] [3]. These previous studies also show that the ethyl acetate extract exhibited the highest antimicrobial activity compared to the methanol and *n*-BuOH extracts against *Staphylococcus aureus*, *Vibrio cholerae*, *Candida albicans* and *Cryptococcus neoformans*. Considering the fact that some microbial species are more virulent than others and show altered susceptibility to conventional antimicrobial drugs [4]-[6], rapid progress has been made in the chemical derivatization of natural products to enhance their antimicrobial activities [7]-[10]. Lupeol is a naturally occurring pentacyclic triterpenoid that is widely distributed in edible fruits, vegetables and medicinal plants. Previous studies have established the pharmacological activities of lupeol, such as anticancer, antioxidant, anti-inflammatory, cholesterol-lowering and antimicrobial activities [11]. Several reactions have been carried out to obtain new bioactive derivatives of lupeol [12]-[14]. In the course of our continuous search for new antimicrobial agents from higher plants able to fight against microbial resistance, we decided to characterize the chemical constituents of the ethyl acetate extract of *G. glandulosum* and the semi-synthetic derivatives obtained after carrying out oxidation reactions on lupeol and then evaluate their antimicrobial activities.

2. Material and Methods

2.1. Plant Material

The collection of plant material was in accordance with the previously described methodology [2].

2.2. Extraction and Isolation

Extraction was performed according to the previously described method [2]. In

the frame of the present study, a portion of the EtOAc extract (28 g) was subjected to silica gel (0.200 - 0.500 mm) column chromatography (φ 80 mm × 600 mm) using *n*-Hexane-EtOAc (100:0→0:100) followed by EtOAc-MeOH (100:0→90:10) gradient elution. A total of 77 fractions of 250 mL each were collected and subsequently combined on the basis of their TLC profiles, resulting in the formation of six distinct fractions labelled G (1 - 5), H (6 - 11), I (12 - 43), J (44 - 50), K (51 - 74) and L (75 - 77). Fraction H (2.5 g) was purified by silica gel (0.063-0.200 mm) column chromatography (φ 20 mm × 600 mm) using *n*-Hexane-EtOAc (95:5) as eluent, resulting in the isolation of compound **1** (1.5 g). Purification of fraction J (3.1 g) on a Sephadex LH-20 column chromatography (φ 20 mm × 400 mm) afforded compounds **2** (10.4 mg) and **3** (35.2 mg). Compounds **4** (22.3 mg) and **5** (500.0 mg) precipitated in fractions I (0.95 mg) and L (1.70 g) respectively and were purified by recrystallisation using ethyl acetate.

2.3. Chromatographic Methods

Column chromatography (CC) was performed with Merck silica gel 60 and gel permeation with Sephadex LH-20. Thin layer chromatography (TLC) was performed on pre-coated silica gel GF_{254} plates with detection by spraying 10% H_2SO_4 followed by heating at 100˚C or by visual inspection under UV lamps at 254 and 365 nm.

2.4. NMR Analysis

One-dimensional proton (^1H) and carbon (^{13}C) nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III 600 spectrometer equipped with a cryoprobe, with ^1H operating at 600 megahertz (MHz) and ^{13}C at 150 MHz. Two-dimensional nuclear magnetic resonance (2D NMR) experiments were performed using standard Bruker microprograms (Xwin-NMR version 2.1 software TopSpin 3.2). The chemical shifts (δ) are reported in parts per million (ppm), using the residual solvent signals as a secondary reference with respect to TMS (δ = 0), and the values of the coupling constants (J) are given in Hertz (Hz).

2.5. Spectrometric Analysis

To obtain the HR-TOFESIMS spectra, an electrospray source was used with a Micromass Q-TOF micro instrument (Manchester, UK). Samples were introduced by direct infusion into a solution of methanol at a rate of 5 mL per minute.

2.6. Semi-Synthesis of Derivative 1a

2.6.1. Preparation of Jones Reagent, Oxidation Reaction and Purification

The preparation of Jones reagent was conducted in accordance with the methodology previously described in the literature [15]. In a 100 mL beaker, 1 g of chromium trioxide, 25 mL of distilled water and 8 mL of sulfuric acid (H_2SO_4) were added. After careful stirring for one minute, the Jones reagent was successfully obtained. A solution of compound **1** (500.0 mg, 1.096 mmol) in methylene

chloride (30 mL) was transferred to a 100 mL flask. Subsequently, 30 mL of Jones reagent was added. The resulting mixture was heated under reflux at 30˚C for a period of 30 minutes. After cooling the reaction mixture, 40 mL of 5% sodium bicarbonate ($NaHCO_3$) was added to neutralise the medium. The mixture was transferred to a separating funnel, and 50 mL of distilled water and 70 mL of ethyl acetate were added to it. Following the separation of the two phases, the organic phase was subjected to evaporation under reduced pressure using a rotavapor, resulting in the production of a residue amounting to 513.7 mg. The purification of this residue was conducted on a silica gel (0.063 - 0.200 mm) column chromatography (φ 10 mm × 200 mm) with *n*-Hexane-EtOAc (97:3) as eluent, leading to the isolation of compound **1a** (480.3 mg, 96.51 %).

2.6.2. Semi-synthesis of Derivative 1b

To a 100 mL flask containing **1a** (200 mg, 0.471 mmol) dissolved in methylene chloride (15 mL), 50 mL of hydrogen peroxide and 20 mL of formic acid were added. The mixture was allowed to stand at room temperature (25˚C) with magnetic stirring. The reaction was monitored on a TLC plate. After 5 h, the reaction mixture was transferred to a separating funnel to which, 20 mL of distilled water and 100 mL of methylene chloride (CH_2Cl_2) were added. After stirring and decantation, the organic phase was recovered and concentrated with a rotavapor to give 217.1 mg of a mixture. This mixture was purified on a silica gel (0.063 - 0.200 mm) column chromatography (φ 10 mm × 200 mm) with *n*-Hexane-EtOAc (95:5) to give compound **1b** (196.5 mg, 91.36%).

2.7. Microorganisms

The microorganisms used to determine antimicrobial activities were selected based on their relevance as human pathogens and included three bacterial strains (*Escherichia coli* S2, *Staphylococcus aureus* ATCC 25923 and *Shigella flexneri* SDINT) and three yeast strains (*Candida tropicalis*, *Candida albicans* ATCC 9002 and *Cryptococcus neoformans* IP95026) from the collection of the Research Unit of Microbiology and Antimicrobial Substances of the Dschang University. The bacterial and fungal species were cultured at 37˚C and maintained on nutrient agar (NA, Conda, Madrid, Spain) and Sabouraud dextrose agar (SDA, Conda) slant, respectively.

2.8. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Microbicidal Concentration (MMC)

Minimum inhibitory concentration (MIC) values were determined using the broth microdilution method previously described by the Clinical and Laboratory Standards Institute [16] [17], with some modifications regarding the final concentration of samples and inoculum solutions. In fact, each sample was dissolved in dimethyl sulphoxide (DMSO) diluted to 10% (v/v). The solution was then added to Mueller-Hinton Broth (MHB) for bacteria or Sabouraud Dextrose Broth (SDB) for yeasts to give a final concentration of 8192 µg/mL (instead of 4000 µg/mL). This was

serially diluted to a concentration range of 0.125 to 4096 µg/mL. 100 µL of each concentration was then added to each well (96-well microplate) containing 95 µL of MHB or SBD and 5 µL of inoculum to give final concentrations ranging from 0.0625 to 2048 µg/mL. The inoculum was standardized to 2.5×10^5 cells/mL for yeasts and 10^6 CFU/mL for bacteria (instead of 1.5×10^6 CFU/mL) using a JENWAY 6105 UV/Vis spectrophotometer. The final concentration of DMSO in each well was < 1% (preliminary analyses with 1% (v/v) DMSO did not inhibit the growth of the test organisms). The negative control well consisted of 195 µL MHB or BSD and 5 µL standard inoculum. The plates were covered with sterile lids, shaken to mix the contents of the wells and incubated at 37˚C for 24 h (for bacteria) or 48 h (for yeasts). The MIC values of the samples were determined by adding 50 µL of a purple solution of *p*-iodonitrotetrazolium at 0.2 mg/mL followed by incubation at 37˚C for 30 minutes. Viable microorganisms reduced the yellow dye to pink. MIC values were defined as the lowest sample concentrations that prevented this colour change, indicating complete inhibition of microbial growth. To determine the MMC values, a portion of the liquid (5 µL) from each well that showed no microbial growth was plated on Mueller Hinton Agar (MHA) or Sabouraud Dextrose agar (SDA) and incubated at 37˚C for 24 hours (for bacteria) or 37˚C for 48 hours (for yeasts). The lowest concentrations that gave no growth after this subculture were used as MMC values. Amphotericin B for yeasts and Ciprofloxacin for bacteria were used as positive controls. All the experiments were performed in triplicate. The experimental results were expressed as the mean ± Standard Deviation (SD). Differences between groups were considered significant when $p < 0.05$. All analyses were performed using the Statistical Package for Social Sciences (SPSS, version 12.0) software.

3. Results and Discussion

3.1. Chemical Analysis

The structural characterization of natural compounds: Lupeol (**1**), Oleanolic acid (**2**), Chrysoeriol (**3**), *N*-methyl-isonicotinamide (**4**) and *β*-sitosterol 3-*O*-*β*-*D*-glucopyranoside (**5**) (Figure 1) as well as semi-synthetic derivatives: Lupenone (**1a**) and (20*R*)-formyloxy-29-*nor*-lupan-3-one (**1b**) (Figure 2) were carried out using a combination of ^{1}H, NMR, ^{13}C NMR, COSY, HSQC and HMBC experiments. Spin systems were identified through the COSY and HSQC experiments. Subsequently these spin systems and the quaternary carbons were connected by correlations found in the HMBC spectra. The NMR data for compounds **1**, **2**, **3**, **4**, **5** and **1a** (Table 1, Table 2 and Table 3) were in perfect agreement with the literature data [18]-[22].

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) nuclear magnetic resonance data for compounds **1**, **1a** and **1b** in $CDCl_3$.

Position	1		1a		1b	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.7	0.90 (*m*); 1.65 (1H, *m*)	39.5	1.90 (*m*); 1.38 (*m*)	39.5	1.91 (*m*); 1.41(*m*)

Continued

2	27.4	1.52 (*m*); 1.67 (*m*)	34.1	2.48 (*m*); 2.41 (*m*)	34.1	2.47 (*m*); 2.44 (*m*)
3	78.8	3.21 (*brs*)	218.1	-	218.2	-
4	38.9	-	47.3	-	47.2	-
5	55.3	0.67 (*m*)	54.9	1.33 (*m*)	54.6	1.33 (*m*)
6	18.5	1.37 (*m*); 1.52 (*m*)	19s.7	1.47 (*m*)	19.7	1.48 (*m*)
7	34.2	1.39 (*m*)	33.5	1.39 (s*m*)	33.5	1.44 (*m*)
8	40.9	-	40.7	-	40.8	-
9	50.4	1.25 (1H, *m*, H-9)	49.7	1.39 (*m*)	49.2	1.39 (*m*)
10	37.2	-	36.8	-	36.7	-
11	21.1	1.20 (*m*,); 1.40 (*m*)	21.5	1.41 (*m*); 1.29 (*m*)	22.6	1.77 (*m*); 1.71 (*m*)
12	25.2	1.06 (*m*); 1.62 (*m*)	25.0	1.71 (*m*); 1.09 (*m*)	26.7	1.55 (*m*); 1.36 (*m*)
13	38.2	1.66 (*m*)	38.0	1.70 (*m*)	37.5	1.74 (*m*)
14	42.9	-	42.8	-	42.9	-
15	27.1	1.05 (*m*); 1.60 (*m*)	27.4	1.69 (*m*); 1.03 (*m*)	27.1	1.70 (*m*); 1.02 (*m*)
16	35.5	1.35 (*m*); 1.45 (*m*)	35.4	1.50 (*m*); 1.38 (*m*)	35.1	1.50 (*m*); 1.34 (*m*)
17	43.0	-	43.0	-	43.0	-
18	48.2	1.36 (*m*)	48.2	1.39 (*m*)	46.8	1.39 (*m*)
19	48.0	2.40 (*m*)	47.9	2.39 (*m*)	44.1	2.76 (*m*)
20	151.0	-	150.8	-	72.4	5.30 (*m*)
21	29.9	1.30 (*m*); 1.91(*m*)	29.8	1.93 (*m*); 1.34 (*m*)	22.6	1.77 (*m*); 1.70 (*m*)
22	40.0	1.18 (*m*); 1.37 (*m*)	40.0	1.34 (*m*); 1.20 (*m*)	39.8	1.39 (*m*); 1.15 (*m*)
23	28.0	0.90 (*s*)	26.6	1.08 (*s*)	26.6	1.08 (*s*)
24	15.5	0.76 (*s*)	21.0	1.04 (*s*)	21.0	1.04 (*s*)
25	16.1	0.83 (*s*)	15.9	0.95 (*s*)	15.9	0.95 (*s*)
26	16.0	1.03 (*s*)	15.7	1.08 (*s*)	15.7	1.08 (*s*)
27	14.8	0.94 (*s*)	14.5	0.97 (*s*)	14.2	0.88 (*s*)
28	18.0	0.79 (*s*)	18.0	0.80 (*s*)	17.9	0.78 (*s*)
29	109.0	4.57 (*d*, 1.9) 4.69 (*d*, 1.9)	109.4	4.70 (*d*, 1.6) 4.59 (*d*, 1.6)	-	-
30	19.5	1.67 (*s*)	19.3	1.70 (*s*)	20.0	1.23 (*d*, 6.4)
1′					161.5	8.14 (*s*)

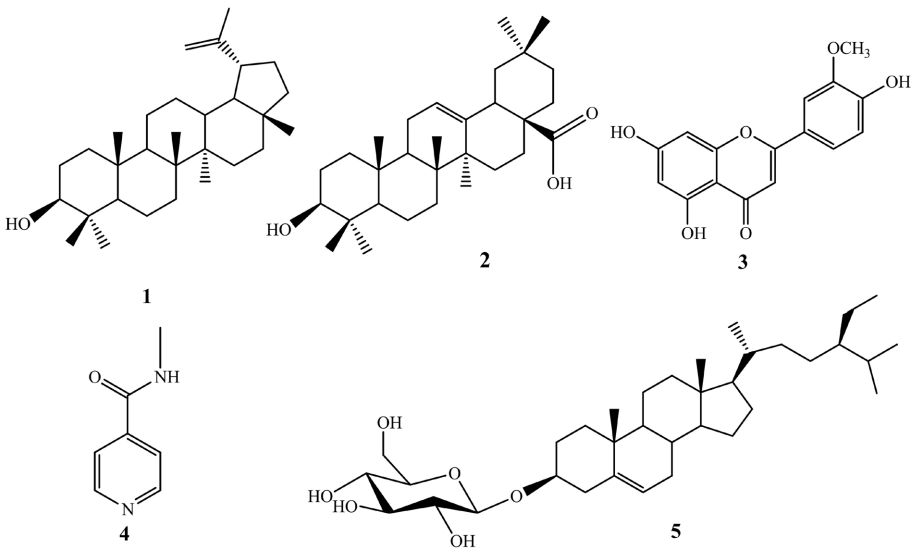


Figure 1. Structures of isolated compounds (**1**-**5**) from *G. glandulosum*.

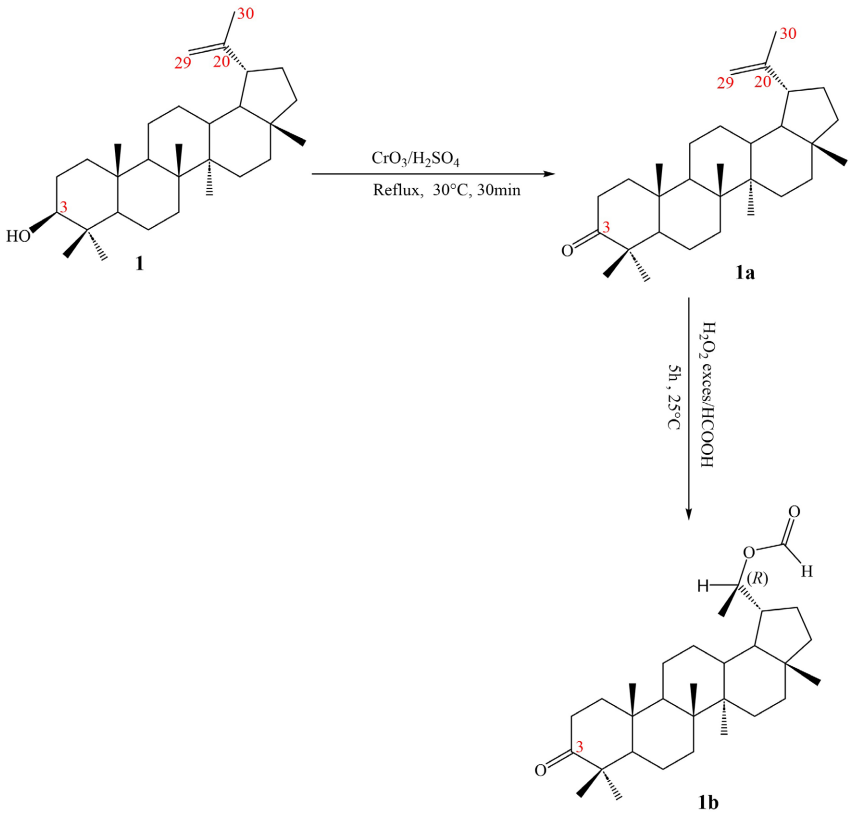


Figure 2. General procedures used for the semi-synthesis of compounds **1a** and **1b**.

Table 2. ^{1}H (600 MHz) and ^{13}C (150 MHz) nuclear magnetic resonance data for compounds **2** (in $CDCl_3$), **3** (in DMSO-d_6) and **4** (in DMSO-d_6).

Position	2		3		4	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	38.8	1.01 (*m*); 1.64 (*m*)	-	-	143.0	7.36 (*d*, 7.6)
2	28.2	1.81 (*m*); 1.79 (*m*)	163.7	-	100.7	5.54 (*d*, 7.6)
3	78.2	3.43 (*brs*)	103.2	6.88 (*s*)	152.1	-
4	39.5	-	181.8	-	100.7	5.54 (*d*, 7.6)
5	55.8	0.87 (*m*)	161.5	-	143.0	7.36 (*d*, 7.6)
6	18.8	1.56 (*m*); 1.39 (*m*)	98.9	6.50 (*d*, 2.0)	165.7	-
7	33.4	1.51 (*m*,); 1.35 (*m*)	164.2		49.0	3.15 (3H, *d*, 3.6)
8	39.8	-	94.1	6.50 (*d*, 2.0)		
9	48.2	1.70 (*m*)	157.4	-		
10	37.4	-	103.7	-		
11	23.8	1.90 (*m*); 1.88 (*m*)				
12	122.0	5.45 (*brs*)				
13	148.9	-				
14	42.1	-				
15	28.2	1.23 (*m*); 2.18 (*m*)				
16	23.8	2.13 (*m*); 1.95 (*m*)				
17	46.7	-				
18	43.0	2.74 (*dd*, 13.8; 4.2)				
19	46.7	1.83 (*m*), 1.32 (*m*)				
20	31.0	-				

Continued

21	34.3	1.46 (*m*); 1.25 (*m*)		
22	33.2	1.82 (*m*); 2.02 (*m*)		
23	28.8	0.75 (*s*)		
24	16.2	0.97 (*s*)		
25	15.6	0.85 (*s*)		
26	17.5	0.68 (*s*)		
27	26.2	1.08 (*s*)		
28	180.1	-		
29	33.2	0.88 (*s*)		
30	23.7	0.87 (*s*)		
1′			121.5	-
2′			110.2	7.54 (1H, *d*, 2.0,)
3′			148.1	-
4′			150.8	-
5′			115.8	6.93 (*d*, 8.4)
6′			120.4	7.56 (*dd*, 2.0; 8.4)
3′-OCH$_3$			56.0	3.89 (*s*)

Table 3. ^{1}H (600 MHz) and ^{13}C (150 MHz) nuclear magnetic resonance data for compound **5** in DMSO-d_6.

Position	5	
	δ_C	δ_H
1	37.6	1.83 (*m*); 1.05 (*m*)
2	30.2	1.87 (*m*); 1.54 (*m*)
3	79.3	3.52 (*brs*)
4	40.2	2.22 (*m*); 2.35 (*m*)
5	140.6	-
6	122.4	5.33 (*m*)
7	32.3	1.92 (*m*); 1.45 (*m*)
8	32.2	1.42 (*m*)
9	50.4	0.94 (*m*)
10	37.1	-
11	21.5	1.41 (*m*); 1.48 (*m*)
12	39.1	1.50 (*m*); 1.98 (*m*)
13	42.6	-
14	57.1	0.98 (*m*)
15	24.5	1.05 (*m*); 1.55 (*m*)
16	29.2	1.85 (*m*); 1.25 (*m*)
17	56.0	1.10 (*m*)
18	12.2	0.70 (*s*)
19	19.5	0.97 (*s*)
20	36.5	1.33 (*m*)
21	19.1	0.88 (*d*, 6.7)
22	34.2	1.31 (*m*); 0.99 (*m*)
23	26.3	1.13 (*s*); 1.24 (*s*)
24	46.1	0.91 (*s*)

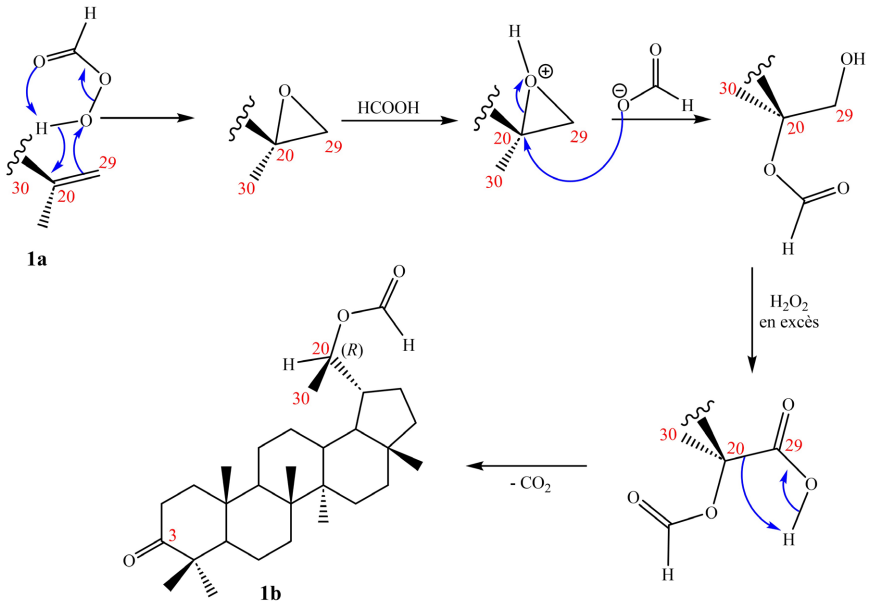
Continued

25	29.4	1.63 (*s*)
26	19.2	0.75 (*d*, 6.2)
27	20.0	0.80 (*d*, 5.1)
28	23.3	1.25 (*m*); 1.20 (*m*)
29	12.3	0.81 (*t*, 7.7)
1′	101.3	4.36 (*d*, 7.0)
2′	73.9	3.19 (*m*)
3′	76.8	3.37 (*m*)
4′	70.6	3.36 (*m*)
5′	76.3	3.24 (*m*)
6′	62.1	3.80 (*dd*, 2.8, 12.0); 3.70 (*dd*, 4.9, 12.0).

Compound **1b** was obtained as a white powder. The yield of synthesis (91.36%) suggests a successful conversion of compound **1a** to compound **1b**. However, the loss of 8.64% was probably due to the purification process. The positive HR-TOFESIMS of this compound showed a sodium adduct peak at *m*/*z* 479.3508 $[M + Na]^+$ (calculated for $C_{30}H_{48}O_3Na$, 479.3501). These MS data and NMR spectra were consistent with a molecular formula of $C_{30}H_{48}O_3$ for compound **1b**. The difference between **1b** and **1a** was observed at the double bond $\Delta^{20\text{-}29}$, which underwent oxidative cleavage with the loss of a carbon atom. This structural modification was reflected in the ^{1}H NMR spectrum, with the disappearance of the signals at δ_H 4.70 (H-29a, *d*; 1.6) and 4.59 (H-29b, *d*, 1.6). This is due to the double bond in compound **1a**, as well as the appearance of an oxymethine proton signal at δ_H 5.30 (H-20, *m*) and that of a formyl group at δ_H 8.14 (H-1′; *s*). This structural modification was corroborated by the ^{13}C NMR spectrum, which revealed the disappearance of the double bond signals at δ_C: 109.4 (C-29) and 150.8 (C-20) present in compound **1a**. Additionally, two signals at δ_C: 161.5 (C-1′) and 72.4 (C-20) were observed. These correspond to the carbon signals of a formyloxy group and an oxymethine, respectively. The position of the formyloxy group was determined by the HMBC experiment, which revealed a correlation between the proton at δ_H 5.30 (H-20) and the carbonyl of the formyloxy group at δ_C 161.5 (C-1′). The absolute configuration *R* of the C-20 stereocentre was determined on the basis of previous work [23] [24]. This work indicated that, the (20*S*) and (20*R*) isomers showed differences in the chemical shifts of C-19, C-20, C-29 and C-30, particularly C-30. Accordingly, compound 1b with C-30 at 20.0 has a 20*R* configuration. Based on the above evidence, the structure of **1b** was determined to be (20*R*)-formyloxy-29-*nor*-lupan-3-one, a novel semi-synthetic lupeol derivative. **Scheme 1** shows the plausible mechanism by which derivative **1b** would have formed.

3.2. Antimicrobial Activities

The evaluation of antimicrobial activities shows that the semi-synthetic derivatives (**1a** and **1b**) and the starting material (**1**) exhibited different minimum inhibitory concentrations (MICs) against the microbial strains tested (**Table 4**).



Scheme 1. Mechanistic steps leading to derivative **1b**.

Table 4. Antimicrobial activities (MIC and MMC in µg/mL) of the substrate and semi-synthetic derivatives as well as antimicrobial reference drugs.

Compounds	Inhibition parameters	*E. coli*	*S. flexneri*	*S. aureus*	*C. tropicalis*	*C. albicans*	*C. neoformans*
1	MIC	32	32	32	128	64	16
	MMC	32	32	32	>256	128	64
	MMC/MIC	1	1	1	nd	2	4
1a	MIC	64	32	32	128	64	32
	MMC	128	32	64	>256	64	32
	MMC/MIC	2	1	2	nd	1	1
1b	MIC	16	32	16	128	32	16
	MMC	32	32	16	256	64	32
	MMC/MIC	2	1	1	2	2	2
Ref*	MIC	32	16	0.5	0.5	1	2
	MMC	32	16	0.5	0.5	1	2
	MMC/MIC	1	1	1	1	1	1

nd: not determined; MIC: Minimum Inhibitory Concentration, MMC: Minimum Microbicidal Concentration; *: amphotericin B for yeasts and ciprofloxacin for bacteria

Indeed, compound **1b** showed the lowest MIC values (16 ≤ MIC ≤ 32 µg/mL) against *E. coli*, *S. aureus* and *C. albicans* compared to the starting material **1** (16 ≤ MIC ≤ 128 µg/mL). None of the **semi-synthetic derivatives** had an MIC lower than the substrate against *C. neoformans*. In addition, the MIC values of the standard drugs were observed to be lower than those of the test samples. Such discrepancies may be due to genetic differences between the microbial strains tested and the structural characteristics of the compounds. With regard to the MIC and MMC values, a lower value of the ratio (MMC/MIC ≤ 4) means that a minimal amount of compounds 1a and 1b is used to kill the microbial species, whereas a

higher value of ratio (MMC/MIC > 4) means that a comparatively larger amount of sample is used to control any microorganism [25]. The analysis of relationships between chemical structure and the activities (SAR) of compounds against microbial strains suggests that antimicrobial activities are primarily associated with functional groups present on rings A and E of the lupeol skeleton. Indeed, it was found that the presence of an oxygenated group at C-3, represented by a hydroxyl or carbonyl group, and the presence of ester group at C-20, increased the antimicrobial activities of lupeol against the microbial strains tested [26] [27]. While the present study highlights the importance of functional groups at C-3 and C-20, systematic modifications of the lupeol scaffold are required to better understand the interactions between the compounds and their microbial targets.

4. Conclusion

The present study was designed to characterize the chemical constituents of the ethyl acetate extract of *Graptophyllum glandulosum* Turrill and the semi-synthetic derivatives generated by the oxidation of lupeol, and then to assess their antimicrobial activities. The overall results indicate that natural compound (**1**) and semi-synthetic derivatives (**1a** and **1b**) presented antimicrobial activities against the tested microorganisms. While the study notes the importance of functional groups at C-3 and C-20, conducting a more detailed SAR study would strengthen the conclusion.

Acknowledgements

The authors are grateful to the "Service Commun d'Analyses" and "Groupe Isolement et Structure" of "Institut de Chimie Moléculaire de Reims" for the spectroscopic and spectrometric analysis.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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