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A New Ceramide (Rumexamide) and Other Chemical Constituents from *Rumex abyssinicus* Jacq (Polygonaceae): Isolation, Characterization, Antibacterial Activities and Chemophenetic Significance

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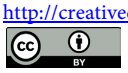
Abstract

The chemical study of *Rumex abyssinicus* Jacp (Polygonaceae) led to the isolation of a new ceramide named (*R*)-2'-hydroxy-*N*-[(2*S*,3*S*,4*R*,16*E*)-1,3,4-trihydroxyhexacos-16-en-2-yl]hexadecanamide (rumexamide) (**1**) together with sixteen known compounds: bis-(2-ethylhexyl) phthalate (**2**), chrysophanol (**3**), physcion (**4**), citreorosein (**5**), emodin (**6**), chrysophanein (**7**), physcionin (**8**), lupeol (**9**), 3*β*,28-dihydroxylup-20(29)-ene (**10**), 3*β*-dihydroxylup-20(29)-en-28-oic acid (**11**), oleanolic acid (**12**), ergosta-6,22-diene-3,5,8-triol (**13**), stigmastane-3,6-dione (**14**), a mixture of *β*-sitosterol (**15**) and stigmasterol (**16**), and stigmasterol 3-*O*-*β*-*D*-glucoside (**17**). Their structures were determined by interpretation of their spectroscopic 1D NMR (^{1}H and ^{13}C NMR), 2D NMR (COSY ^{1}H-^{1}H, HSQC and HMBC) data in conjunction with mass spectrometry (TOFESIMS and HR-TOFESIMS) and by comparison with those reported in the literature. Among all the known compounds, twelve (**2**, **5**, **8**-**17**) were firstly isolated from *Rumex abyssinicus*, seven (**2**, **10**-**14** and **17**) from the genus *Rumex* and three (**13**, **14**, **17**) from family Polygonaceae. The *in vitro* anti-



bacterial activities of extracts (MeOH, *n*-BuOH and EtOAc), as well as compounds **9**, **11**, **12**, **15** + **16** and **17** against pathogenic bacteria (*Staphylococcus aureus* ATCC 43300, *Shigella flexneri* NR 518, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC 25922), were performed using the broth microdilution method and the results show that, extracts were not active (MIC > 1000 μg/mL) while compounds were weakly or not active (MIC ≥ 500 μg/mL) against all bacteria strains. Furthermore, the chemophenetic relationships of the isolated compounds and their significances were discussed.

Keywords

Polygonaceae, *Rumex abyssinicus*, Rumexamide, Antibacterial Activities, Chemophenetic Significance

1. Introduction

Since their discoveries, antibiotics have been essential in the treatment of diseases caused by pathogens (especially bacterial infections) which have helped in extending the average life expectancy. However, their overexploitation has caused the appearance and increase in the rate of resistance of microorganisms to said antibiotics [1]. It is well established that this resistance is nowadays responsible for nearly 7 million deaths per year and it is estimated that it will reach to 10 million deaths per year by 2050 [2]. Thus, in order to overcome this state of affairs, efforts are being made to find new antimicrobial agents such as medicinal plants [3]. *Rumex abyssinicus* is a large annual herbaceous plant with generally sagittate leaves, highly branched inflorescence and light brown hazelnuts and locally named in Amharic "mekmako" [4]. It is a medicinal plant widely distributed in the highlands of tropical Africa and distributed throughout North Africa and Ethiopia [5]. Indeed, *Rumex abyssinicus* is used in traditional medicine to treat several diseases like rheumatism, malaria, typhoid and hepatitis [5] [6]. Members of the genus *Rumex* have been reported to produce a wide range of secondary metabolites such as anthraquinones [7], flavonoids [8], stilbenoids [9] [10], tannins [11], triterpenoids [12], steroids and saponins [13]. Some of them possess several pharmacological activities such as antioxidant, antitumour, antimicrobial, antiparasitic, antiviral [14]. Previous pharmacological studies carried out on *R. abyssinicus* revealed its antimicrobial activities against *Salmonella typhimurium*, *Listeria monocytogenes* (ATCC 29211), *Escherichia coli* (ATCC25922), *Staphylococcus aureus* (ATCC 4944) [15] and antiplasmodial activity against chloroquine-sensitive *Plasmodium falciparum* strain (3D7) [16]. In our continuous search for bioactive secondary metabolites from Cameroonian medicinal plants [17] [18] [19] [20], we report in this paper the isolation, characterization, antibacterial activities and Chemophenetic significance of secondary metabolites from the whole plant of *R. abyssinicus*.

2. Materials and Methods

2.1. General Experimental Procedures

Bruker IR-Alpha spectrometer apparatus was used for scanning IR (Infrared) spectroscopy using KBr pellets. Column chromatography was carried out on silica gel 230 - 400 mesh, Merck (Merck, Darmstadt, Germany), 70 - 230 mesh (Merck) and/or gel permeation on Sephadex LH-20 (Sigma-Aldrich, Munich, Germany). High resolution mass spectra were obtained with QTOF (Quadrupole Time of Flight) Compact Spectrometer (Bruker, Germany) equipped with a HRESI source. The spectrometer was operated in positive and negative modes (mass range: 50 - 1500, with a scan rate of 1.00 Hz) with automatic gain control to provide high-accuracy mass measurements within 0.4 ppm deviation using Na formate as calibrant. The following parameters were used for experiments: spray voltage of 4.5 kV, capillary temperature of 200˚C. Nitrogen was used as sheath gas (4 L/min). The ^{1}H and ^{13}C Nuclear Magnetic Resonance (NMR) spectra were recorded on Bruker DRX 500 MHz and 600 MHz NMR spectrometers and on a Bruker Avance III 600 and 500 spectrometers equipped with a cryo-platform. 2D NMR experiments were performed using standard Bruker microprograms (XwinNMR version 2.1 software) in deuterated solvents. Chemical shifts (δ) are reported in parts per million (ppm) using the residual solvent signals as secondary reference relatively to TMS (Tetramethylsilane) ($\delta = 0$), while the coupling constants (J values) are given in Hertz (Hz). Thin-layer chromatography (TLC) was carried out on Merck pre-coated silica gel (60 F_{254}) aluminium foil (Merck) with detection accomplished by spraying with diluted sulfuric acid (50% H_2SO_4, Riedel-de Haen AG, 95% - 97%) followed by heating at 100˚C, or by visual inspection under Ultraviolet (UV) lamp at 254 and 365 nm.

2.2. Plant Material

In the present study the whole plant of *Rumex abyssinicus* was collected in February 2018 in Dschang Western Region of Cameroon, with GPS data of: latitude: N 5˚27'5.94828" and longitude: E 10˚3'17.39556". The botanical identification was carried out by Victor Nana, a botanist of the National Herbarium of Cameroon, where a specimen was deposited under the voucher number N˚ 50551/HNC.

2.3. Extraction and Isolation

The air-dried plant material (4.5 kg) was powdered and extracted at room temperature with methanol (3 × 20 L, 72 h) to yield 200 g of crude methanolic extract after evaporation of solvent under reduced pressure. A part of this crude extract (195 g) was dissolved in water (H_2O, 300 mL) followed by a liquid-liquid extraction with ethyl acetate (EtOAc, 500 mL) and *n*-butanol (*n*-BuOH, 500 mL) yielding respectively 50 g and 18 g after solvents evaporation under vacuum (at 40˚C). A part of the EtOAc fraction (45 g, i.e 90% of the mass obtained) was subjected to silica gel column chromatography using *n*-hexane-EtOAc (95:5 → 80:20, v/v) followed by EtOAc-MeOH (95:5 → 70:30, v/v) for a gradient elution.

Seventy (70) fractions of 400 mL were collected and combined on the basis of their TLC profiles to give 8 fractions A-H (A: 1 - 3; B: 4 - 10; C: 11 - 22; D: 23 - 28; E: 29 - 35; F: 36 - 44; G: 45 - 63; H: 64 - 70). Compounds **1** (30.8 mg), **17** (15.5 mg) [21] and **2** (6.8 mg) [22] were obtained after purification of fraction F (2 g) on silica gel column chromatography using *n*-hexane-EtOAc (40:60 → 00:100, v/v). Fraction A (4 g) was purified on silica gel column chromatography with *n*-hexane-EtOAc as eluent (95:5, v/v) to yield compounds **9** (10.2 mg) [23], **3** (15 mg) [24] and **4** (17 mg) [25]. Fraction B (5 g) was also purified on silica gel column chromatography eluted with *n*-hexane-EtOAc (95:5 → 90:10, v/v) to give a mixture of two compounds (**15** and **16**) (12.5 mg) [26] and compound **10** (6.4 mg) [27]. Sephadex LH-20 gel column chromatography of fraction C (1.9 g) led to two sub-fractions labelled C_1 and C_2. The purification of the sub-fraction C_1 (500 mg) by silica gel column chromatography using *n*-hexane-EtOAc, (90:10 → 80:20, v/v) as mobile phase afforded to compounds **14** (4.1 mg) [28], **11** (7.6 mg) [27], **12** (8.2 mg) [29] and **13** (15 mg) [30]. Sub-fraction C_2 (300 mg), was purified on Sephadex LH-20 gel column chromatography using MeOH as eluent to give compound **6** (40 mg) [25]. Fraction D (3.74 g) was also subjected to Sephadex LH-20 gel column chromatography using isocratic elution with MeOH to obtain three sub-fractions D_1, D_2 and D_3. Purification of D_3 (400 mg) sub-fraction on silica gel column chromatography with *n*-hexane–EtOAc (85:15, v/v) gave compound **5** (11 mg) [31]. Recrystallization of fraction G (5 g) afforded a mixture of two compounds **7** and **8** (10 mg) [32].

2.4. Methanolysis of Compound 1

Compound **1** (5.0 mg, 7.3421 μMol) was dissolved in 5% HCl-MeOH (3 mL) (HCl, 37% produce by Fisher Scientific and MeOH obtained from SOLEVO-Cameroon) and refluxed for 14 h at 70˚C. The reaction was monitored using TLC analysis. At the end of the reaction, the mixture obtained was extracted with methylene chloride (CH_2Cl_2 obtained from SOLEVO-Cameroon) after neutralization with dilute potassium carbonate (K_2CO_3, produce by Fisher Scientific with 99.9% purity). The organic phase was separated and concentrated to yield 2.1 mg.

2.5. Methodology of Antibacterial Assay

2.5.1. Bacteria Strains and Sample Preparation

The microorganisms used in this study were consisted of four bacterial strains namely: *Staphylococcus aureus* NR 43300, *Shighella flexineri* NR 518, *Klebsiella, pneumonia* ATCC 700603, *Escherichia coli* ATCC 25922 obtained from our local stocks. They were cultivated in petri dishes containing Mueller Hinton Agar (MHA) followed by an incubation period of 24 hours at 37˚C. Each microorganism was sub-cultured in a new agar plate and incubated in the same above mentioned conditions prior each experiment. Extracts and compounds were weighed and dissolved in different solvents (pyridine, DMSO) for a final of 100 mg/mL and 20 mg/mL for extracts and compounds respectively. Positive control Ci-

profloxacin was prepared at 1 mg/mL. Extracts, compounds and the reference drugs were screened at 1000 µg/mL, 500 µg/mL and 119 µg/mL, respectively.

2.5.2. Determination of Minimal Inhibitory Concentration (MIC)

The antimicrobial activity of each product was done as previously described by the CLSI, (2012) using the broth microdilution method in 96-wells microplates. Briefly, 196 µL and 190 µL of culture media (Nutrient Broth (NB) for extracts and compounds respectively were introduced in plates and 4 µL and 10 µL of test products were added only in wells belonging to the first line. After a gentle homogenization, serial two-fold dilutions of test products were made by transferring 100 µL of the mixture from the first well to the second up to the last. 100 µL of the microbial inoculum standardized at 5.105 UFC/Ml (With 0.5 McFarland standard) were respectively introduced in wells. Wells containing the culture medium only served as sterility control, those containing the microorganisms and the culture medium were the Negative control corresponding to one hundred percent growth (100% growth). Positive control Ciprofloxacin was distinctively screened in same conditions. All the experiments were performed in duplicate and twice. The plates were incubated for 24 hours at 37˚C. 20 microliters of resazurin (0.15 mg/mL in PBS) were then added in duplicate wells and plates were further incubated for 2 hours. MICs were determined as the lowest concentration in which no visible growth (blue colored wells) was observed in wells after the incubation period. Test concentrations were 1000 - 15.625 µg/mL and 500 - 7.875 µg/mL for extracts and compounds respectively.

3. Results and Discussion

The structures of compounds **2**-**17** were determined on the basis of the spectroscopic and mass spectrometric data as bis-(2-ethylhexyl) phthalate (**2**) [22], Chrysophanol (**3**) [24], physcion (**4**), citreorosein (**5**) [31], emodin (**6**) [25], chrysophanein (**7**) and physcionin (**8**) [32], lupeol (**9**) [23], 3*β*, 28-dihydroxylup-20(29)-ene (**10**) [27], 3*β*-dihydroxylup-20(29)-en-28-oic acid (**11**) [27], oleanolic acid (**12**) [29], ergosta-6,22-diene-3,5,8-triol (**13**) [30], stigmastane-3,6-dione (**14**) [28], *β*-sitosterol (**15**) and stigmasterol (**16**) [26], stigmasterol 3-*O*-*β*-D-glucoside (**17**) [21] (**Figure 1**).

Compound **1** was obtained as white powder in *n*-hexane-EtOAc (30:70, v/v). Its molecular formula $C_{42}H_{83}NO_5$ was determined on the basis of its HR-TOFESIMS spectrum which showed a sodium adduct peak $[M+Na]^+$ at *m/z* 704.6133 (calcd. $C_{42}H_{83}NO_5Na$, 704.6163), indicating two degrees of insaturation. The IR spectrum displayed characteristic vibration bands for hydroxy group (3315 cm^{-1}), olefinic group (1626 cm^{-1}) and amide carbonyl group (1655 cm^{-1}). The ^1H NMR spectrum indicated signals of (-NH) at δ_H 8.61 (1H, *d*, *J* = 9.1 Hz), oxygenated methylene at δ_H 4.54 (1H, *m*, H-1a) and 4.45 (1 H, *m*, H-1b), three oxymethine protons at δ_H 4.65 (1H, *m*, H-2'), 4.39 (1H, *m*, H-3) and 4.32 (1H, *m*, H-4),

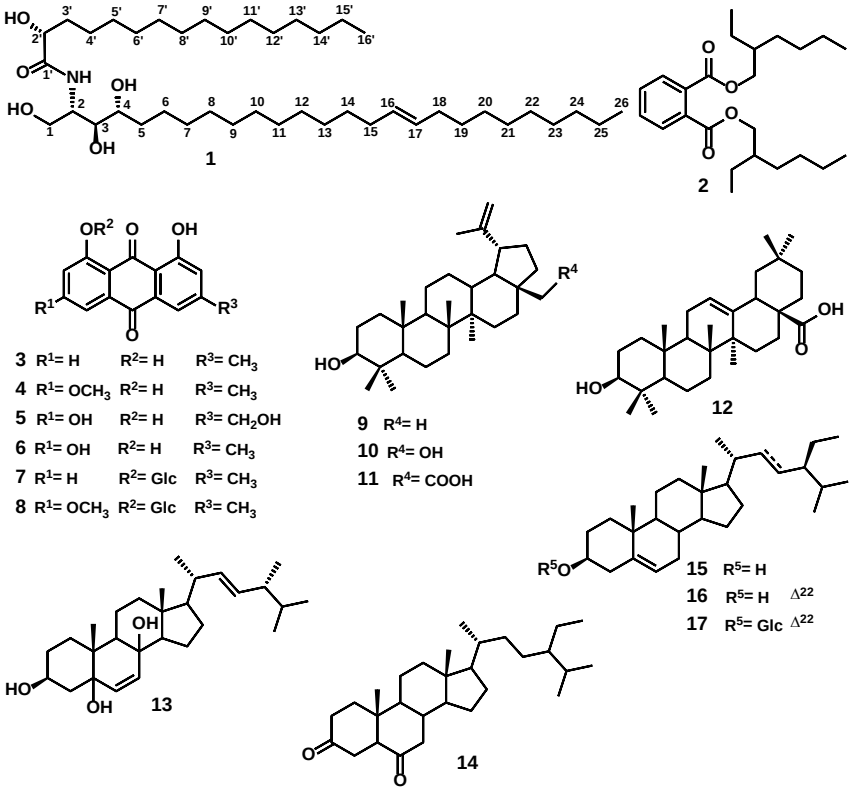


Figure 1. Structures of compounds **1-17** isolated from EtOAc soluble fraction of crude extract of *Rumex abyssinicus*.

two terminal methyls at δ_H 0.88 (6H, *t*, *J* = 7.0 Hz, H-16 and H-26). A set of signals appearing as broad in the range of δ_H 1.52 - 1.20 corresponding to the methylenes (-CH_2-) associated to the two long chains, and a downfield signal at δ_H 5.14 (1H, *m*, H-2) assigned to the aminomethine H-2 of sphingosine were also observed [33]. All the above spectral data (**Table 1**) revealed that compound 1 was a phytosphingosine type sphingolipid [34]. In addition, its ^{1}H NMR spectrum exhibited two olefinic protons at δ_H 5.57 (1H, *m*, H-16) and 5.51 (1H, *m*, H-17).

A concomitant analysis of its ^{13}C-NMR, DEPT and HSQC spectra was in agreement with the ^{1}H-NMR spectrum and allowed to identify an amide carbonyl carbon at δ_C 175.5 (C-1'), two olefinic methine carbons resonated at δ_C 131.2 (C-16) and 131.1 (C-17), an oxymethylene carbon resonated at δ_C 62.4 (C-1), four sp^3 methine carbon signals in which, three oxymethines at δ_C 77.2 (C-3), 73.3 (C-4), 72.8 (C-2'), and an aminomethine at δ_C 53.2 (C-2). It also revealed aliphatic methylenic carbons in the range of δ_C 36.1 - 23.2, and two terminal methyl carbons at δ_C 14.6 ppm. The *trans* (*E*) configuration of the double bond was confirmed by the chemical shifts of the allylic carbons at δ_C 33.7 (C-15) and 33.6 (C-18) [35] [36]. The correlation observed on its COSY ^{1}H-^{1}H (**Figure 2**) spectrum between protons: H-1a/H-1b and H-2, H-2 and H-3, H-3 and H-4 allowed us to identify the position of four hydroxy groups. This was further confirmed by the HMBC correlations between the protons at δ_H 4.54

Table 1. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR data of rumexamide (**1**) in pyridine-d_5 (δ in ppm, J in Hz, TMS as internal standard).

Position	δ_H (nH, mult; J in Hz)	δ_C	(mult)	HMBC
Long chain base				
NH	8.61 (1H, *d*, J = 9.1)	-		-
1a, 1b	4.54 (1H, *m*) 4.45 (1H, *m*)	62.4	(CH_2)	2; 3
2	5.14 (1H, *m*)	53.2	(CH)	1'; 1; 3
3	4.39 (1H, *m*)	77.2	(CH)	1; 2; 4; 5
4	4.32 (1H, *m*)	73.3	(CH)	5
5	2.00 (2H, *m*)	34.2	(CH_2)	-
6 - 14	1.52 - 1.20 (18H, *brs*)	32.5 - 27.0	$(CH_2)_9$	-
15	2.19 (2H, *m*)	33.7	(CH_2)	16
16	5.57 (1H, *m*)	131.2	(CH)	15
17	5.51 (1H, *m*)	131.1	(CH)	18
18	2.02 (2H, *m*)	33.6	(CH_2)	17
19 - 24	1.52 - 1.20 (10H, *brs*)	32.5 - 27.0	$(CH_2)_5$	
25	1.26 (1H, *m*)	23.3	(CH_2)	-
26	0.88 (3H, *t*, J = 7.0)	14.6	(CH_3)	25
N-acyl moiety				
1'	-	175.5	(C)	-
2'	4.65 (2H, *dt*, J = 7.7, 3.7)	72.8	(CH_2)	
3'	2.26 (1H, *m*) 2.06 (1H, *m*)	36.1	(CH_2)	- 2'
4' - 14'	1.52 - 1.20 (22H, *brs*)	32.5 - 27.0	$(CH_2)_{11}$	-
15'	1.26 (1H, *m*)	23.3	(CH_2)	-
16'	0.88 (3H, *t*, J = 7.0)	14.6	(CH_3)	15'

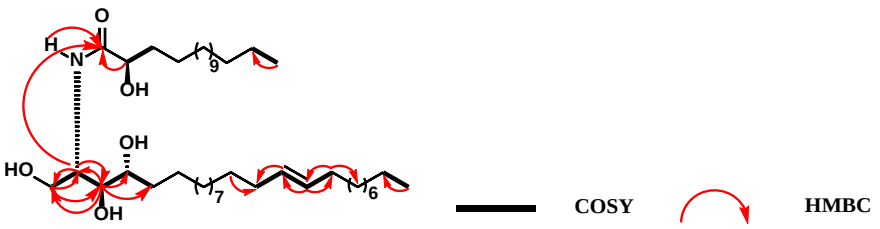


Figure 2. Selected COSY and HMBC correlations for compound **1**.

(H-1a), 4.45 (H-1b) and the carbons at δ_C: 77.2 (C-3), 53.2 (C-2) ; between the proton at δ_H 4.39 (H-3) and the carbons at δ_C: 73.3 (C-4), 62.3 (C-1), 53.2 (C-2), 34.2 (C-5) and between the proton of hydroxymethine at δ_H 4.65 (H-2') and

carbonyl at δ_C 175.5 (C-1'). In addition, correlation observed between the proton of aminomethine at δ_H 5.14 (H-2) and carbons at δ_C 77.2 (C-3), 62.4 (C-1) and 175.5 (C-1') allowed to conclude that, the two chains (acid and basic) are linked. The absolute configuration of the stereocenters C-2, C-3, C-4 and C-2', were assigned to be 2*R*, 3*S*, 4*R*, 2'*R* on the basis of their carbon chemical shifts which are very close to those of vernoguinamide [37].

The length of the acid chain (ALC) was determined using the ESI (-) mass spectrum where characteristic fragment ions are observed at *m/z*: 255 $[CH_3(CH_2)_{13}CH(OH)CO]^-$, 227 $[CH_3(CH_2)_{13}CH(OH)]^-$, 199 $[HNCOCH(OH)(CH_2)_9]^-$, 170 $[CH_3(CH_2)_{11}+H]^-$, 156 $[CH_3(CH_2)_{10}+H]^-$. Concerning the basic chain, the length was deduced from the mass of the entire molecule by subtracting that of the acid chain. Thus, said basic chain has 426 for mass, corresponding to $[CH_3(CH_2)_8CH=CH(CH_2)_{11}CH(OH)\ CH(OH)CH(NH)CH_2(OH)]$ having a double bond. Note that this number of carbon atoms of the acid and basic chains was further confirmed by the methanolysis of said compound which led to a fatty acid methyl ester unit (FAME; **1a**) and a long chain phytosphingosine unit (LCB; **1b**) (Scheme 1).

The LC-MS analysis of the organic phase showed that, the methanolysis of compound **1** led to the formation of methyl (*R*) 2-hydroxyhexadecanoate (**1a**), (*m/z* 309.1 $[M+Na]^-$) and (2*S*,3*S*,4*R*,16*E*)-2-aminohexacos-16-ene-1,3,4-triol (**1b**), (*m/z* 468.5 $[M+CH_3CN]^+$ and *m/z* 451.3 $[M+H+Na]^+$) (Scheme 1) indicating 16 carbon atoms for acid chain (**1a**) and 26 carbon atoms with one double bond for basic chain (**1b**). The position of the double bond was deduced from its ESI (+) mass spectrum on which ions fragments are observed at *m/z* 554 $[M-C_9H_{19}]^+$ and *m/z* 152 $[C_{11}H_{21}-H]^+$ (Figure 3). Accordingly, the structure of compound **1** was established as (*R*)-2'-hydroxy-*N*-[(2*S*,3*S*,4*R*,16*E*)-1,3,4-trihydroxyhexacos-16-en-2-yl] hexadecanamide, trivially named rumexamide.

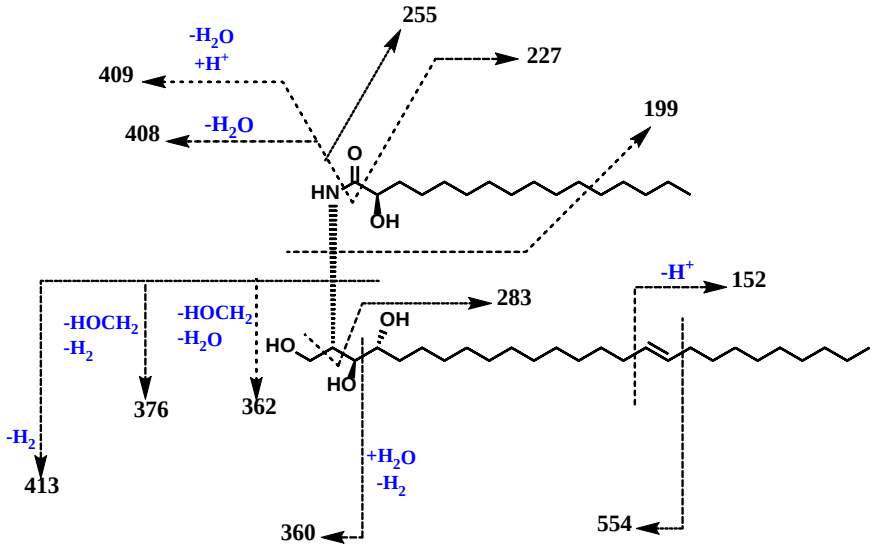
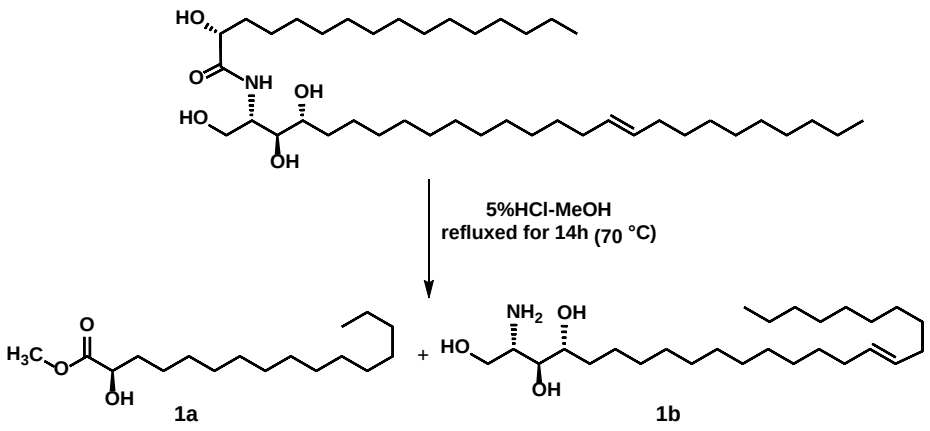


Figure 3. Mass fragmentation pattern for compound **1**.



Scheme 1. Methanolysis of **1**.

The antibacterial activities of MeOH, EtOAc and *n*-BuOH extracts as well as compounds **1**, **9**, **11**, **12**, **15** + **16** and **17** against pathogenic bacteria are presented in Table 2. In general, the test samples demonstrated weak variation degrees of inhibitory activity against the bacteria. The antibacterial activity of the plant extract can be classified as significant (MIC < 100 µg/mL), moderate (100 < MIC ≤ 625 µg/mL) and weak (MIC > 625 µg/mL) whereas antimicrobial activity of pure compound can be classified as significant (MIC < 10 µg/mL), moderate (10 < MIC ≤ 100 µg/mL) and weak (MIC > 100 µg/mL) [38]. Base on this, extract were not active (MIC > 1000 µg/mL) while compounds were weakly or not active (MIC ≥ 500 µg/mL) against all bacteria strains.

4. Spectroscopic Data of Compounds 1-17

Bis-(2-ethylhexyl) phthalate (2)—White powder; ($C_{24}H_{38}O_4$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ_H: 7.70 (*dd*, 5.7, 3.3Hz, H-3), 7.52 (*dd*, 5.7, 3.3Hz, H-4), 4.22 (*m*, H-1'), 1.68 (*p*, 6.1Hz, H-2'), 1.41 (*m*, H-7), 1.34 (*m*, H-3'), 1.32 (*m*, H-4'), 1.31 (*m*, H-5'), 0.92 (*m*, H-8'), 0.89 (*m*, H-6'); ^{13}C-NMR (100 MHz, $CDCl_3$) δ_C:167.9 (C-1), 132.6 (C-2), 130.9 (C-4), 128.9 (C-3), 68.3 (C-1'), 38.9 (C-2'), 30.5 (C-3'), 29.1 (C-4'), 23.9 (C-7'), 23.1 (C-5'), 14.2 (C-6'), 11.1 (C-8').

Chrysophanol (3)—Yellow powder; ($C_{15}H_{10}O_4$): ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 12.08 (*s*, 1-OH), 11.97 (*s*, 8-OH) 7.84 (*d*, 7.5 Hz, H-5), 7.77 (*br s*, H-6), 7.69 (*br s*, H-4), 7.30 (*d*, 8.4Hz, H-7), 7.12 (*br s*, H-2), 2.48 (*s*, -CH_3); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 192.5 (C-9), 182.1 (C-10), 162.7 (C-1), 162.4 (C-8), 149.3 (C-3), 137.0 (C-6), 133.6 (C-11), 133.2 (C-14), 124.5 (C-7), 124.3 (C-2), 121.3 (C-4), 119.9 (C-5), 115.8 (C-12), 113.7 (C-13), 22.4 (-CH_3).

Physcion (4)—Yellow powder; ($C_{16}H_{12}O_5$): ^{1}H-NMR (600 MHz, $CDCl_3$) δ_H: 12.34 (*s*, 1-OH), 12.15 (*s*, 8-OH), 7.65 (*br s*, H-5), 7.39 (*d*, 2.5 Hz, H-4), 7.10 (*br s*, H-7), 6.70 (*d*, 2.5 Hz, H-2), 3.95 (*s*, OCH_3), 2.46 (*s*, -CH_3); ^{13}C-NMR (150 MHz, $CDCl_3$) δ_C: 190.8 (C-9), 182.1 (C-10), 166.6 (C-3), 165.2 (C-1), 162.5 (C-8), 148.5 (C-6), 135.2 (C-14), 133.2 (C-11), 124.6 (C-7), 121.4 (C-5), 113.7 (C-12), 110.3 (C-13), 108.3 (C-4), 106.8 (C-2), 56.1 (-OCH_3), 22.2 (-CH_3).

Table 2. Antimicrobial activities of extracts, some isolated compounds and ciprofloxacin.

Samples	Parameters (µg/mL)	Bacteria strains			
		Sa	***Sf***	***Kp***	***Ec***
EtOAc extract	MIC	>1000	>1000	>1000	>1000
n-BuOH extract	MIC	>1000	>1000	>1000	>1000
MeOH extract	MIC	>1000	>1000	>1000	>1000
1	MIC	>500	500 ± 000	>500	>500
9	MIC	>500	>500	>500	>500
11	MIC	>500	>500	>500	>500
12	MIC	>500	>500	>500	>500
15 + **16**	MIC	>500	>500	>500	>500
17	MIC	>500	>500	>500	>500
Ciprofloxacin	MIC	0.232 ± 000	0.232 ± 000	0.464 ± 000	0.232 ± 000

Sa: *Staphylococcus aureus* ATCC 43300; ***Sf***: *Shighella flexineri* NR 518; ***Kp***: *Klebsiella pneumonia* ATCC 700603; ***Ec***: *Escherichia coli* ATCC 25922; **MIC:** Minimum Inhibitory Concentration; **Ciprofloxacin:** reference drug.

Citreorosein (5)—Red powder; **($C_{15}H_{10}O_6$)**: ^{1}H-NMR (600 MHz, CD_3OD) δ_H: 7.28 (*br s*, H-2), 7.75 (*br s*, H-4), 7.20 (*br s*, H-5), 6.54 (*br s*, H-7), 4.70 (*s*, -OCH_2-); ^{13}C-NMR (150 MHz, CD_3OD) δ_C: 191.5 (C-9), 183.4 (C-10), 169.1 (C-8), 166.7 (C-6), 163.7 (C-1), 152.9 (C-3), 136.9 (C-11), 135.0 (C-14), 122.2 (C-2), 118.4 (C-4), 115.9 (C-13), 111.1 (C-5), 109.2 (C-7), 108.5 (C-12), 64.1 (-OCH_2-).

Emodin (6)—Red powder; **($C_{15}H_{10}O_5$)**: ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H: 12.1 (*s*, 3-OH), 12.0 (*s*, 8-OH) 7.48 (*d*, 0.7Hz, H-5), 7.16 (*d*, 0.7Hz, H-7) 7.11 (*d*, 2.4 Hz, H-4) 6.59 (*d*, 2.4Hz, H-2), 2.41 (*s*, -CH_3); ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C: 190.2 (C-9), 181.9 (C-10), 166.1 (C-1), 164.9 (C-3), 161.9 (C-8), 148.7 (C-6), 135.6 (C-14), 133.3 (C-11), 124.6 (C-7), 120.9 (C-5), 113.8 (C-12), 109.4 (C-13), 109.3 (C-4), 108.4 (C-2), 21.9 (-CH_3).

Chrysophanein (7)—Yellow powder; **($C_{21}H_{20}O_9$)**: ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H: 13.1 (*s*, 1-OH), 7.88 (*m*, H-5), 7.86 (*m*, H-6) 7.71 (*d*; 7.9Hz, H-7) 7.51 (*br s*, H-4) 7.21 (*br s*, H-2) 5.20 - 3.10 (Glu), 2.44 (*s*, 3-CH_3); ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C: 188.0 (C-9), 182.6 (C-10), 162.2 (C-1), 158.7 (C-8), 148.1 (C-3), 136.4 (C-6), 135.2 (C-11), 132.6 (C-14), 124,5 (C-2), 122.9 (C-7), 121.0 (C-5), 119.8 (C-4), 115.3 (C-12), 115.2 (C-13), 101.0 (C-1'), 77.8 (C-5'), 77.0 (C-3'), 73.7 (C-2'), 70.0 (C-4'), 61.1 (C-6'), 21,9 (-CH_3).

Physcionin (8)—Yellow powder; **($C_{22}H_{22}O_{10}$)**: ^{1}H-NMR (600 MHz, DMSO-d_6) δ_H: 12.8 (*s*, 1-OH), 7.50 (*br s*, H-4), 7.37 (*d*, 2.3Hz, H-5) 7.19 (*d*, 2.3 Hz, H-7) 7.18 (*br s*, H-2), 5.20 - 3.10 (Glu), 3.97 (*s*, -OCH_3), 2.42 (*s*, -CH_3); ^{13}C-NMR (150 MHz, DMSO-d_6) δ_C: 186.9 (C-9), 182.4 (C-10), 165.2 (C-6), 162.1 (C-1), 161.2 (C-8), 147.6 (C-3), 135.1 (C-11), 132.5 (C-14), 124.7 (C-2), 119.7 (C-4), 114.96 (C-13), 114.95 (C-12), 107.9 (C-7), 106.9 (C-5), 101.1 (C-1'), 77.9 (C-5'), 77.1 (C-3'), 73.8 (C-2'), 70.3 (C-4'), 61.3 (C-6'), 56.6 (-OCH_3) 21,8 (-CH_3).

Lupeol (9)—White powder; **($C_{30}H_{50}O$)**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ_H: 4.73

(*s*, H-29b), 4.62 (*s*, H-29a), 3.23 (*m*, H-3), 2.04 (*m*, H-19), 1.73 (*s*, 30-CH_3), 1.07 (*s*, 26-CH_3), 1.01 (*s*, 23-CH_3), 0.99 (*s*, 27-CH_3), 0.87 (*s*, 25-CH_3), 0.81 (*s*, 24-CH_3), 0.69 (*m*, H-5); ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C: 18.0 (C-28), 150.6 (C-20), 109.9 (C-29), 79.0 (C-3), 42.8 (C-17), 55.3 (C-5), 50.5 (C-9), 48.3 (C-18), 48.0 (C-19), 43.0 (C-14), 40.6 (C-8), 38.9 (C-4), 38.7 (C-1), 38.1 (C-13), 37.2 (C-10), 40.8 (C-22), 34.8 (C-7), 35.2 (C-16), 28.0 (C-15), 29.9 (C-21), 27.5 (C-23), 27.4 (C-2), 25.2 (C-12), 21.0 (C-11), 18.4 (C-6), 19.3 (C-30), 16.0 (C-25), 16.2 (C-26), 15.4 (C-24), 14.6 (C-27).

3*β*,28-dihydroxylup-20(29)-ene (10)—White powder; ($C_{30}H_{50}O_2$): ^{1}H-NMR (300 MHz, $CDCl_3$) δ_H: 4.73 (*s*, H-29b), 4.63 (*s*, H-29a), 3.85 (*m*, H-28), 3.36 (*m*, H-3), 1.73 (*s*, 30-CH_3), 1.07 (*s*, 27-CH_3), 1.03 (*s*, 26-CH_3), 1.00 (*s*, 23-CH_3), 0.87 (*s*, 25-CH_3), 0.75 (*s*, 24-CH_3); ^{13}C-NMR (75 MHz, $CDCl_3$) δ_C: 38.7 (C-1), 27.4 (C-2), 79.0 (C-3), 39.0 (C-4), 55.3 (C-5), 18.3 (C-6), 34.3 (C-7), 40.9 (C-8), 50.4 (C-9), 37.4 (C-10), 20.7 (C-11), 25.3 (C-12), 37.2 (C-13), 42.7 (C-14), 27.1 (C-15), 29.2 (C-16), 47.8 (C-17), 47.8 (C-18), 48.8 (C-19), 150.5 (C-20), 29.7 (C-21), 34.0 (C-22), 28.0 (C-23), 15.2 (C-24), 16.1 (C-25), 15.5 (C-26), 14.9 (C-27), 60.5 (C-28), 109.7 (C-29), 19.0 (C-30).

3*β*-dihydroxylup-20(29)-en-28-oic acid (11)—White powder; ($C_{30}H_{48}O_3$): ^{1}H-NMR (500 MHz, Methanol-d_5) δ_H: 4.73 (*s*, H-29b), 4.61 (*s*, H-29a), 3.14 (*dd*, 11.4 Hz/4.7 Hz, H-3), 3.04 (*m*, H-19), 2.32 (*m*, H-13), 2.25 (*m*, H-16), 1.95 (*m*, H-15a), 1.91 (*m*, H-22), 1.74 (*m*, H-12), 1.71 (*s*, 30-CH_3), 1.65 (*m*, H-18), 1.62 (*m*, H-1), 1.59 (*m*, H-2), 1.56 (*m*, H-6), 1.49-1.44 (*m*, H-11), 1.40 (*m*, H15b), 1.45 - 1.39 (*m*, H-7), 1.34 (*m*, H-9), 1.19 (*m*, H-21), 1.02 (*s*, 27-CH_3), 0.99 (*s*, 26-CH_3), 0.97 (*s*, 23-CH_3), 0.88 (*s*, 25-CH_3), 0.77 (*s*, 24-CH_3), 0.73 (*m*, H-5); ^{13}C-NMR (125 MHz, Methanol-d_5) δ_C: 178.7 (C-28), 150.6 (C-20), 108.8 (C-29), 78.3 (C-3), 56.1 (C-17), 55.5 (C-5), 50.6 (C-9), 49.0 (C-18), 47.2 (C-19), 42.2 (C-14), 40.6 (C-8), 38.7 (C-4), 38.6 (C-1), 38.3 (C-13), 36.9 (C-10), 36.7 (C-22), 34.2 (C-7), 31.9 (C-16), 30.3 (C-15), 29.4 (C-21), 27.2 (C-23), 26.6 (C-2), 25.5 (C-12), 20.7 (C-11), 18.1 (C-6), 18.0 (C-30), 15.3 (C-25), 15.2 (C-26), 14.7 (C-24), 13.7 (C-27).

Oleanolic acid (12)—White powder; ($C_{30}H_{48}O_3$): ^{1}H NMR (300 MHz, $CDCl_3$) δ_H: 5.31 (*m*, H-12), 3.23 (*m*, H-3), 2.84 (*m*, H-19), 2.80 (*m*, H-18), 0.98 (*s*, 23-CH_3), 0.75 (*s*, 24-CH_3), 0.92 (*s*, 25-CH_3), 0.77 (*s*, 26-CH_3), 1.13 (*s*, 27-CH_3), 0.90 (*s*, 29-CH_3), 0.92 (*s*, 30-CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C: 182.9 (C-28), 143.7 (C-13), 122.8 (C-12), 79.0 (C-3), 55.3 (C-5), 47.8 (C-9), 46.6 (C-17), 46.0 (C-19), 41.1 (C-14), 41.7 (C-18), 39.4 (C-8), 38.9 (C-4), 38.5 (C-1), 37.2 (C-10), 33.9 (C-21), 33.2 (C-29), 32.7 (C-7), 32.7 (C-22), 32.7 (C-20), 27.3 (C-23), 27.8 (C-15), 28.2 (C-2), 26.1 (C-27), 23.5 (C-30), 23.1 (C-16), 23.7 (C-11), 18.4 (C-6), 17.3 (C-26), 15.6 (C-24), 15.4 (C-25).

Ergosta-6,22-diene-3,5,8-triol (13)—White powder; ($C_{28}H_{46}O_3$): ^{13}C-NMR (150 MHz, $CDCl_3$): δ_C 135.5 (C-6), 135.3 (C-22), 132.4 (C-23), 130.9 (C-7), 82.3 (C-5), 79.6 (C-8), 66.6 (C-3), 56.3 (C-17), 51.8 (C-14), 51.2 (C-9), 44.7 (C-13), 42.9 (C-24), 39.9 (C-20), 39.4 (C-11), 37.1 (C-10), 37.0 (C-4), 34.8 (C-1), 33.2 (C-25), 30.2 (C-2), 28.8 (C-15) 23.5 (C-12), 21.0 (C-21), 20.8 (C-16), 20.1 (C-26),

19.8 (C-28), 18.3 (C-19), 17.7 (C-27), 13.0 (C-18).

Stigmastane-3,6-dione (14)—White powder; ($C_{29}H_{48}O_2$): ^{1}H-NMR (500 MHz, Pyridine-d_5) δ_H: 2.64 (*m*, H-5), 2.29 (*m*, H-4a), 2.42 (*m*, H-4b), 2.37 (*m*, H-7a), 2.08 (*m*, H-1b), 2.05 (*m*, H-2a), 2.05 (*m*, H-12a), 2.00 (*m*, H-7b), 1.77 (*m*, H-16a), 1.73 (*m*, H-8), 1.64 (*m*, H-2a), 1.64 (*m*, H-12b), 1.64 (*m*, H-25), 1.60 (*m*, H-11a), 1.43 (*m*, H-23a), 1.37 (*m*, H-9), 1.30 (*m*, H-11b), 1.26 (*m*, H-20), 1.25 (*m*, H-1a), 1.25 (*m*, H-17), 1.21 (*m*, H-22a), 1.18 (*m*, H-16b), 1.16 (*m*, H-14), 1.14 (*m*, H-28), 1.08 (*m*, H-15), 1.00 (*m*, H-23b), 0.92 (*m*, H-24), 0.91 (*d*; 6.4 Hz, 29-CH_3), 0.90 (*m*, H-22b), 0.87 (*br s*, 21-CH_3), 0.83 (*s*, 18-CH_3), 0.82 (*d*; 3.2 Hz, 26-CH_3), 0.80 (*br s*, 27-CH_3), 0.66 (*s*, 19-CH_3); ^{13}C-NMR (125 MHz, Pyridine-d_5) δ_C: 211.8 (C-3), 209.4 (C-6), 56.6 (C-5), 55.8 (C-17), 55.3 (C-14), 52.5 (C-9), 45.8 (C-7), 45.1 (C-24), 42.3 (C-13), 40.6 (C-10), 38.6 (C-1), 37.4 (C-8), 37.2 (C-12), 36.7 (C-4), 36.2 (C-2), 35.3 (C-20), 33.1 (C-22), 28.4 (C-25), 27.7 (C-16), 25.3 (C-15), 23.3 (C-23), 22.3 (C-28), 20.9 (C-11), 19.0 (C-26), 18.2 (C-27), 17.9 (C-21), 11.7 (C-29), 11.3 (C-19), 11.2 (C-18).

***β*-sitosterol (15) + stigmasterol (16)**: **White crystal from methanol**: mp: 135˚C - 137˚C [lit. 134˚C - 136˚C] [39].

Stigmasterol 3-*O*-*β*-D-glucoside (17)—White powder; ($C_{35}H_{58}O_6$): ^{1}H-NMR (600 MHz, Pyridine-d_5) δ_H: 5.22 (*m*, H-22), 5.09 (*m*, H-23), 5. 05 (*d*, 7.7Hz, H-1'), 4.58 (*m*, H-6 á), 4.40 (*m*, H-6 b), 4.33 (*m*, H-3), 4.26 (*m*, H-4 '), 4.05 (*m*, H-2 '), 3.99 (*m*, H-3 '), 3.96 (*m*, H-5 '), 2.05 (*m*, H-20), 2.04 (*m*, H-2), 2.03 (*m*, H-7a), 1.98 (*m*, H-12), 1.82 (*m*, H-14), 1.81 (*m*, H-16), 1.74 (*m*, H-7b), 1.70 (*m*, H-1),1.60 (*m*, H-15), 1.59 (*m*, H-24), 1.55 (m, H-8), 1.54 (*m*, H-25), 1.43 (*m*, H-28), 1.26 (*m*, H-17), 1.24 (*m*, H-9), 1.22 (*m*, H-4), 1.21 (*m*, H-11), 1.09 (*d*, 6.5Hz, 21-CH_3), 0.92 (*d*, 6.6Hz, 27-CH_3), 0.90 (*d*, 7.3 Hz, H-29), 0.87 (*d*; 7.1 Hz, 26-CH_3), 0.74 (*s*, 19-CH_3), 0.59 (*s*, 18-CH_3); ^{13}C-NMR (150 MHz, Pyridine-d_5) δ_C: 139.9 (C-5), 139.1 (C-22), 130.0 (C-23), 118.2 (C-6), 102.6 (C-1 '), 79.1 (C-3), 78.9 (C-3 '), 77.5 (C-5 '), 75.8 (C-2 '), 72.2 (C-4 '), 63.3 (C-6 '), 56.7 (C-17), 55.9 (C-14), 51.2 (C-24), 49.9 (C-9), 43.8 (C-13), 41.5 (C-20), 40.5 (C-4), 39.9 (C-12), 37.9 (C-1), 35.1 (C-10), 34.9 (C-2), 32.6 (C-25), 30.4 (C-7), 30.1 (C-8), 29.3 (C-16), 25.5 (C-28), 23.7 (C-15), 22.1 (C-11), 22.0 (C-21), 21.7 (C-26), 19.6 (C-27), 13.4 (C-19), 12.9 (C-29), 12.6 (C-18).

5. Chemophenetic Significance

In this study, seventeen secondary metabolites (Figure 1) were isolated from *Rumex abyssinicus* including one new ceramide (**1**), one phthalate derivative (**2**), six anthraquinones (**3**-**8**), four triterpenes (**9**-**12**) and five steroids (**13**-**17**). From all these isolated metabolites, it appears that compounds **2**, **5**, **8**-**17** are reported here for the first time from *R. abyssinicus.* All the anthraquinones described here have already been isolated from other species of the genus *Rumex*: Compounds **3**, **4** and **6** were isolated from *R. abysinica* [40] *R. japonicus* [24], *R. acetosa*., *R. acetosella* L., *R. confertus* Willd., *R. crispus* L., *R. hydrolapathum* Huds. *R. obtusifolius* [41] and *R. aquaticus* [42], compounds **5** and **7** from *R. japonicus* [43], *R.*

nepalensis [44], compound **8** from *R. obtusifolius* [45] and compounds **7** and **8** from *R. acetosa* [46]. Base to the fact that, anthraquinones represent one of the main classes of secondary metabolites isolated from the genus *Rumex* [14] [47], their presence in all of the above species allowed us to conclude that *R. abyssinicus* and the other species mentioned exhibit very close chemotaxonomic relationships. The isolation of a new ceramide (**1**) from this plant was not a surprising since the works carried out by Watanabe *et al.*, (2011) reported glucosylceramides in *R. obtusifolius*. Although compounds **15** and **16** have already been isolated from the genus *Rumex* [26] [48] [49] [50] [51], stigmasterol 3-O-*β*-*D*-glucoside (**17**), stigmastane-3,6-dione (**14**), ergosta-6,22-diene-3,5,8-triol (**13**) have never been isolated from the family Polygonaceae. Bis-(2-ethylhexyl) phthalate (DEHP) (**2**) isolated for the first time from *Rumex* genus has already been reported from Polygonaceae family especially from *Polygonum runcinatum* [52].

6. Conclusion

In conclusion, the chemical study of the whole plant of *R. abyssinicus* led to the isolation of one previously undescribed ceramide (**1**) and sixteen known (**2**-**17**) compounds which enriched the chemical diversities of the plant, genus *Rumex* and Polygonaceae family. Compounds **10**-**12** may demonstrate here the relationship between *F. aubertii* and other species belonging to other genera herbs of Polygonaceae. Unfortunately, the extracts showed no antibacterial activity against the strains tested while compounds were either not active or weakly active. It will be interesting in future studies to test these extracts as well as the compounds isolated on other microbial strains.

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Conflicts of Interest

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

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