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Characterization of bioactive compounds from *Ficus vallis-choudae* Delile (Moraceae)

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ABSTRACT

Ficus vallis-choudae Delile has been reported to exhibit antifungal, anticonvulsant, anti-inflammatory and antinociceptive activities. Herein, we report the first ever pharmacochemical studies on the figs of *Ficus vallis-choudae* Delile resulting in the isolation of a new ceramide named nkwenamide (**1**). In addition, seven known compounds including the binary mixture of β-amyrin palmitate (**2**) and lupeol palmitate (**3**), olean-12-en-3-one (**4**), *n*-hexacosan-1-ol (**5**), β-sitosterol (**6**), and mixture of β-amyrin (**7**) and lupeol (**8**) were isolated. Their structures were elucidated using spectroscopic methods. The methanol extract from the figs of this plant exhibited urease, and α-glucosidase inhibitory activities and showed DPPH radical scavenging potency with IC_{50} values, 61.7, 73.7 and 87.4 µg/mL, respectively. It also showed a weak chemiluminescence activity as compared to ibuprofen. The mixture of **2** and **3** exhibited maximum urease inhibitory activity with IC_{50}=23.9 µg/mL while the mixture of **7** and **8** showed the maximum α-glucosidase inhibition with an IC_{50} value of 44.0 µg/mL. All the isolates showed weak chemiluminescence activity.

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1. Introduction

Medicinal plants have long been used in traditional medicines for the treatment of many illnesses. This situation has pushed researchers to screen a variety of plants for their biological activities and their chemical constituents (Mohammadhosseini, 2017; Mohammadhosseini et al., 2017). In this context, we carried out the study of the figs of *Ficus vallis-choudae* Delile, a Cameroonian medicinal plant of the family Moraceae.

Ficus vallis-choudae D. is a tropical and subtropical shrub or tree of the Moraceae family, with white latex (Evans, 1996). It is distributed in Tropical Africa from Senegal to Cameroon, from Sudan to Ethiopia and Malawi (Vivien and Faure, 1996). The decoctions of leaves and young leafy stems are used as local drug for jaundice, nausea, bronchial and gastrointestinal disorders (Oliver, 1960). The figs are edible and are really appreciated by children (Vivien and Faure, 1996). Its bark extract has been reported to possess antifungal and anticonvulsant activities (Adekunle et al., 2005; Malami et al., 2010) as well as anti-inflammatory and antinociceptive effects (Lawan et al., 2008).

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Helicobacter pylori bacteria is recognized as class 1 carcinogen by the World Health Organization (WHO), consequently efforts are being focused worldwide for its eradication through the application of several therapies. Treatment of *H. pylori* using synthetic compounds is associated with several problems such as high pretreatment cost, pretreatment bacterial resistance and adverse side effects (Yesilada et al., 1999; Huang et al., 2017). Therefore, exploration of some safer urease inhibitors derived from medicinal plants is becoming important as an alternate therapy against *H. pylori* based infections (Yesilada et al., 1999; Huang et al., 2017). According to chemical literature survey, about 80-90% plants found in nature has great potential against a large number of naturally occurring antimicrobial agents and these agents are the best sources for the treatment of *H. pylori* (Yesilada et al., 1999).

Intestinal α-glucosidase is a key enzyme for carbohydrate digestion, located at the epithelium of the small intestine. α-Glucosidase has been recognized as a therapeutic target for the modulation of postprandial hyperglycemia, which is the earliest metabolic abnormality that occurs in Type II diabetes (Yao et al., 2010). Natural products are still the most available source of α-glucosidase inhibitors. Therefore, screening of α-glucosidase inhibitors in medicinal plants has received much attention.

Inhibition of reactive oxygen species (ROS) can be used for the regulation of the inflammatory responses in the innate immune system. Reactive oxygen species play a key role in several inflammatory disorders, such as cancer, atherosclerosis, and ischemic heart diseases (Rimess et al., 2016).

Previous phytochemical studies of plants of *Ficus* genus have revealed the occurrence of ceramides, cerebrosides, steroids, pentacyclic triterpenes, flavonoids and phenolic compounds (Bankeu et al., 2010; Bankeu et al., 2011; Fongang et al., 2015). Even though a preliminary phytochemical analysis on the bark of *F. vallis-choudae* D. revealed the presence of flavonoids, glycosides, alkaloids, tannins and saponins (Lawan et al., 2008), no attempt has been made so far to isolate its compounds. Therefore, the purpose of present study was to isolate and characterize compounds from the methanolic extract of *F. vallis-choudae* D. figs and to assess their DPPH radical scavenging and chemiluminescence activities as well as their inhibitory effect on urease and α-glucosidase.

2. Experimental

2.1. Plant material

The figs of *F. vallis-choudae* D., Moraceae, were collected in March, 2014 from Nkwen, Bamenda, North West Region of Cameroon and identified by two botanists from the Department of Biological Sciences, Faculty of Science, The University of Bamenda, Cameroon, and compared with voucher specimens formerly kept at the National Herbarium of Cameroon under the registration number of HNC N° 5115 SRF/Can (YA).

2.2. Chemicals

For this study, the following reagents and technical and laboratory grade solvents (Fisher) were used: methanol was used for the extraction of the plant material; *n*-hexane, dichloromethane, ethyl acetate and methanol were used as pure or binary mixtures at different concentrations for purification of compounds. Column chromatography was performed on silica gel (230-400 mesh). Fractions were monitored by TLC using Merck pre-coated silica gel sheets (60 F_{254}), and the identification of spots on the TLC plate was carried out by spraying ceric sulfate reagent solution and heating the plate at about 80 °C. Depending on the solubility of the isolated compound, deuterated solvents including $CDCl_3$ and MeOD were used for ^1H and ^{13}C NMR experiments. Phenol, sodium nitroprusside, dipotassium hydrogen phosphate trihydrate, EDTA, lithium chloride and thiourea, all purchased from Sigma were used for the urease assay. Potassium phosphate, *p*-nitrophenyl-α-glucopyranoside, dimethylsulfoxide, glycine and 1-deoxynojirimycin (Sigma) were used for α-glucosidase assay while 1,1'-diphenyl-2-picrylhydrazyl, ethanol and butylated hydroxyanisole (Sigma) were used for DPPH. Hanks balanced salt solution and luminol (Sigma) were used for chemiluminescence assay, as well.

2.3. Apparatus

Melting points were obtained on a Büchi M-560 melting point apparatus. Optical rotations were measured with a JASCO DIP-360 polarimeter. UV spectra were recorded on a Hitachi UV 3200 spectrophotometer. A JASCO 320-A spectrophotometer was used for scanning IR spectroscopy using KBr pellets. 1D and 2D NMR spectra of the isolates were run on three Bruker spectrometers operating at 75, 100, 150, 400, 500 and 600 MHz, respectively where chemical shifts (δ) were expressed in ppm with reference to the TMS. EI-MS spectra were obtained on Varian MAT 311A mass spectrometer operating at 300 °C. FAB-MS spectra were measured on a JEOL JMS-HX-110 mass Spectrometer. These spectrometers use a magnetic sector and an electric sector analyzer.

2.4. Procedures

2.4.1. Extraction and isolation of compounds

The figs of *F. vallis-choudae* D. (3.8 kg) were harvested from a planted tree, chopped and air-dried under shade and ground in a locally made mill

and then extracted with MeOH (methanol) (15 L) (72 hours, repeated three times) at room temperature (24 ºC). The extract was then concentrated to dryness under vacuum at low temperature (40 ºC) to give 129 g of grey crude extract. Part of the extract (128 g) was subjected to medium pressure liquid column (Buchner funnel: 13 cm diameter, 10 cm height) chromatography over silica gel (200 g) (Merck, 230-400 mesh) eluting with mixtures of *n*-hexane/EtOAc (ethyl acetate) and EtOAc/MeOH of increasing polarities. One hundred and fifty subfractions, each containing 500 mL, were collected and combined according to their TLC profiles on pre-coated silica gel 60 F_{254} plates developed with *n*-hexane/EtOAc and $CHCl_3$/MeOH mixture to give 3 fractions (F1-3).

Fraction F1 (78 g) was subjected to column chromatography (CC) over silica gel (Merck 230-400 mesh), eluted with *n*-hexane/EtOAc mixture starting from 100% *n*-hexane to 50% of the mixture. This resulted in the isolation of a (1:1) mixture of β-amyrin palmitate (**2**) and lupeol palmitate (**3**) (5.05 g) (1:99 *n*-hexane/EtOAc), olean-12-en-3-one (**4**) (500 mg) (2:98 *n*-hexane/EtOAc) and *n*-hexacosan-1-ol (5) (3.0 mg) (1:99 *n*-hexane/EtOAc). Fraction 2 (22 g) was also subjected to successive CC using the same quality of silica gel and eluted with a mixture of EtOAc and *n*-hexane (varying from 1:9 *n*-hexane/EtOAc to 100% EtOAc) to give: β-sitosterol (**6**) (14 mg) (10:90 *n*-hexane/EtOAc), mixture of β-amyrin (**7**) and lupeol (**8**) (250 mg) (15:85 *n*-hexane/EtOAc). Fraction 3 (18 g) was eluted with the same mixture of solvents with different polarity to yield nkwenamide (**1**) (5.5 mg) (98:2 $CHCl_3$/MeOH).

2.4.2. Methanolysis of **1**

Compound **1** (2 mg) was heated with 5% HCl in MeOH (1 mL) at 70 ºC for 12 h in a sealed small-volume vial. The reaction was monitored by TLC analysis. On completion of the reaction, the solution was extracted with *n*-hexane. The *n*-hexane layer (0.8 mg) was then separated and concentrated for further analysis using GC-MS, to yield methyl 2-hydroxydocosanoate (**1a**) (*m/z* 370 $[M]^+$) (Bakhat et al., 2014).

2.4.3. Bioassays

2.4.3.1. Urease inhibition assay

Urease activity was determined by measuring ammonia production using the indophenol method described by Weatherburn (1967) with little modification (Pervez et al., 2016). Reaction mixtures with 25 µL of enzyme (Jack bean Urease purchased from Sigma) solution and 55 µL of buffers containing 100 mM urea were incubated with 5 µL of test samples (extract and compounds **1**-**8**) (1 mM concentration) at 30 ºC for 15 min in 96-well plates. Briefly, 45 µL each of phenol reagent (1% w/v phenol and 0.005% w/v sodium nitroprusside) and 70 µL of alkali reagent (0.5% w/v NaOH and 0.1% active chloride NaOCl) were added to each well. The absorbance at 630 nm was measured after 50 min, using a microplate reader (Molecular Device, USA). All reactions were performed in triplicate in a final volume of 200 µL. The results (change in absorbance per min) were processed by using Soft Max Pro software (Molecular Device, USA). All the assays were performed at pH 8.2 (0.01 M K_2HPO_4•$3H_2O$, 1 mM EDTA and 0.01 M LiCl). Percentage inhibitions were calculated from the formula (Eqn. 1):

$$U_{rease\ inhibition}(\%) = 100-(OD_{test} / OD_{control})\times 100 \quad \text{(Eqn. 1)}$$

Where OD stands for optical density. Thiourea was used as the standard inhibitor of urease.

2.4.3.2. α-Glucosidase inhibition assay

The enzyme inhibition assay is based on the breakdown of substrate to produce a coloured product, followed by measuring the absorbance over a period of time (Kurihara et al., 1994). In brief, α-glucosidase (Sigma, type III, from yeast) was dissolved in buffer A (0.1 mol/L potassium phosphate, 3.2 mmol/L-$MgCl_2$, pH=6.8) (0.1 units/mL). *p*-Nitrophenyl-α-D-glucopyranoside dissolved in buffer A at 6 mmol/L was used as substrates. 102 µL buffer B (0.5 mol/L potassium phosphate, 16 mmol/L-$MgCl_2$, pH=6.8), 120 µL sample solution (extract or compound 0.6 mg/mL in dimethyl sulfoxide: DMSO), 282 µL deionized water and 200 µL substrate were mixed. This mixture was incubated in a water-bath at 37 ºC for 5 min and then 200 µL enzyme solution was added and mixed. The enzyme reaction was carried out at 37 ºC for 30 min and then 1.2 mL of 0.4 mol/L glycine buffer (pH=10.4) was added to terminate the reaction. Enzymatic activity was quantified by measuring the absorbance at 410 nm. The crude extract showed various colours due to pigments, so the background absorption of every sample was considered. 1-Deoxynojirimycin hydrochloride (DNJ) was used as standard inhibitor of α-glucosidase. The percent inhibition was calculated using the following equation (Eqn. 2):

$$\text{Inhibition } \% = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100 \quad \text{(Eqn. 2)}$$

2.4.3.3. DPPH radical scavenging activity

The free radical scavenging activity was measured using 1,1'-diphenyl-2-picryl-hydrazyl (DPPH) (Gulcin et al., 2005). An alcoholic solution of DPPH (0.3 mM) was prepared in ethanol. Five microlitres of each sample of different concentrations (62.5 µg-500 µg) were mixed with 95 µL of DPPH solution in ethanol. The mixture was dispersed in 96 well plate and incubated in dark at 37 ºC for 30 min. The absorbance at 515 nm was measured by

microtitre plate reader (Spectramax plus 384 Molecular Device, USA) and percent radical scavenging activity was determined in comparison with the methanol treated control (Eqn. 3). Butylated hydroxyanisole (BHA) was used as standard.

$$\text{DPPH scavenging effect (\%)} = \frac{Ac - As}{Ac} \times 100 \quad \text{(Eqn. 3)}$$

Where Ac and As, respectively account for the absorbances of control (DMSO treated) and sample.

2.4.3.4. Chemiluminescence assay

Luminol enhanced chemiluminescence assays were performed to study the effect of compounds on reactive oxygen species (ROS) from phagocytes (Yamamura et al., 1992). Briefly, 25 µL of diluted whole human blood [1:50 dilution in sterile Hanks balanced salt solution ($HBSS^{++}$)] was incubated with 25 µL of serially diluted compounds with concentration ranges between 10, 50, 100 and 250 µg/mL. Control wells received $HBSS^{++}$ and cells but no compounds. Serum-opsonized zymosan-A (SOZ) 25 µL, followed by 25 µL luminol (7×10^{-5} M) along with $HBSS^{++}$, was added to each well to obtain a 100 µL volume/well. Tests were performed in white 96-well plates, which were incubated at 37 ºC for 30 min in the thermostated chamber of the luminometer. Results were measured as relative light unit (RLU) reading, with peak and total integral values set with repeated scans at 60-second intervals and 1-second point measuring time.

2.4.3.5. Statistical analysis

The resulting data are shown as mean ± SD of three independent assays. One way analysis of variance (ANOVA) was carried out for the determination of difference between groups (GraphPad Prism 5.0, USA). $P>0.05$ was considered as significant.

Table 1

^1H (400 MHz) and ^{13}C (100 MHz) NMR data for compound **1** in $CD_3OD+CDCl_3$ (δ in ppm, J in Hz, TMS as internal standard).

Atom	δ_C	Mult.	δ_H (J in Hz)	HMBC
1	60.9	CH_2	3.70, dd, (4.5, 11.5) 3.63, dd, (4.5, 11.5)	3
2	51.4	CH	3.98-3.99, m	1, 3
3	75.5	CH	3.43, overlapped dd	4
4	72.2	CH	3.44-3.45, m	3
5	34.2	CH_2	1.69-1.72, m 1.43-1.47, m	
6-13	29.2-29.5	CH_2	1.14-1.20, brs	
14	31.8	CH_2	1.85-1.87, m	15, 16
15	129.7	CH	5.29-5.30, m	14, 17
16	130.6	CH	5.29-5.30, m	14, 17
17	31.8	CH_2	1.85-1.87, m	15, 16
18	25.1	CH_2	1.30-1.32, 2H, m	
19	22.5	CH_2	1.14-1.20, 2H, m	
20	13.9	CH_3	0.77, t, (7.0)	
1′	175.7	C		
2′	71.8	CH	3.94, dd, (3.0, 8.0)	1′
3′	32.5	CH_2	1.60-1.56, m 1.30-1.32, m	
4′	25.7	CH_2	1.14-1.20, brs	
5′-20′	29.2-29.5	CH_2	1.14-1.20, brs	
21′	22.5	CH_2	1.14-1.20, brs	
22′	13.9	CH_3	0.77, t, (7.0)	

2.5. MS and NMR data of the isolates

Nkwenamide (**1**): (*R*)-2-hydroxy-N-((2*S*,3*S*,4*R*,*E*)-1,3,4-trihydroxyicos-15-en-2-yl) docosanamide, colorless gummy solid. $[\alpha]_D^{24}$ +51.2 (c 0.05, MeOH); UV λ_{max} (MeOH) nm: 208 (6.5). IR (KBr) cm^{-1}: 3510-3338 (O-H/N-H), 2920 (C-H), 1657, 1538 (HN-C=O), 1636 (C=C), 722 (aliphatic Cs); ^1H (500 MHz, $CD_3OD+CDCl_3$) and ^{13}C NMR (125 MHz, $MeOD+CDCl_3$): see Table 1; EI-MS: *m/z* (%)=681 (1.9) $[M]^+$, 663 (38) $[M-H_2O]^+$, 647 (29), 645 (17) $[M-2H_2O]^+$, 408 (44), 384 (100), 339 (85), 283 (10), 281 (10), 223 (4), 111 (27), 97 (48), 83 (68), 57 (86), 43 (73); HR-FAB-MS: *m/z* 682.6350 (calcd 682.6344 for $C_{42}H_{84}NO_5$ $[M+H]^+$).

β-Amyrin palmitate (**2**): colourless solid; EI-MS *m/z* (rel. int. %): 664 $(M)^+$ (16.6), 649 $(M-CH_3)^+$ (5.9), 409 (22.9), 218 (100.0), 203 (70.1), 189 (67.3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C 38.3 (C-1); 26.9 (C-2); 80.6 (C-3); 37.8 (C-4); 55.4 (C-5); 18.3 (C-6); 32.6 (C-7); 39.8 (C-8); 47.6 (C-9); 36.9 (C-10); 23.6 (C-11); 121.7 (C-12); 145.2 (C-13); 41.7 (C-14); 26.1 (C-15); 26.1 (C-16); 32.5 (C-17); 47.2 (C-18); 46.8 (C-19); 31.1 (C-20); 34.9 (C-21); 37.1 (C-22); 28.1 (C-23); 16.8 (C-24); 15.6 (C-25); 16.8 (C-26); 26.0 (C-27); 28.4 (C-28); 33.3 (C-29); 23.7 (C-30), 173.7 (C-1′), 31.9 (C-2′), 25.2 (C-3′), 29.2-29.8 (C-4′ - C-14′), 22.7 (C-15′), 14.1 (C-16′).

Lupeol palmitate (**3**): colourless solid; EI-MS *m/z* (rel. int. %): 664 $(M)^+$ (16.6), 649 $(M-CH_3)^+$ (5.9), 409 (22.9), 218 (100.0), 203 (70.1), 189 (67.3); ^{13}C-NMR (75 MHz, $CDCl_3$): δ_C 38.4 (C-1); 27.4 (C-2); 80.6 (C-3); 37.8 (C-4); 55.4 (C-5); 18.2 (C-6); 34.7 (C-7); 40.9 (C-8); 50.4 (C-9); 37.1 (C-10); 21.0 (C-11); 25.1 (C-12); 38.1 (C-13); 42.8 (C-14); 27.4 (C-15); 35.6 (C-16); 43.0 (C-17); 48.3 (C-18); 48.0 (C-19); 151.0 (C-20); 29.8 (C-21); 40.0 (C-22); 28.0 (C-23); 16.0 (C-24); 16.6 (C-25); 16.2 (C-26); 14.5 (C-27); 18.0 (C-28); 109.3 (C-29); 19.3 (C-30), 173.7 (C-1′), 31.9 (C-2′), 25.2 (C-3′), 29.2-29.8 (C-4′ - C-14′), 22.7 (C-15′), 14.1 (C-16′).

Olean-12-en-3-one (**4**): colourless polymorph solid; mp 166-167, IR (KBr) ν_{max} 1701 cm^{-1}; EI-MS *m/z* (rel. int. %): 424 $(M)^+$ (9.1), 409 $(M-CH_3)^+$ (7.0), 218 (100.0), 205 (16.0),189 (15.7); ^{13}C-NMR (150 MHz, $CDCl_3$): δ_C 39.2 (C-1); 34.2 (C-2); 217.7 (C-3); 47.4 (C-4); 55.3 (C-5); 19.6 (C-6); 32.1 (C-7); 39.7 (C-8); 46.8 (C-9); 36.6 (C-10); 23.6 (C-11); 121.4 (C-12); 145.2 (C-13); 41.8 (C-14); 26.1 (C-15); 26.9 (C-16); 32.5 (C-17); 47.2 (C-18); 46.7 (C-19); 31.0 (C-20); 34.7 (C-21); 37.1 (C-22); 26.4 (C-23); 21.5 (C-24); 15.2 (C-25); 25.8 (C-26); 16.7 (C-27); 28.4 (C-28); 33.3 (C-29); 23.6 (C-30).

n-Hexacosan-1-ol (**5**): waxy colourless solid; EI-MS *m/z* (rel. int. %): 364 $(M-H_2O)^+$ (3.6), 336 (5.2), 153 (23.0), 139 (28.6), 125 (42.5), 111 (83.1), 97 (100.0), 83 (88.3), 71 (48.1), 57 (61.3); ^1H-NMR (500 MHz, $CDCl_3$): δ_H 5.15 (1H,

brs, OH), 3.87 (2H, t, *J*=6.5 Hz, H-1), 1.77-1.72 (2H, m, H-2), 1.53-1.47 (2H, m, H-3), 1.30-1.24 (44H, brs, H-4 to H-25), 0.85 (3H, t, *J*=6.5 Hz, H-26).

β-Sitosterol (**6**): colourless solid; EI-MS *m/z* (rel. int. %): 414 $(M)^+$ (6.1), 399 $(M-CH_3)^+$ (12.3), 397 (97), 396 (100.0), 381 (16.5), 255 (19.2), 147 (26.2); ^{1}H-NMR (600 MHz, $CDCl_3$): δ_H 3.53-3.47 (1H, m, H-3), 5.33 (1H, dd, *J*=1.8, 4.8 Hz, H-6), 0.66 (3H, s, H-18), 0.99 (3H, s, H-19), 0.90 (3H, d, *J*=6.6 Hz, H-21), 0.79 (3H, d, *J*=6.6 Hz, H-26), 0.82 (3H, d, *J*=6.6 Hz, H-27), 0.86 (3H, t, *J*=7.2 Hz, H-29).

β-Amyrin (**7**): colourless solid; EI-MS *m/z* (rel. int. %): 426 $(M)^+$ (26.2), 411 $(M-CH_3)^+$ (8.8), 218 (100.0), 203 (67. s1),189 (31.8); ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C 38.6 (C-1); 27.2 (C-2); 79.0 (C-3); 38.8 (C-4); 55.2 (C-5); 18.4 (C-6); 32.7 (C-7); 39.8 (C-8); 47.6 (C-9); 37.0 (C-10); 23.5 (C-11); 121.7 (C-12); 145.2 (C-13); 41.7 (C-14); 26.2 (C-15); 26.0 (C-16); 32.5 (C-17); 47.2 (C-18); 46.8 (C-19); 31.1 (C-20); 34.7 (C-21); 37.1 (C-22); 28.1 (C-23); 15.5 (C-24); 15.6 (C-25); 16.8 (C-26); 26.9 (C-27); 28.4 (C-28); 33.3 (C-29); 23.7 (C-30).

Lupeol (**8**): colourless solid; EI-MS *m/z* (rel. int. %): 426 $(M)^+$ (26.2), 411 $(M-CH_3)^+$ (8.8), 218 (100.0), 203 (67.1),189 (31.8); ^{13}C-NMR (100 MHz, $CDCl_3$): δ_C 38.7 (C-1); 27.5 (C-2); 79.0 (C-3); 38.9 (C-4); 55.3 (C-5); 18.3 (C-6); 34.3 (C-7); 40.8 (C-8); 50.4 (C-9); 37.2 (C-10); 20.9 (C-11); 25.2 (C-12); 38.1 (C-13); 42.8 (C-14); 27.4 (C-15); 35.6 (C-16); 43.0 (C-17); 48.3 (C-18); 48.0 (C-19); 151.0 (C-20); 29.9 (C-21); 40.0 (C-22); 28.0 (C-23); 15.4 (C-24); 16.1 (C-25); 16.0 (C-26); 14.5 (C-27); 18.0 (C-28); 109.3 (C-29); 19.3 (C-30).

3. Results and Discussion

The MeOH extract of the figs of *F. vallis-choudae* D. was fractionated and subjected to repeated column chromatography on silica gel to afford eight compounds including nkwenamide (**1**), β-amyrin palmitate (**2**), lupeol palmitate (**3**), olean-12-en-3-one (**4**), *n*-hexacosan-1-ol (**5**), β-sitosterol (**6**), β-amyrin (**7**) and lupeol (**8**), respectively. The previously reported compounds **4**, **5** and **6** were identified by comparison of their physical and spectral data with literature (Nakane et al., 2002; Ahmad et al., 2012; Chaturvedula and Prakash, 2012). The triterpenes **2**/**3** and **7**/**8** (Fig. 1) obtained as inseparable binary mixtures were identified by shifting of peaks in ^{13}C NMR spectra and subsequent comparison with literature (Mahato and Kundu, 1994; Barreiros et al., 2002; Lakshmi et al., 2014).

3.1. Structure elucidation

Compound **1** was isolated as a gummy colorless solid, mp 88-90 °C, $[\alpha]_D^{24}$ +51.2 (c 0.05, MeOH). The molecular formula, $C_{42}H_{83}NO_5$, implying two degrees of unsaturation, was deduced from the detailed analysis of one- and two-dimensional NMR data, the EI-MS fragmentation pattern and the positive mode HR-FAB-MS which showed a pseudomolecular ion peak $[M+H]^+$ at *m/z* 682.6350 (calcd 682.6344 for $C_{42}H_{84}NO_5$). The UV spectrum in MeOH exhibited absorption bands at λ_{max} 208 and 226 nm, suggesting a ceramide skeleton (Bakhat et al., 2014). The IR spectrum showed absorption bands for amide, hydroxy (3200-3500 cm^{-1}), and secondary amide (1657 cm^{-1}) functionalities (Bakhat et al., 2014).

The ^{1}H NMR spectrum (Table 1) exhibited resonances for two olefinic protons at δ_H 5.30-5.29 (2H, m, H-15 and H-16), two oxymethylene protons at δ_H 3.70 (1H, dd, *J*=11.5, 4.5, Hz, H-1a) and 3.63 (1H, dd, *J*=4.5, 11.5 Hz, H-1b) and two methyl protons at δ_H 0.78 (6H, t, *J*=7.0 Hz, H-20 and H-22′), a downfield methine proton between δ_H 3.99-3.98 (1H, m, H-2), three oxymethine protons at δ_H 3.94 (1H, dd, *J*=3.0, 8.0 Hz, H-2′), 3.43 (1H, overlapped dd, H-3), 3.45-3.44 (1H, m, H-4) four methylene groups between δ_H 1.31-1.91, and the rest of the methylene protons between δ_H 1.53-1.20 (br, s, 28×CH_2). These signals confirmed the basic skeleton of

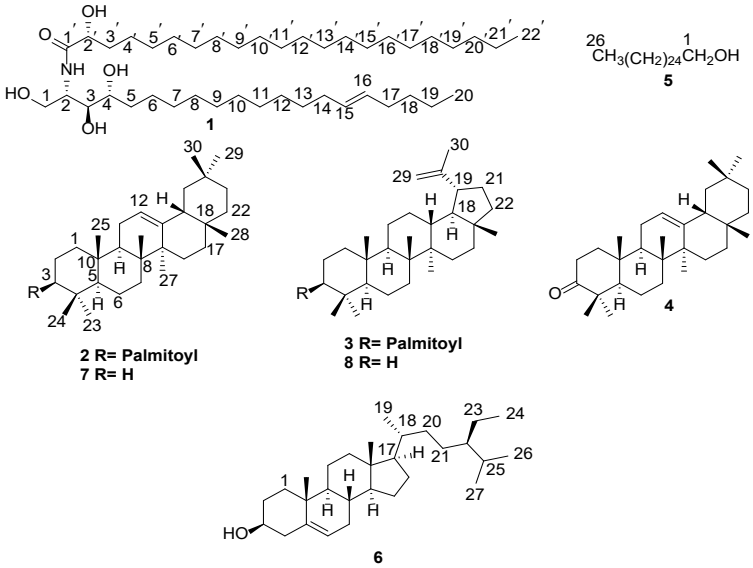

Fig. 1. Structures of the isolates.

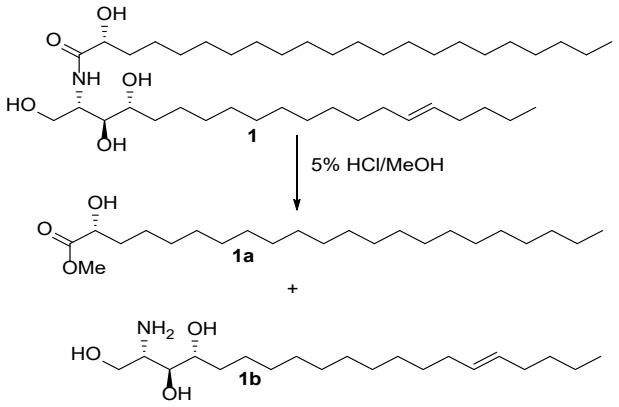

Fig. 2. Methanolysis of **1**.

1 to be a sphingolipid (Bakhat et al., 2014).

The broad band decoupled ^{13}C NMR spectrum (Table 1) displayed carbon signals, which were sorted by DEPT and HSQC techniques into a quaternary carbon of an amide carbonyl at δ_C 175.7 (C-1′), two olefinic methine carbons at δ_C 129.7 (C-15) and 130.6 (C-16), four methine carbons at δ_C 51.4 (C-2), 75.5 (C-3), 72.2 (C-4) and δ 71.8 (C-2′) and an oxymethylene carbon at δ_C 60.9 (C-1). It also exhibited signals for aliphatic methylenic carbons in the range of δ_C 22.5-34.2, while the two terminal methyl carbons were observed at δ_C 13.9.

Analysis of the ^1H-^1H COSY (Fig. 2), HSQC and HMBC (Fig. 2) spectra led to the assignment of proton and carbon signals for **1**. The ^1H-^1H COSY spectrum exhibited the correlation between the oxygenated methylene hydrogens at δ_H 3.70 (H-1a) and 3.63 (H-1b) with the azomethine hydrogen between δ_H 3.99-3.98 (H-2) which in turn was connected to the oxymethine proton H-3 at δ_H 3.43. The proton H-3 at δ_H 3.43 also correlated with the oxymethine proton H-4 at δ_H 3.44-3.45 confirming the position of C-3 and C-4 for the two hydroxyl groups, respectively (Fig. 2). The position of the third hydroxyl group was located at C-2′, based on the HMBC correlation observed between the proton H-2′ at δ_H 3.94 and the amide carbonyl carbon (δ_C 175.7).

The geometry of the double bond was assigned as *trans* based on the chemical shift of C-14 (31.8) and C-17 (31.8). Typically, the signals of a carbon next to a *trans* double bond appear at δ≈32, while those of a *cis* double bond appear at δ≈27 (Bankeu et al., 2010). The stereochemistry at different stereocenters was further confirmed through NOESY spectrum, which showed correlation of the azomethine hydrogen at δ_H 3.98-3.99 (H-2) with H-4 at δ_H 3.44-3.45 and H-2′ at δ_H 3.94 similar to that of pakistamide C which has already been established (Bakhat et al., 2014).

The number of carbons in the fatty acid chain of the sphingolipid was determined to be 22 based on the prominent ion peak observed on the EI-MS spectrum of 1 at *m/z* 339 (85%) corresponding to the cleavage between the nitrogen atom and the carbonyl carbon

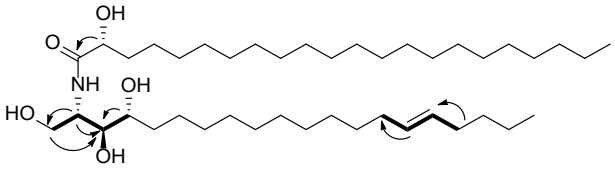

Fig. 3. Important COSY (——) and HMBC (⟶) correlations of **1**.

(Bakhat et al., 2014). This was further confirmed by the methanolysis of compound which yielded methyl 2-hydroxydocosanoate (*m/z* 370 $[M]^+$) as the fatty acid methyl ester; therefore, the long-chain base length was composed of 20 carbons with double bond located in the base chain. In the same way, the position of the olefinic double bond was determined at C-15 due to appearance of prominent ion peaks in EI-MS at *m/z* 57 (86) and 83 (68) (Fig. 3) relating to the fragment ion $C_4H_9^+$ and $C_6H_{11}^+$, respectively from the long-chain base branch. On the basis of these evidences the structure of nkwenamide (**1**) was determined as (*R*)-2′-hydroxy-N-((2*S*,3*S*,4*R*,*E*)-1,3,4-trihydroxyicos-15-en-2-yl) docosanamide.

3.2. Methanolysis of **1**

The methanolysis of compound **1** led to the formation of methyl 2-hydroxydocosanoate (**1a**) (*m/z* 370 $[M]^+$) as the fatty acid methyl ester indicating that the long-chain base (**1b**) length was composed of 20 carbons with the double bond located in the base chain.

3.3. Biological activities

3.3.1. Urease enzyme inhibition

The methanol extract of *F. vallis-choudae* D. and some isolated compounds showed considerable anti-urease activity with IC_{50} values ranging from 23.9 to 61.7 µg/mL (Table 2). The binary mixture of **2** and **3** was the most potent among all the isolated compounds and as compared to the reference, thiourea with an IC_{50} value of 21.7 µg/mL (Table 2). This finding is in line with the traditional use of *F. vallis-choudae* D. In fact, leaves and

Table 2
DPPH radical scavenging, urease inhibition and glucosidase inhibition activities of some isolates and the MeOH extract.

Compounds	DPPH radical scavenging activity	Urease inhibition activity	Glucosidase inhibition activity
	IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)
2 and **3**	Nil	23.9 ± 0.32[a]	87.4 ± 0.85[a]
4	Nil	32.7 ± 0.11[b]	55.3 ± 0.22[b]
7 and **8**	< 500	43.6 ± 0.85[c]	44.0 ± 0.31[c]
MeOH extract	87.4 ± 0.10[a]	61.7 ± 0.32[d]	73.7 ± 0.43[d]
BHA	44.2 ± 0.09[b]	-	-
Thiourea	-	21.7 ± 0.32[a]	-
DNJ	-	-	3.42 ± 1.7[e]

Values are shown as mean ± SD of three independent assays. Superscript letters within the same column indicate significant differences (P<0.05).

young leafy stems are used to treat gastrointestinal disorder (Oliver, 1960; Yesilada et al., 1999). This activity could be justified by the presence of urease inhibitors, an enzyme found in *H. pylori*.

3.3.2. α-Glucosidase enzyme inhibition

In the present study, we investigated the *in vitro* α-glucosidase inhibitory activity by using some compounds isolated from figs of *F. vallis-choudae* D. The tested compounds produced a weak α-glucosidase enzyme inhibition with IC_{50} values between 44.0 and 87.4 µg/mL while the control DNJ had an IC_{50} value of 3.42 µg/mL with the mixture of **7** and **8** being the most potent. B-amyrin isolated from *Memecylon umbellatum* showed significant inhibition of α-glucosidase (Sridevi et al., 2015) as well as lupeol showed 13.1% inhibition of this enzyme at 20 µg/mL (Kakarla et al., 2016).

3.3.3. DPPH radical scavenging assay

In this assay, only the extract showed a potent antioxidant activity against DPPH radical scavenging with an IC_{50} value of 87.4 µg/mL while all the tested isolates were inactive (Table 2). The synergetic action of secondary metabolites of the methanol extract could justify the poor activity of resulting compounds. As far as we are concerned, this is the first report of the antioxidant activity of *F. vallis-choudae* D. However, the antioxidant activity of *Ficus* species such as *F. carica* have been reported previously (Ali et al., 2012).

3.3.4. Chemiluminescence assay

A cellular and a cell-free luminol-enhanced chemiluminescence assays were used to evaluate the antioxidant and ROS scavenger effects of compounds isolated from figs of *F. vallis-choudae* D. All the compounds exerted a weak inhibition, olean-12-en-3-one (**4**) and nkwenamide (**1**) were more active than other isolates as shown in Table 3.

These results were in accordance with previously reported pharmacological activities of the methanol stem bark extract *F. vallis-choudae* D. such as anti-inflammatory and antinociceptive (Lawan et al., 2008). In fact, the inhibition of the production of ROS is in connection with the control of oxidative stress and inflammation. In addition, some traditional uses such as the use of leaves and young leafy stems for gastrointestinal troubles (Oliver, 1960; Yesilada et al., 1999) can probably be explained since the extract and some isolates inhibited urease enzyme found in *H. pylori* which is involved in such disorder.

Table 3
Effect of isolates on human whole blood assayed by luminol-amplified chemiluminescence.

Compounds	IC_{50} values (µg/mL)
1	122.67 ± 3.67^{a}
2 and **3**	243.71 ± 6.33^{b}
4	102.91 ± 2.37^{a}
5	139.35 ± 2.45^{a}
6	225.45 ± 5.06^{b}
7 and **8**	173.47 ± 3.37^{c}
MeOH extract	270.22 ± 4.79^{d}
Ibuprofen	14.42 ± 0.35^{e}

Values are shown as mean ± SD of three independent assays. Superscript letters within the same column indicate significant differences ($P<0.05$).

4. Concluding remarks

The phytochemical study of the figs of *Ficus vallis-choudae* D. led to the isolation of a new ceramide, nkwenamide (**1**) and seven known compounds. Though methanol extract displayed urease, and α-glucosidase inhibitory activities and antioxidant potency, majority of isolated compounds only inhibited the activity of urease and α-glucosidase and the reactive oxygen species. The results obtained in this study support the use of *Ficus vallis-choudae* D. in Cameroonian pharmacopeia.

Conflict of interest

The authors declare that there is no conflict of interest.

Acknowledgments

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