Chemical Constituents and Antiulcer Activity of *n*-Hexane Extract of *Sanchezia nobilis* Hook F. Leaves from Vietnam

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Received: 28 April 2019;

Accepted: 10 June 2019;

Published online: 31 July 2019;

AJC-19510

Five compounds (**1-5**) were isolated from the leaves of *Sanchezia nobilis* collected in Nam Dinh province by chromatographic methods. These compounds were identified as: mangiferin (**1**), β -sitosterol (**2**), margaric acid (**3**), ursolic acid (**4**), oleanolic acid (**5**). Their structures were elucidated by spectroscopic methods, including mass spectrometry and nuclear magnetic resonance. These compounds were isolated from the leaves of *Sanchezia nobilis* for the first time. Screening of gastric and duodenal antiulcer effects on indomethacin induced gastric ulcer models showed that *n*-hexane fraction produced the highest antiulcer activity. Percentage inhibition of gastric ulceration of misoprostol was 22.86 %, while that of *n*-hexane fraction was 28.57 % ($p < 0.05$). Evaluation of gastric and duodenal antiulcer effects on acetylsalicylic acid induced gastric ulcer models showed that this fraction was effective against gastric and duodenal ulcer (83.3 %), improved ulcer damage (54.17 %), significantly reduced the number of mean ulcer and ulcer index (2.00 ± 1.28) but it did not change the area of the ulcer.

Keywords: *Sanchezia nobilis*, Mangiferin, β -Sitosterol, Margaric acid, Ursolic acid, Oleanolic acid.

INTRODUCTION

In the world, the genus *Sanchezia* (Acanthaceae) includes more than 50 species, occurring in the tropical and subtropical regions such as Mediterranean, India, Africa, Australia, USA and some Southeast Asian countries. Most of the species have long been found in the tropical rainforests of Central and South America (Ecuador) [1]. In Vietnam, the genus *Sanchezia* is found in many places such as Tuyen Quang, Quang Nam, Da Nang and other provinces such as Nam Dinh, Vinh Phuc, Phu Tho, Thai Nguyen, etc. [2].

Some biological activity, chemical constituents of this plant have been previously reported in the world. Parvin *et al.* [3] showed that the results of brine shrimp lethality bioassay on *n*-hexane and ethylacetate fractions of *Sanchezia nobilis* Hook. F. leaves were safer than vincristine sulphate [3]. Paydar *et al.* [4] tested the antioxidant and anticancer effects of methanolic fraction of the extracts of *Sanchezia nobilis* leaves. The anticancer effect on Hela cells from *Sanchezia nobilis* roots gave good results by MTT assay of Shaheen *et al.* [5]. The

antibacterial, antifungal and insecticidal activities of the extracts of *Sanchezia speciosa* Hook.F. gave a very positive result [6]. Similarly, the study of antioxidant effect by DPPH and anti-inflammatory by the inhibition of albumine denaturation assay was reported by Thanh *et al.* [7].

Some specific substances were isolated as five matsutake alcohol compounds, four compounds were isolated for the first time from the family Acanthaceae and one another compound was isolated from a nature sources [3,8,9]. Ellah *et al.* [10] isolated six compounds from the methanol extracts of leaves and roots of *Sanchezia nobilis*. In Vietnam, *S. nobilis* has been known as a valuable herbal medicine to treat gastritis for a long time. However, there is scarcely any research on the chemical composition of *S. nobilis* and its biological effects. Therefore, it is necessary to study the phytochemical and pharmacological activities of this plant. In this study, the antigastric ulcer effect of *n*-hexane fraction from the extracts of *Sanchezia nobilis* Hook.F. leaves and its chemical composition of this fraction were studied.

EXPERIMENTAL

The leaves of *Sanchezia nobilis* Hook.F. were collected in Nam Dinh province during January, 2018 and authenticated by the School of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam. A voucher specimen (No: 190DV18 SMP-VNU) has been deposited in the herbarium of the University.

Melting points were measured on Mikroskopheiztisch PHMK-50 (VEB Waegetechnik Rapido, Germany). The FT-IR spectra were recorded on an IMPACT-410FT-IR spectrometer (CARL ZEISS JENA). The NMR [^1H (500 MHz), ^{13}C (125 MHz) and DEPT-90 and 135 MHz] spectra were recorded on an AVANCE spectrometer AV 500 (Bruker, Germany) in the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam. The chemical shifts were reported in ppm downfield from TMS with J in Hz. Electrospray Ionization Mass Spectra (ESI-MS) were recorded on a Varian Agilent 1100 LCMSD mass spectrometer. Optical rotation was measured on WXG-4 disc polarimeter. Analytical TLC was performed on Kieselgel 60 F₂₅₄ (Merck) plates (silica gel, 0.25 mm layer thickness) and RP-18 F₂₅₄ (Merck) plates (0.25 mm layer thickness). Spots were visualized using ultraviolet radiation (at $\lambda = 254$ and 365 nm) and by spraying with 10 % H_2SO_4 , followed by heating with a heat gun. Column chromatography was performed on silica gel (70-230 and 230-400 mesh, Merck). Organic solvents were of analytical grade. Optical densities were read on an ELISA plate reader (Bio-rad).

Extraction and isolation: The fresh leaves were washed, sun dried and cut into small pieces. The plant powder (2.5 kg) was extracted with ethanol 80 % (8 L) for 3 days at room temperature. Repeated the extraction twice by adding more solvent to make ingredient inside the solvent at least 2-3 cm (8 L/time), got the 2nd and 3rd aqueous extract.

The extracts were filtered with filter paper, combined and evaporated under low pressure resulting in a semi-solid crude extract (150 g). The ethanol crude extract (100 g) was suspended in water and successively partitioned with *n*-hexane and ethyl acetate (3 × 500 mL, each solvent/30 min). Combined solvent *n*-hexane and ethyl acetate were then evaporated under low pressure to obtain *n*-hexane and ethyl acetate fractions, respectively. The remaining water extract was evaporated to remove the organic solvent by heat to give water fraction denoted by N (26.6 g). Fraction H (9.2 g) was separated on a silica gel column chromatography, eluting with a gradient solvent system of *n*-hexane:CHCl₃ (15:1; v/v) to give three fractions H1-H3. The elution liquid to be caught into tubes and test by thin layer chromatography-TLC, combine tubes from 2-16 to obtain fraction H1, combine tubes from 17-20 to obtain fraction H2, similar to obtain fraction H3. Carry out the separated by silica gel 60 columns for fraction H1, with *n*-hexane:CHCl₃ solvent (10:1; v/v), check tubes of elution liquid by TLC, combine all tubes with the same components and evaporative solvent to obtain 4 small fraction including: H1.1, H1.2, H1.3, H1.4. Fraction H1.3 was chromatographed on a silica gel column, eluting with trichloromethane:methanol (3:1; v/v) to yield compound **1** (26 mg). Fraction H1.4 was separated on a silica gel column chromatography, eluting with *n*-hexane:ethyl acetate (5:2; v/v) to yield compound **2** (21 mg).

Fraction H2 was chromatographed on a silica gel column, eluting with a gradient solvent system of *n*-hexane:ethyl acetate (10:1, 5:1; v/v) to give one fraction H2.1 (320 mg), H2.2 (260 mg). Fraction H2.1 was separated on a silica gel column chromatography, eluting with *n*-hexane:chloroform (5:2; v/v) to yield compound **3** (31 mg). Fraction H2.2 was separated on a silica gel column chromatography, eluting with *n*-hexane:ethyl acetate (4:1; v/v) to yield compound **4** (21 mg).

Fraction H3 was chromatographed on a silica gel column, eluting with a solvent system of *n*-hexane:ethyl acetate (10:1 v/v) to give one fraction H3.1 (420 mg). Fraction H3.1 was further separated on a silica gel column chromatography, eluting with chloroform:ethyl acetate (4:1; v/v) to yield compound **5** (26 mg).

Evaluation of antiulcer activity: Antipeptic ulcer activity of total extracts, hexane fraction, ethyl acetate fraction and water fraction from *S. nobilis* was evaluated on a single oral administration of indomethacine (40 mg/kg) induced gastric ulcer models in adult Wistar albino rats [11,12].

The adult Wistar albino rats were randomized into 7 groups of 11 rats with both the same sex ratio each.

Group 1 (normal control): Distilled water (10 mL/kg).

Group 2 (ulcerated control): Distilled water (10 mL/kg) + INDO 40 mg/kg.

Group 3 (misoprostol): Misoprostol 50 µg/kg + INDO 40 mg/kg.

Group 4 (Sample A): Total extracts (60 mg/kg (equivalent dose for people, calculating with six conversion coefficients)) + INDO 40 mg/kg.

Group 5 (Sample B): *n*-Hexane fraction (3.68 mg/kg (equivalent dose for people, calculating with six conversion coefficients)) + INDO 40 mg/kg.

Group 6 (Sample C): Ethyl acetate fraction (11.52 mg/kg (equivalent dose for people, calculating with six conversion coefficients)) + INDO 40 mg/kg.

Group 7 (Model D): Water fraction (10.64 mg/kg (equivalent dose for people, calculating with six conversion coefficients)) + INDO 40 mg/kg.

Treatments with the reference drug and extracts lasted for 6 days, on the 7th day, after 1 h they were orally administered, Groups **2**, **3**, **4**, **5**, **6** and **7** rats were orally administered once daily with INDO (40mg/kg). Rats were kept fasting before administering with INDO for 18 h. After 24 h administration of the last dose of INDO, rats were anesthetized with thiopental to evaluate results.

Ulcers were examined under 10-fold binocular magnification to assess lesions. Severity of gastric ulcer was assessed according to the point scale as reported earlier [12].

Evaluation index: The ulcer index (UI) was calculated by the mean ulcer scores in each group. Similarly, percentage inhibition of gastric ulceration was calculated by the formula:

$$\text{Ulcer inhibition (\%)} = \frac{\text{UI}_{\text{control}} - \text{UI}_{\text{test}}}{\text{UI}_{\text{control}}} \times 100$$

Antiduodenal ulcer activity of *n*-hexane fraction: Gastric and duodenal antiulcer activity of *n*-hexane fraction from *S. nobilis* was evaluated on cysteamine induced gastric and duodenal ulcer models in adult Wistar albino rats with two oral doses of cysteamine (400 mg/kg) at an interval of 4 h [13,14].

The adult Wistar albino rats were randomized into five groups with both the same sex ratio each.

Group 1 (normal control): Distilled water (10 mL/kg)

Group 2 (ulcerated control): Distilled water (10 mL/kg) + cysteamine 400 mg/kg

Group 3 (ranitidine): Ranitidine 50 mg/kg + cysteamine 400 mg/kg

Group 4 (*n*-hexane fraction): *n*-Hexane fraction (3.68 mg/kg (equivalent dose for people, calculating with six conversion coefficients)) + cysteamine 400 mg/kg

Treatments with the reference drug and extracts lasted for 9 days. On the 10th day, after 1 h they were orally administered, Groups 2, 3 and 4 rats were administered with cysteamine (400 mg/kg), two doses orally at an interval of 4 h. Rats were kept fasting before administering with cysteamine for 18 h. After 24 h of administration of the last dose of cysteamine, the rats were anesthetized with thiopental to evaluate results. Ulcers were examined under 10-fold binocular magnification to assess lesions. Severity of gastric ulcer was assessed [15]. The ulcer index was calculated by the following equation:

$$UI = UN + US + UA \times 0.1$$

where UI is the ulcer index, UN is the ulcer number, US is the ulcer score and UA is the ulcer surface area.

Statistical analysis: All results are expressed as mean $\pm$ SEM. Serial measurements were analyzed by using Two-way ANOVA with Tukey's post hoc test using SigmaStat 3.5 program and figures were performed by using SigmaPlot 10.0 program (Systat Software Inc.). The critical significance level α was 0.050 and, then statistical significance was defined as $p < 0.05$.

RESULTS AND DISCUSSION

Mangiferin (compound 1): Compound 1 was isolated as yellow crystals, soluble in methanol, hot ethanol and *n*-hexane. ^1H and ^{13}C NMR data are given in Table-1. Compound 1 gave a molecular formula of $\text{C}_{19}\text{H}_{18}\text{O}_{11}$ based on the ESI-MS spectrum, revealing a molecular ion peak at m/z 423.16 $[\text{M}+\text{H}]^+$. The NMR spectrum showed that the compound had a C-glucoside xanthone structure. Analysis of ^1H NMR spectrum exhibited signals of three aromatic protons at low field, which were δ_{H} 6.36 (s, H-8); 6.79 (s, H-11) and 7.38 (s, H-14). The proton signal at δ_{H} 13.86 of the characteristic hydroxyl group attached to C-5 position. Besides, the absence of signal of an anomeric proton of sugar was determined at δ_{H} 4.59 (1H, dd, $J = 9.5$ Hz, H-1') corresponding to the double bond. Analysis of ^{13}C NMR and DEPT spectra indicated its presence of 19 carbon atoms, inclu-

Structures of compounds

TABLE-1
 NMR DATA OF COMPOUND **1** AND REFERENCE DATA [Ref. 16]

Position C	DEPT	δ_c^1 (ppm)	$\delta_c^{Qa,b}$ (ppm)	δ_H^1 (ppm) (Mult, $J = \text{Hz}$)	$\delta_H^{Qa,c}$ (ppm) (Mult, $J = \text{Hz}$)	HMBC (H→C)
2	C	151.2	151.1			
3	C	108.2	110.9			
4	C	179.5	178.9			
5	C	164.3	161.7			
6	C	108.1	107.3			
7	C	164.2	163.8			
8	CH	101.6	103.2	6.36 (s)	6.39 (s)	6, 7, 9, 10
9	C	156.9	156.1			
10	C	101.6	101.2			
11	C	101.7	102.2	6.79 (s)	6.88 (s)	2, 3, 12, 13
12	C	154.5	155.5			
13	C	144.5	144.1			
14	C	108.4	107.5	7.38 (s)	7.39 (s)	2, 4, 12, 13
1'	CH	82.3	73.1	4.59 (d; 9.5)	4.59 (d; 8.0)	5, 6, 7, 2', 5'
2'	CH	73.5	70.2	4.06 (t; 9.5)	4.06 (s)	1', 3'
3'	CH	71.2	79.0	3.20 m	3.17 (d)	
4'	CH	70.7	70.6	3.15 m	3.17 (d)	3', 5', 6'
5'	CH	79.5	81.5	3.17 m	3.17 (d)	
6'	CH ₂	61.9	61.5	3.68 brd (11.0; 2.5) 3.31 (dd; 11.0; 6.0)	3.69 (d; 8.0) 3.21 (dd; 16)	4'

^aRecorded in CDCl₃, ^b100 MHz, ^c300 MHz, ^Qreference data of mangiferin

ding 10 quaternary carbons, 8 methine carbons and 1 methylene carbon. In which, there were three methine carbons of aromatic ring; 10 quaternary carbons of xanthone skeleton.

Compound **1** also showed signals of the anomeric proton with 4 anomeric protons and 1 methylene carbon assigned to a characteristic sugar molecule. Comparing NMR spectral data of compound **1** with data of mangiferin [16] showed the similarities in corresponding positions (Table-1). The structure of compound **1** was further confirmed by the key HMBC and HSQC correlations. The HMBC spectrum showed the H-8 proton signal resonated at δ_H 6.79 (C-8, δ_C 101.6) in the form of a singlet, this was due to the aromatic ring with five positions. The H-11 proton signal resonated at δ_H 6.79 (C-11, δ_C 101.7) appeared as a singlet; The H-14 proton signal resonated at δ_H 7.38 (C-14, δ_C 108.4) also appeared as a singlet. On the other hand, correlations of H-11 with C-2 (δ_C 151.2); C-3 (δ_C 108.2); C-12 (δ_C 154.5); C-13 (δ_C 144.5) and correlations of H-14 with C-2 (δ_C 151.2); C-4 (δ_C 179.5); C-12 (δ_C 154.5); C-13 (δ_C 144.5) in the HMBC spectrum made the signal of two protons of the aromatic ring with four positions. In addition, according to calculations related number of carbon atoms in sugar and aglycon in the structure of compound **1**, there were 13 carbon atoms in the aglycon part, this aglycon was completely confirmed to be xanthone skeleton. The location of glucose-binding site was determined based on correlations of H-1' of the sugar with C-5 of aglycon in the HMBC spectrum. Thus, compound **1** was identified as mangiferin.

β -Sitosterol (compound 2): White amorphous powder; m.p.: 140-143 °C; IR (KBr, ν_{max} , cm⁻¹): 3440, 2924, 1684, 1397, 1259. ¹H and ¹³C NMR data are given in Table-2. The ESI-MS spectrum of compound **2** exhibited a molecular ion peak at m/z : 437.15 [M+Na]⁺ indicating the molecular formula as C₂₉H₅₀ONa (437.38). The IR spectra of the compound showed absorption bands at around 3440 and 1684 cm⁻¹ due to OH and C=C groups, respectively. The ¹H NMR spectra of compound **2** indicated a

 TABLE-2
 NMR DATA OF COMPOUND **1** AND
 REFERENCE DATA [Ref. 17]

Position C	DEPT	δ_c^1 (ppm)	$\delta_c^{Ra,b}$ (ppm)	δ_H^2 (ppm) (Mult, $J = \text{Hz}$)	$\delta_H^{Rb,c}$ (ppm) (Mult, $J = \text{Hz}$)
1	CH ₂	37.2	37.5		
2	CH ₂	31.9	31.9		
3	CH-OH	71.7	72.0	3.51 (m)	3.53 (tdd; 4.5; 4.2; 3.8)
4	CH ₂	42.3	42.5		
5	C	140.7	140.9		
6	CH	121.5	121.9	5.35 (d; 5.0)	5.36 (d; 6.4)
7	CH ₂	31.9	32.1		
8	CH	31.9	32.1		
9	CH	50.0	50.3		
10	C	36.5	36.7		
11	CH ₂	21.2	21.3		
12	CH ₂	39.7	39.9		
13	C	42.3	42.6		
14	CH	56.8	56.9		
15	CH ₂	24.4	26.3		
16	CH ₂	28.3	28.5		
17	CH	56.0	56.3		
18	CH ₃	11.9	12.0	1.02 (s)	1.01 (s)
19	CH ₃	19.9	19.0	0.69 (s)	0.68 (s)
20	CH	36.2	36.3		
21	CH ₃	18.8	19.2	0.94 (d; 6.5)	0.93 (d; 6.5)
22	CH ₂	33.9	34.2		
23	CH ₂	26.0	26.3		
24	CH	45.7	46.1		
25	CH	29.1	29.4		
26	CH ₃	19.1	20.1	0.83 (d; 7.0)	0.83 (d; 6.4)
27	CH ₃	19.5	19.6	0.81 (d; 7.0)	0.81 (d; 6.4)
28	CH ₂	23.0	23.3		
29	CH ₃	12.2	12.2	0.85 (t; 7.5)	0.84 (t; 7.2)

^aRecorded in CDCl₃, ^b125 MHz, ^c500 MHz, ^Rreference data of β -sitosterol

doublet of olefinic proton at δ_H 5.35 (1H, d, $J = 5.0$ Hz, H-6); a signal of oxymethine proton at δ_C 3.51 (1H, m, H-3). There were also 6 methyl signals in the spectrum as 2 methyl singlets at δ_H 0.69 (3H, s, H₃-18) and 1.02 (3H, s, H₃-19); three methyl doublets that appeared at δ_H 0.94 (3H, d, $J = 6.5$ Hz, H₃-21); 0.83 (3H, d, $J = 7.0$ Hz, H₃-26); 0.81 (3H, d, $J = 7.0$ Hz, H₃-27); and a methyl triplet at δ_H 0.85 (3H, t, $J = 7.5$ Hz, H₃-29).

Analysis of ^{13}C NMR and DEPT spectra showed the presence of 29 carbon resonances, distinguishing into 6 methyl resonances (δ_C 11.9, 19.9, 18.8, 19.1, 19.5, 12.2) were ascribed at C-18, C-19, C-21, C-26, C-27, C-29, respectively and the two olefinic carbons signals at δ_C 140.7 and 121.5 were assigned to C-5 and C-6, respectively. Thus, structure of compound **2** was assigned as β -sitosterol that was consistent with the reported literature values [17].

Margaric acid (compound 3): White solid, m.p.: 60-63 °C; $R_f = 0.86$ (TLC, silica gel, *n*-hexane:acetone 5:1, v/v), purple colour with vanillin-sulfuric acid reagent (vanillin:sulfuric acid 1 %). Compound **3** gave a molecular formula of $\text{C}_{17}\text{H}_{34}\text{O}_2$ based on the ESI-MS spectrum, revealing a molecular ion peak at m/z 271.12 $[\text{M}+\text{H}]^+$. In ^1H NMR spectrum, the resonance signal of methylene group at δ_H 2.33 (2H, t, $J = 7.5$ Hz, H-2) directly attached to the carbonyl group corresponding to the carbon signal at δ_C 33.9 (C-2) in ^{13}C NMR spectrum and also showed another methylene group, was adjacent to this group at δ_H 1.64 (2H, quartet, H-3). The presence of a carbonyl group of acid was easily recognized in ^{13}C NMR spectrum based on resonant signal at δ_C 179.1 (C-1). The terminal methyl group gave the signal at δ_H 0.87 (3H, t, $J = 7.0$ Hz, H-17) and δ_C 14.2 (C-17). The overlap of 26H within δ_H 1.26-1.32 corresponds to thirteen long-chain methylene groups (H-4 $\rightarrow$ H-16). ^1H and ^{13}C NMR spectra (Table-3) that compound **3** contained 15 methylene groups, one methyl group and one carboxyl group.

TABLE-3
NMR DATA OF COMPOUND **3** AND
REFERENCE DATA [Ref. 18]

Position C	DEPT	δ_C^3 (ppm)	$\delta_C^{\text{Sa,b}}$ (ppm)	δ_H^3 (ppm) (Mult, $J = \text{Hz}$)	$\delta_H^{\text{Sa,c}}$ (ppm) (Mult, $J = \text{Hz}$)
1	C	179.1	180.6		
2	CH ₂	33.9	34.1	2.33 (t; 7.5)	2.34 (t; 7.2)
3	CH ₂	24.6	24.7	1.64 (quartet)	1.63 (m)
4	CH ₂	29.2	29.1		1.26 (br)
5	CH ₂	29.4	29.4		1.26 (br)
6	CH ₂	29.7	29.7		1.26 (br)
7	CH ₂	29.7	29.7		1.26 (br)
8	CH ₂	29.7	29.7		1.26 (br)
9	CH ₂	29.7	29.7		1.26 (br)
10	CH ₂	29.7	29.7		1.26 (br)
11	CH ₂	29.7	29.7		1.26 (br)
12	CH ₂	29.7	29.7		1.26 (br)
13	CH ₂	29.7	29.7		1.26 (br)
14	CH ₂	29.4	29.4		1.26 (br)
15	CH ₂	31.8	31.9		1.26 (br)
16	CH ₂	22.6	22.7		1.26 (br)
17	CH ₃	14.2	14.1	0.87 (t, 7.0)	0.88 (t, 6.6)

^aRecorded in CDCl_3 , ^b125 MHz, ^c500 MHz ^dreference data of margaric

acid or margaric acid. This compound was first isolated from the leaves of *Sanchezia nobilis*.

Ursolic acid (compound 4): White solid, m.p.: 290-292 °C; $R_f = 0.50$ (TLC, silica gel RP-18, $\text{CH}_3\text{OH}:\text{H}_2\text{O}$, 8:1, v/v), purple color with vanillin-sulfuric acid reagent (1 %). The ESI-MS spectrum showed the ion of compounds **4** at m/z 456.1 $[\text{M}]^+$ ($\text{C}_{30}\text{H}_{48}\text{O}_3$) and the fragments at m/z 248.1; 203.1 and 189.0 corresponds to retro-Diels Alder fragments in triterpene urs-12-en bearing OH group in A ring. Analysis of ^1H and ^{13}C NMR spectra (Table-4) showed the signal for hydroxymethyl group at δ_H 3.18 ppm (1H, t, $J = 16.5$ Hz, H-3) corresponds to the carbon signal at δ_C 78.6 (C-3), carbonyl group shifted to the low field at δ_C 180.6 (C-28). Correlations of δ_H 5.21 (1H, t, $J = 7.5$ Hz, H-12) with C-12 (δ_C 125.1) made the location of olefinic methyl groups very clear. Combining analysis of ^{13}C NMR and DEPT spectra indicated that compound **4** displayed the presence of thirty carbon atoms with seven methine groups, seven methyl groups, nine methylene and seven quaternary carbon. Based on the aforementioned data and by comparison with the reported literature, the structure of compound **4** was confirmed to be 3 β -hydroxyurs-12-en-28-oic acid or ursolic acid.

TABLE-4
NMR DATA OF COMPOUND **4** AND
REFERENCE DATA [Ref. 19]

Position C	DEPT	δ_C^4 (ppm)	$\delta_C^{\text{Ta,b}}$ (ppm)	δ_H^4 (ppm) (Mult, $J = \text{Hz}$)	$\delta_H^{\text{Ta,c}}$ (ppm) (Mult, $J = \text{Hz}$)
1	CH ₂	38.6	38.8		
2	CH ₂	26.8	27.0		
3	CH	78.6	76.9	3.18 (t; 16.5)	3.17 (s)
4	C	38.4	38.4		
5	CH	55.1	54.8		
6	CH ₂	18.1	18.0		
7	CH ₂	32.9	37.7		
8	C	39.5	40.2		
9	CH	47.4	47.1		
10	C	36.6	36.6		
11	CH ₂	23.1	22.9		
12	CH	125.1	124.6	5.21 (t; 7.5)	5.12 (s)
13	C	138.1	138.2		
14	C	41.8	41.7		
15	CH ₂	29.5	27.6		
16	CH ₂	24.1	23.8		
17	C	47.6	46.9		
18	CH	52.6	52.4	2.18 (d; 11.0)	2.1 (d; 11.0)
19	CH	38.9	38.6		
20	CH	38.7	38.5		
21	CH ₂	30.6	30.2		
22	CH ₂	36.8	36.4		
23	CH ₃	27.9	28.3	0.71 (s)	0.67 (s)
24	CH ₃	15.4	15.3	0.78 (s)	0.89 (s)
25	CH ₃	15.4	16.1	0.91 (s)	0.86 (s)
26	CH ₃	16.9	17.0	0.98 (s)	0.74 (s)
27	CH ₃	23.4	23.3	1.08 (s)	1.03 (s)
28	C	180.6	178.3		
29	CH ₃	16.8	16.9	0.82 (d; 6.5)	0.81 (d; 6.4)
30	CH ₃	21.0	21.1	0.91 (s)	0.90 (d; 6.0)

^aRecorded in CDCl_3 & CD_3OD , ^b125 MHz, ^c500 MHz ^dreference data of ursolic acid

Based on the aforementioned data and combined with reference [18], compound **3** was identified as heptadecanoic

Oleanolic acid (compound 5): White solid, m.p.: 306-308 °C; $R_f = 0.45$ (TLC, silica gel RP-18, methanol:water, 8:1,

v/v), purple color with vanillin-sulfuric acid reagent (1 %). ^1H and ^{13}C NMR spectrum of compound **5** were found to be similar to compound **4**. This was consistent that compound **5** was triterpenoids with five six-membered ring with hydroxymethyl group δ_{H} 3.19 (1H, dd, $J = 11.5$; 5.0 Hz, H-3), olefinic proton δ_{H} 5.27 (1H, t, $J = 7.5\text{Hz}$, H-12) (Table-5). The presence of 30 carbon atoms with 5 methine carbons, 10 methylene carbons, 7 methyl carbons and 7 quaternary carbons was confirmed by ^{13}C NMR and DEPT spectra. The difference observed between compounds **4** and **5** was that of methyl group at position 29. In the ^1H NMR spectrum of compound **4**, methyl group gave the doublet signal at δ_{H} 0.86 ($J = 6.5$ Hz) due to its bound directly to C-19 (δ_{C} 38.9) and interacted with protons at C-20 (δ_{C} 38.7). While in the spectrum of compound **5**, this methyl group only gave the singlet signal δ_{H} 0.91, which could be explained by the fact that methyl group has shifted its position to C-20 directly (δ_{C} 31.3) as a quaternary carbon and C-19 (δ_{C} 46.8) was a methylene group. Based on the above analysis as well as the published spectral data [20], it was possible to conclude that the isolated substance was oleanolic acid, which was a constituent of compound **4**.

TABLE-5
NMR DATA OF COMPOUND **5** AND
REFERENCE DATA [Ref. 20]

Position C	DEPT	$\delta_{\text{C}}^{\text{s}}$ (ppm)	$\delta_{\text{C}}^{\text{Ua,b}}$ (ppm)	$\delta_{\text{H}}^{\text{s}}$ (ppm) (Mult, $J = \text{Hz}$)	$\delta_{\text{H}}^{\text{Ua,c}}$ (ppm) (Mult, $J = \text{Hz}$)
1	CH ₂	39.6	39.0	-	-
2	CH ₂	27.7	28.2	-	-
3	CH	79.6	78.1	3.19 (dd; 11.5; 5.0)	3.23 (dd; 11.2; 4.4)
4	C	37.8	39.4	-	-
5	CH	56.4	55.8	-	-
6	CH ₂	19.5	18.8	-	-
7	CH ₂	33.4	33.3	-	-
8	C	40.2	39.8	-	-
9	CH	48.6	48.2	-	-
10	C	37.8	37.4	-	-
11	CH ₂	23.8	23.8	-	-
12	CH	123.4	122.6	5.27 (t; 3.5)	5.27 (d; 3.5)
13	C	144.9	144.8	-	-
14	C	42.6	42.2	-	-
15	CH ₂	28.6	28.4	-	-
16	CH ₂	24.3	23.8	-	-
17	CH	47.3	46.7	-	-
18	CH	42.3	42.0	2.85 (dd; 14.0; 4.0)	2.82 (dd; 13.2; 3.6)
19	CH ₂	46.8	46.5	-	-
20	C	31.3	31.0	-	-
21	CH ₂	34.7	34.3	-	-
22	CH ₂	33.8	33.2	-	-
23	CH ₃	28.7	28.8	0.98 (s)	1.24 (s)
24	CH ₃	15.9	16.6	0.81 (s)	1.02 (s)
25	CH ₃	16.1	15.6	0.93 (s)	0.93 (s)
26	CH ₃	17.6	17.5	0.98 (s)	1.04 (s)
27	CH ₃	26.4	26.2	1.14 (s)	1.13 (s)
28	C	181.5	180.2	-	-
29	CH ₃	33.4	33.3	0.91 (s)	0.97 (s)
30	CH ₃	23.8	23.8	0.96 (s)	1.02 (s)

^aRecorded in CDCl₃, ^b125 MHz, ^c500 MHz ^Ureference data of oleanolic acid.

Antilulcer activity: Antipeptic ulcer activity of total extracts, *n*-hexane, ethyl acetate and water fractions from the extracts of *Sanchezia nobilis* Hook.F. leaves are shown in Table-6.

TABLE-6
PERCENTAGE OF RATS WITH ULCER IN THE STUDY GROUPS

	Percentage of mice with ulcer images	Percentage of mice without ulcer images
Normal control group	0	100
Ulcerated control group	100	0
Misoprostol group	63.6	36.4
Total extract group	100	0
<i>n</i> -Hexane group	72.7	27.3
Ethylacetate group	81.8	18.2
Water group	81.8	18.2

*Significantly different from Ulcerated control group at $p < 0.05$.

Thus, it can be concluded that percentage of rats with ulcers in the ulcerated control group were 100 %. While misoprostol significantly reduced the rate of INDO induced ulcer as compared to ulcerated control group. The effect produced by misoprostol was statistically significant with $p(X > \chi^2) = 0.027 < \alpha = 0.05$.

Percentage of rats with ulcers in the control group of rats rats was administrated with total extracts were 100 %. Thus, there was no significant differences with the ulcerated control group. However, *n*-hexane fraction (72.7 % ulceration), ethyl acetate fraction (81.8 % ulceration) and water fraction (81.8 % ulceration) reduced the rate of INDO induced ulcer as compared to ulcerated control group. But, there is no statistically significant with *p*-values of these fractions, where were found to be 0.062; 0.138 and 0.138, for *n*-hexane, ethyl acetate and water fractions, respectively.

Effects of drug samples on the ulcer index: It was found that treatment with misoprostol (50 $\mu\text{g/kg}$) significantly ($p < 0.05$) reduced the ulcer index as compared to ulcerated control group. Inhibitory effect of misoprostol was found to be 22.86 % (Table-7). However, total extracts and water fraction did not showed any effect of antagastric ulcer compared to ulcerated control group. But *n*-hexane and ethyl acetate fractions significantly ($p < 0.05$) reduced the ulcer index as compared to ulcerated control group.

TABLE-7
EFFECTS OF DRUG SAMPLES ON THE ULCER INDEX

Group (n = 11)	Ulcer index	Ulcer inhibition (%)
Group: Ulcerated control	1.05 $\pm$ 0.16	-
Group: misoprostol	0.81 $\pm$ 0.23*	22.86
Group A: Total extracts	1.28 $\pm$ 0.23*	-
Group B: <i>n</i> -hexane fraction	0.75 $\pm$ 0.39*	28.57
Group C: ethyl acetate fraction	0.88 $\pm$ 0.19*	16.19
Group D: water fraction	1.18 $\pm$ 0.39	-

*Significantly different from ulcerated control group at $p < 0.05$.

Therefore, the results of effect of antiulcer of *n*-hexane, ethyl acetate and water fractions on indomethacine (40 mg/kg) induced gastric ulcer models can be summarized as: (a) treatment with the total extracts at an equivalent dose was not effective against gastric ulcer on inducing by indomethacine (40 mg/kg)

in adult Wistar albino rats; (b) Treatment with *n*-hexane and ethyl acetate fractions at an equivalent dose on-human clinical trials improved ulcer index, percentage inhibition of gastric ulceration and tended to reduce the percentage of rats with ulcers when compared with ulcerated control group; (c) treatment with the water fraction at an equivalent dose on-human clinical trials reduced percentage of rats with ulcers when compared to ulcerated control group. However, it did not change the ulcer index.

Antipeptic ulcer activity of *n*-hexane fraction on cysteamine induced gastric and duodenal ulcer models: The results shown in Table-8 indicate that the normal control group did not effect any rats with its an image of ulcers, but ranitidine significantly reduced the rate of cysteamine induced ulcer when compared to ulcerated control group. The effect produced at by ranitidine was statistically significant with $p(X > \chi^2) = 0.044$ ($\alpha = 0.05$). The rat group orally administered with *n*-hexane fraction (83.3 %) was found to be lower that of ulcerated control group (100 %). However, this was not statistically significant with $p = 0.125$.

TABLE-8
PERCENTAGE OF MICE WITH ULCER
IMAGES IN THE STUDY GROUPS

	Percentage of mice with ulcer images	Percentage of mice without ulcer images
Normal control group	0	100
Ulcerated control group	100	0
Ranitidine group	72.7*	27.3
<i>n</i> -Hexane group	83.3	16.7

*Significantly different from ulcerated control group at $p < 0.05$

Effect of drug samples on the severity of gastric and duodenal ulcer: The results are shown in Table-9, which indicated that (a) In ulcerated control group: the ulcerative lesions including single ulcer, deep ulcer perforated ulcers were observed, in which deep ulcer (62.50 %) was the major lesion; (b) rat group which was orally administered with ranitidine 50 mg/kg: shows ulcerative lesions including single and deep ulcers, in which single ulcer (57.14 %) was the major lesion; (c) rate of deep ulcer of the group was decreased 1.46 times when orally administered with ranitidine (42.86 %) as compared to the ulcerated control group (62.50 %); (d) when orally administered with *n*-hexane fraction, the rat group consist of ulcerative lesions including single and deep ulcers, but no perforated ulcer. The deep ulcer was greater in this sample (54.17 %).

TABLE-9
EFFECT OF DRUG SAMPLES ON THE
SEVERITY OF GASTRIC AND DUODENAL ULCER

	Perforation	Deep ulcer	Surface ulcer
Normal control group	0	0	0
Ulcerated control group	6.25	62.50	31.25
Ranitidine group	0	42.86	57.14
<i>n</i> -Hexane group	0	54.17	45.83

Effect of drug samples on the number of mean ulcers: When treated with ranitidine (50 mg/kg), the number of mean

ulcers was significantly reduced as compared to ulcerated control group, which was statistically significant ($p = 0.001$). However, when orally administered with *n*-hexane fraction, no significant differences in the number of mean ulcers of rat group with the ulcerated control group ($p = 0.398$) was observed (Table-10).

TABLE-10
EFFECT OF DRUG SAMPLES ON
THE NUMBER OF MEAN ULCERS

Group	N	Number of mean ulcers
Group: Ulcerated control	13	2.46 ± 0.52
Group: Ranitidine	11	$1.27 \pm 0.90^{***}$
Group: <i>n</i> -hexane fraction	12	2.00 ± 1.28

* $p < 0.05$; *** $p < 0.001$ compare with ulcerated control group (Mann-Whitney test).

Effect of drug samples on the mean area of ulcer: No difference in the area of ulcer between ranitidine and *n*-hexane fractions was found when compared with the ulcerated control group ($p > 0.05$) (Table-11).

TABLE-11
EFFECT OF DRUG SAMPLES ON
THE MEAN AREA OF THE ULCER

Group	Area of ulcer (mm ²)
Group 2: Ulcerated control	5.28 ± 1.79
Group 3: Ranitidine	4.01 ± 2.91
Group 4: <i>n</i> -hexane fraction	4.29 ± 2.46

Effect of drug samples on ulcer index: When treated with ranitidine (50 mg/kg), the rat group significantly reduced the ulcer index as compared to ulcerated control group. This was statistically significant ($p = 0.001$). In case of *n*-hexane fraction, the rat group tended to reduce the ulcer index, however, this was not statistically significant with $p = 0.265$ (Table-12).

TABLE-12
EFFECT OF DRUG SAMPLES ON THE ULCER INDEX

Group	Ulcer index
Group 2: Ulcerated control	8.07 ± 2.21
Group 3: Ranitidine	$3.77 \pm 2.63^{***}$
Group 4: <i>n</i> -Hexane fraction	6.11 ± 3.94

* $p < 0.05$; *** $p < 0.001$ compare with ulcerated control group (Mann-Whitney test).

Conclusion

Five compounds (**1-5**) were isolated from the leaves of *Sanchezia nobilis* collected in Nam Dinh province of Vietnam by chromatographic methods for the first time. These compounds were identified as: mangiferin (**1**), β -sitosterol (**2**), margaric acid (**3**), ursolic acid (**4**) and oleanolic acid (**5**). The antipeptic ulcer activity of total extracts, *n*-hexane, ethyl acetate and water fractions were studied on indomethacine (40 mg/kg) induced gastric ulcer models. The *n*-hexane and ethyl acetate fractions observed to be the most pharmacologically active antiulcer fractions. The results showed that *n*-hexane fraction of *Sanchezia nobilis* Hook.F. leaves with an equivalent dose on-human clinical trials was effective against gastric and duodenal ulcer, improved ulcer damage, reduced the number of mean ulcers and ulcer index, but it did not change the area of ulcer.

ACKNOWLEDGEMENTS

The research was supported by financially by Vietnam National University, Hanoi, Vietnam with grants number: QG.18.20.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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