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Research Article

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Phytochemical and anti-inflammatory effect from the leaf of *Sanchezia speciosa* Leonard growing in Vietnam

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ABSTRACT

*Sanchezia speciosa is an evergreen shrub in the dogbane family Apocynaceae and planted throughout the tropical and subtropical region. It has been showed that Sanchezia speciosa has high amount of cardiac glycoside, flavonoids compounds and also the antioxidant, anti-inflammatory and anticancer activities. In this study, we aim to study the phytochemical component of ethanolic extract of Sanchezia speciosa leaf and investigate the anti-inflammatory effect of Sanchezia speciosa leaf extract. Leaf of Sanchezia Speciosa Leonard was extracted with 80% ethanol. Compounds were isolated using on silica gel normal and reverse phase and preparative glass-backed TLC plates. The compound's structure were characterized on the basis of spectroscopic data, including IR, MS and NMR and by comparing their physicochemical and spectral data with those published in literatures. Paw edema was induced in the mice using 0.05 ml of 1% Carrageenan sodium salt to investigate the anti-inflammatory effect of Sanchezia speciosa leaf extract. From ethanolic extract of Sanchezia speciosa leaf we have isolated four compounds. Their structures were identified as (**1**) Quercetin 3-O-α-L-rhamnopyranosid (quercitrin), (**2**) Quercetin 3-O-β-D-galactopyranosid (hyperosid), (**3**) sitosterol-3-O-β-D-glucopyranosid (daucosterol), (**4**) 3-Methyl-1H-benz[f]indole-4,9-dione. Our data showed that S.specsiosa leaves extract (dose 1.5 g/kg b.w) reduced significantly paw edema induced by Carrageenan. This is first time compound (**1**), (**2**), (**4**) were isolated from leaves of Sanchezia speciosa. S.specsiosa leaf extract have been showed strong effect in inhibit carrageenan-induced paw edema in mice.*

Keywords: *Sanchezia speciosa*, quercitrin, hyperosid, daucosterol, anti-inflammatory.

INTRODUCTION

Vietnam has the tropical climate with diversity of plant species. It is estimated that Vietnam has more than 12000 plant species of which nearly 4000 species can be used in traditional medicine. *Sanchezia speciosa* is a member of Acanthaceae plant family. In Vietnam, *Sanchezia speciosa* grown mostly in North Vietnam and have been commonly used to treat gastritis disease. Literature survey reveals that *Sanchezia speciosa* had antioxidant and anticancer [1], antibacterial, antifungal and insecticidal effects [2]. Currently, there is scarcely research on the chemical composition of *S.speciosa* and biological effects of them for gastritis treatment. Therefore, it is necessary to study about the phytochemical and pharmacology activity of this plant. In this study, we have isolated and identified four compounds from ethanolic extract of *Sanchezia speciosa* leaf grown in Vietnam, including quercitrin, hyperosid, daucosterol, 3-methyl-1H-benz[f]indole-4,9-dione. We also investigated the anti-inflammatory effect of *Sanchezia speciosa* leaf extract on carrageenan-induced paw edema in mice.

EXPERIMENTAL SECTION

Chemicals, reagents and solvents

All chemicals used were of analytical grade and include: carrageenan (Sigma-Aldrich, Germany), ethanol, methanol, chloroform, ethyl acetate (Sigma-Aldrich, Germany), indomethacin (Sigma-Aldrich, Germany), distilled water, normal saline.

Plant material

Leaf of *Sanchezia Speciosa* Leonard were collected in Tuyen Quang province, Vietnam during 2015 and authenticated by Department of Pharmacognosy and Traditional Pharmacy, School of Medicine and Pharmacy, Vietnam National University, Hanoi, Vietnam (SMP-VNU). A voucher specimen (DL-SMP-16) has been deposited in the SMP-VNU.

General experimental procedures

Melting points were measured on Mikroskopheiztisch PHMK-50 (VEB WaegetechnikRapido, Germany). The FT-IR spectra were recorded on an IMPACT-410FT-IR spectrometer (CARL ZEISS JENA). The NMR [^{1}H (500 MHz), ^{13}C (125 MHz), and DEPT-90 and 135 MHz)] spectra were recorded on an AVANCE spectrometer AV 500 (Brucker, Germany) in the Institute of Chemistry, Vietnam Academy of Science and Technology (VAST). 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 LC-MSD mass spectrometer. Analytical TLC was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness) and RP-18 F_{254} (Merck) plates (0.25 mm layer thickness). Spots were visualized using ultraviolet radiation (at 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.

Extraction and isolation

Dry leaf powder of *Sanchezia specsiosa* (1.5kg) was extracted with 80% ethanol (3 L) by ultrasonic at 40^{o}C for three hours for three times. The extracts were filtered, combined and evaporated under low pressure to afford the EtOH extract (125 g). The EtOH extract was suspended in water and successively partitioned with *n*-hexane and ethyl acetate (3 x 700mL, each solvent). Combined solvent was then evaporated under low pressure to obtain the *n*-hexane fraction (6.2 g), and ethyl acetate fraction (25.8 g).

The ethyl acetate fraction was separated over silica gel (Φ 85mm-90mm) using *n*-hexane – ethyl acetate gradient solvent system (5:1→1:1, v/v, 600mL each), then with chloroform – methanol (10:1→ 1:1, v/v, 500mL) to give seven fractions (P1~P7).

Fraction P5 (8.3 g) was furthermore separated to silica gel column (Φ 45mm-350mm) and eluted with chloroform – methanol (15:1, v/v, 1.5 L) to give six subfractions (P5.1~P5.6). Furthermore, P5.1 was applied on a RP-18 column eluted with methanol – water (3:1, v/v, 1,5L) to yield compound **1** (21mg), compound **2** (13mg) and subfraction (P5.1.4). Subfraction P5.1.4 was separated on silica gel TLC preparative with *n*-hexane – ethyl acetate (3:1, v/v) to yield compound **3** (9mg) and compound **4** (14mg).

Determination of anti-inflammatory activity

Swiss mice were purchased from National Institute Of Hygiene And Epidemiology (Vietnam). Mice of either sex weighing 20g were divided into four groups with nine mice of each group. All experimental procedures were reviewed and approved by the Ethical Committee of the Vietnam National University, Hanoi. Procedures were performed according to the guidelines of School of Medicine and Pharmacy, Vietnam National University, Ha Noi on the ethical use of animals. Mice were maintained in standard conditions (a good ventilation room, 28 ± 0,5^{o}C, relative humidity 55 ± 5% and 12 h light/dark cyles).

- Group 1 (control group) was given normal saline.
- Group 2 was treated orally with 5mg/kg body weight of indomethacin 30 minutes before the carrageenan injection.
- Group 3, 4 was treated orally with the extract of *Sanchezia specsiosa* leave extract at the dose 3 g/kg and 1.5 g/kg body weight, respectively, at 60 minutes, 30 minutes before and 30 minutes after the injection of carrageenan.

Paw edema was induced in the mice using 0.05 ml of 1% carrageenan sodium salt. Carrageenan was injected into the right hind foot of each mouse under the plantar aponeurosis.

Measurements of foot volume were performed immediately before and 2h, 4h, 6h, 24h after the injection of

Carrageenan.

The percentage increase in paw volume was calculated using the fontaine formula:

$$X\ (\%) = \frac{\mathrm{Vt} - \mathrm{Vo}}{\mathrm{Vo}} \times 100$$

where Vt is the paw volume before Carrageenan injection
Vo is the paw volume after Carrageenan injection

Inhibitory activity was calculated using the following formula:

$$\mathrm{I}\% = \frac{\mathrm{Xc} - \mathrm{Xt}}{\mathrm{Xc}} \times 100$$

where Xc is the average percentage increase in paw volume of control mice,
Xt is the average percentage increase in paw volume of treated mice.

Statistical analysis

All data were analyzed using Sigma Plot 2010 USA. Data are expressed as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine significance among groups. Statistical significance was set at $p < 0.05$.

RESULTS

Isolation and Chemistry

Compound 1: Quercetin 3-*O*-*α*-L- rhamnopyranoside (quercitrin); Yellow crystal; melting point 182-183^{o}C; R_f = 0,4 ($CHCl_3$–MeOH, 85:15); ESI-MS *m/z:* 449 $[M+H]^+$ and 447 $[M-H]^-$ (calculated *m/z* for $C_{21}H_{20}O_{11}$ is 448); The ^{1}H-NMR (500 MHz, $CDCl_3$-d6, δ, ppm, J/Hz), ^{13}C-NMR (125 MHz, $CDCl_3$-d6, δ, ppm, J/Hz) and DEPT data for compound **1** are presented in table 1.

Compound 2: Quercetin 3-*O*-*β*-D-galactopyranoside *(hyperoside;* Yellow crystal; melting point 232-233^{o}C; R_f = 0,3 ($CHCl_3$–MeOH, 85:15);ESI-MS *m/z:* 465 $[M+H]^+$ and 463 $[M-H]^-$ (calculated *m/z* for $C_{21}H_{20}O_{12}$ is 464); The ^{1}H-NMR (500 MHz, DMSO-d6, δ, ppm, J/Hz), ^{13}C-NMR (125 MHz, DMSO-d6, δ, ppm, J/Hz) and DEPT data for compound **2** are presented in table 1.

Compound 3: *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (daucosterol); White crystal; melting point 285^{o}C; FT-IR *(KBr, cm^{-1})*ν_{max}: 3430 (OH), 2938 (C-H), 1635 (C=CH), 1077 (C-O-C), 1021 (C-O-C). *ESI-MS: m/z* 599 $[M+Na]^+$. The ^{1}H-NMR (500 MHz, $CDCl_3$-d6, δ, ppm, J/Hz), ^{13}C-NMR (125 MHz, $CDCl_3$-d6, δ, ppm, J/Hz) data for compound **3** are presented in table 2.

Compound 4: 3-Methyl-1H-benz[f]indole-4,9-dione; Yellow, solid powder, melting point 248-249^{o}C; R_f= 0,44 (n-hexane-CH_2Cl_2 25/75, v/v). (+)-ESI-MS: m/z 210 $[MH]^+$, (+)-ESI-MS: m/z 212 [M+H]+; FT-IR (KBr) ν_{max} (*cm^{-1}*): 3429, 3322, 2938, 1647, 1588, 1507, 1403, 1238, 1031, 932, 714; UV (MeOH) λ_{max} nm (log ε): 258 (4,07); 330 (3,32). The ^{1}H-NMR (500 MHz, $CDCl_3$-d6, δ, ppm, J/Hz), ^{13}C-NMR (125 MHz, $CDCl_3$-d6, δ, ppm, J/Hz) data for compound **4** are presented in table 3.

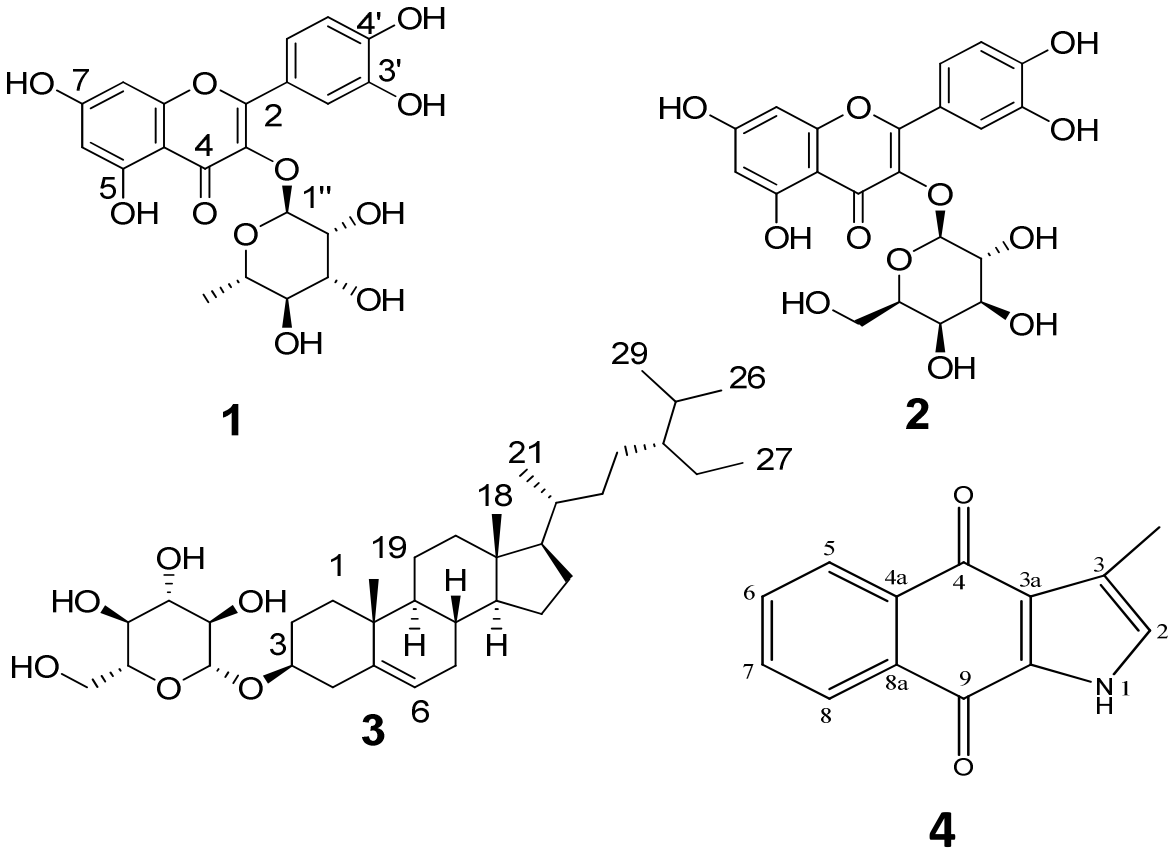

Figure 1. Chemical structures of compounds (1-4)

Table 1. ^{1}H-NMR, ^{13}C-NMR and DEPT Data for compound 1 and 2

Position C	Compound 1			Compound 2		
	DEPT	δ_H (ppm, *J*: Hz)	δ_C (ppm)	DEPT	δ_H (ppm, *J*: Hz)	δ_C (ppm)
2	C	-	158.5	C	-	156.3
3	C	-	136.2	C	-	133.5
4	C	-	179.6	C	-	177.5
5	C	-	163.1	C	-	161.2
6	CH	6.22 *d* (1.5)	99.8	CH	6.20 *d* (2.0)	98.6
7	C	-	165.8	C	-	164.2
8	CH	6.38 *d* (1.5)	94.7	CH	6.40 *d* (2.0)	93.5
9	C	-	158.5	C	-	156.2
10	C	-	105.9	C	-	103.9
1'	C	-	123.0	C	-	121.1
2'	CH	7.36 *d* (2.0)	116.4	CH	7.53 *d* (2.0)	115.2
3'	C	-	146.4	C	-	144.8
4'	C	-	149.7	C	-	148.5
5'	CH	6.92 *d* (8.5)	117.0	CH	6.82 *d* (8.5)	115.9
6'	CH	7.33 *dd* (2.0; 8.5)	123.0	CH	7.67 *dd* (2.0; 8.5)	121.9
1"	CH	5.37 *d* (1.0)	103.5	CH	5.37 *d* (7.5)	101.8
2"	CH	4.25 *brd* (3.0)	72.1	CH	3.46 *dd* (7.5; 9.0)	71.2
3"	CH	3.77 *dd* (3.0; 9.0)	72.0	CH	3.55*	73.2
4"	CH	4.87*	73.3	CH	3.30-3.40*	67.9
5"	CH	3.33 *m*	71.9	CH	3.30-3.40*	75.8
6"	CH_3	0.97 *d* (6.0)	17.6	CH_2	3.30-3.40*	60.1
5-OH					12.65 *s*	

Table 2. ^{1}H-NMR, ^{13}C-NMR Data for compound 3

Position C	Compound 3	
	δ_C (ppm)	δ_H (ppm, *J*: Hz)
1	36.8	
2	31.3	
3	76.9	3.56 *sextet* (7.0)
4	39.3	
5	140.3	
6	121.1	5.36 *d* (5.0)
7	31.4	
8	31.3	
9	49.6	
10	36.1	
11	20.5	
12	38.3	
13	41.8	0.69 *s*
14	55.4	
15	25.5	
16	29.2	
17	56.2	
18	11.7	
19	19.0	
20	35.4	0.93 *d* (6.5)
21	18.5	
22	33.3	
23	27.7	
24	45.1	
25	28.7	0.79 *m*
26	19.6	
27	18.9	
28	22.5	0.87 *m*
29	11.7	
1'	100.8	4.13 *d* (8.0)
2'	76.7	3.45 *m*
3'	73.4	3.24 *m*
4'	70.0	3.31 *m*
5'	76.6	3.47 *m*
6'	61.0	3.76 d (12.0)

Table 3. ^{1}H-NMR, ^{13}C-NMR Data for compound 4

Position C	Compound 4	
	δ_C (ppm)	δ_H (ppm, *J*: Hz)
1	NH	12.65 (1H, br s);
2	125.5	7.13 (1H, m);
3	122.8	
3a	125.1	
4	175.7	
4a	133.4	
5	126.5	8.18 (1H, m);
6	133.2	7.76 (1H, m);
7	133.2	7.76 (1H, m);
8	126.0	8.18 (1H, m);
8a	134.5	
9	182.3	
9a	132.7	
10	11.0	2.31 (3H, s).

Anti-inflammatory activity

Carrageenan has been widely used for inducing paw edema in mice to measure anti-inflammatory capacity and for the screening of new anti-inflammatory drugs [3]. As we can be seen from Table 4, *Sanchezia specsiosa* leaves extract at the dose 3 g/kg b.w and 1.5 g/kg b.w reduced significantly paw edema Carrageenan-induced after 24 h when compared to the control group ($p<0,05$), but not statistically significant at 2h, 4h, 6h ($p>0.05$). Indomethacin, as a positive control, at dose 5mg/kg, decreased significantly paw edema after 2 h ($p<0.001$).

Table 4: Anti-inflammatory activity of *Sanchezia specsiosa* leave on carrageenan induced paw edema

Group	Percentage inhibition (I%)			
	2 h	4h	6h	24h
Control group	1.25	1.24	1.14	1.25
Indomethacin: 5mg/kg	56.43 *	46.08*	48.59*	58.41*
Sanchezia specsiosa: 3 g/kg	3.03	3.17	1.6	32.45*
Sanchezia specsiosa: 1.5 g/kg	2.84	2.72	2.34	17.83*

**: Significantly difference with Control group.*

DISCUSSION

Compound **1** was isolated as a yellow crystal, its positive and negative EIMS showed a molecular ion peak $[M+H]^+$ and $[M-H]^-$ at *m/z* 449 và 447, consistent with the molecular formula $C_{21}H_{20}O_{11.}$ The ^{1}H-NMR data of the aglycone of **1** were consistent with a quercetin derivative. The ^{1}H-NMR spectrum also showed the presence of a rhamnosyl unit with an α-linkage characterized of an anomeric proton at δ_H 5.37 (1H, d, *J* = 1.0 Hz, H-1"). The presence of a methyl doublet at δ_H 0.96 (3H, d, *J* = 6.5 Hz, H-6") together with the small coupling constant value at H-1' (*J* = 1.0 Hz) indicated the presence of a rhamnopyranosyl moiety. The proton signals at δ_H 7.35 (1H, d, *J* = 2.0 Hz, H-2'), 6.93 (1H, d, *J* = 8.5 Hz, H-5'), 7.32 (1H, dd, *J* = 2.0, 8.5 Hz, H-6'), 6.21 (1H, d, *J* = 1.5 Hz, H-6), 6.37 (1H, d, *J* = 1.5 Hz, H-8) were characterized of a quercetin moiety. The ^{13}C-NMR spectrum indicated the presence of 21 carbon atoms, the carbon signal at δ_C 179.6 was suggested to a carbonyl carbon at C-4, two carbon signals as 165.8 and 163.1 indicated the presence of hydroxyl group located at C-7 and C-5, two carbon signals at 146.3 and 149.7 showed oxygenated carbons of B-ring at C-3' and C-4'. Six carbon signals at 103.5, 72.1, 71.9, 73.2, 71.8, and 17.6 were assigned to a glucopyranosyl unit. As compare the proton and carbon data of **1** to those of related published [4, 5], compound **1** were identified as quercitrin. This compound was first time isolated from *Sanchezia Speciosa* Leonard.

Compound **2** was isolated as a yellow crystal. The ESI-MS showed *m/z* 465 $[M+H]^+$ and 463 $[M-H]^-$ revealed the molecular formula to be $C_{21}H_{20}O_{12}$. The ^{1}H-NMR spectrum of 2 showed three aromatic protons signals at *δ* 7.52 (1H, d, *J* = 2.0 Hz, H-2'), 6.81 (1H, d, *J* = 8.4 Hz, H-5') and 7.66 (2H, dd, *J* = 2, 8.5 Hz) in the form of an ABX spin-system suggesting a flavonol with 3',4'- disubstituted B-ring and showed a pair of meta coupling proton signals at 6.20 (d, *J* = 2.0 Hz, H-6) and 6.39 (d, *J* = 2.0 Hz, H-8) for the A-ring. The sugar moiety in **2** was deduced to be *β*-D-glucopyranosyl unit because of the anomeric carbon and proton signals at δ_C 101.8 (C-1") and δ_H 5.36 (d, *J* = 7.6 Hz, H-1"). The ^{13}C-NMR spectrum showed 21 signals including 15 carbon signals of quercetin unit at δ_C 156.2 (C-2), 133.4 (C-3), 177.4 (C-4), 161.2 (C-5), 98.6 (C-6), 164.1 (C-7), 93.5 (C-8), 156.3 (C-9), 103.8 (C-10), 121.1 (C-1'), 115.2 (C-2'), 144.8 (C-3'), 148.4 (C-4'), 115.9 (C-5'), 121.9 (C-6') and 6 carbon signal of sugar unit at δ_C 101.8 (C-1"), 73.2 (C-2"), 75.8 (C-3"), 71.2 (C-4"), 67.9 (C-5"), 60.1 (C-6"). By comparing these data with literature [6], compound **2** was identified as hyperoside. This compound was first time isolated from *Sanchezia Speciosa* Leonard .

Compound **3** was obtained as white crystal. The molecular formula was established to be $C_{35}H_{60}O_6$ from a molecular ion peak at *m/z* 599 $[M+Na]^+$. The IR spectrum showed an absorption bands arising from hydroxyl (3430 cm^{-1}), olefinic (2938), carbonyl group (1635 cm^{-1}), –CH aliphatic asymmetric stretching of $–CH_3$, $–CH_2–$ and > CH_2 groups (2900-2850 cm^{-1}). The ^{1}H-NMR spectrum of 3 showed the following signals: three tertiary methyl group at δ_H 0,69 (3H, s), 1,01 (3H, s); four secondary methyl group at δ_H 0.79-0.87 (9H, m, Me-26, Me-27 Me-29), 0.93 (3H, d, *J* = 6.5 Hz, Me-21), one olefinic proton at δ_H 5.36 (1H, d, *J* = 5.0 Hz, 1H, H-6) and an oxygenated proton at δ_H 3.56 (1H, sextet, *J* = 7.0 Hz, 1H, H-3). Based on the above evidences and by compared to those of published data [7, 8], compound 3 was assigned to be *β*-sitosterol 3-*O*-*β*-D-glucopyranoside.

Compound **4** was obtained as a yellow, solid powder. The molecular formula was established as $C_{13}H_9O_2N$ based on a molecular ion peak at *m/z* 212,0710 $[M+H]^+$. The IR spectrum showed typical absorption bands arising from NH (ν_{max} 3429 cm^{-1}) and carbonyl C=O (ν_{max} 1647 cm^{-1}).

The ^{1}H-NMR spectrum of compound **4** exhibited the presence of 5 signals proton of aromatic ring at δ_H 8.08 (1H, m, H-5); 8.01 (1H, m, H-8), 7.59 (2H, m, H-6 and H-7) and 6.83 (1H, m, H-2); and 1 methyl group at δ_H 2.31 (3H, s). The ^{1}H-NMR spectrum of compound **4** also exhibited the presence of a proton NH signal at δ_H 12.65 (1H, br s, H-1). The ^{13}C-NMR spectra of compound **4** indicated the presence of 13 carbon atoms in the molecule. There are a methyl group; 5 methine sp^2 groups; and 2 carbonyls group at δ_C 182.3 (C-9) và 175.7 (C-4) and 5 quaternary carbons in aromatic ring at δ_C 122.8 (C-3), 125.1 (C-3a), 133.4 (C-4a), 134.5 (C-8a) and 132.7 (C-9a).

The signal in COSY spectra allowed determining the correlation from H-3 to H-8. HMBC spectrum showed the correlation between H-5 and H-8 with C-4a and C-8a, confirmed the presence of benzen A ring. Also there are correlations between 2 carbonyls C-4 and C-9 with H-5 and H-8, respectively, in HMBC spectrum. It allowed

confirming the C-4 and C-9 linked to ring A at position C-4a and C-8a. The pyrole C ring also is determined by HMBC spectrum: H-2 correlates with C-3, C-3a and C-9a; CH_3-10 correlates with C-2 and C-3a. The methyl group position at C-3 in pyrole C ring is also confirmed by NOE correlation between H-2 and CH_3-10. Based on the above evidence and the literature data [9], compound **4** was identified as 3-methyl-1H-benz[f]indole-4,9-dione. This compound was first time isolated from *Sanchezia Speciosa* Leonard.

Inflammation is the response of the immunological defense system to microbial infections, burns, allergens and other stimuli. The pathogenesis of many diseases, including diabetes, cardiovascular, neurodegenerative, cancer and other life-threatening diseases involve the inflammation. Inflammation is a complex series of cascade reactions, including enzyme activation, release of chemical mediators, effusion of fluids, cell migration, and tissue damage and repair [10]. The carrageenan-induced mouse paw edema is a practicable model for evaluating the anti-inflammatory effect of natural products. The acute and local inflammatory response is induced by carrageenan injection into the mice paw. The model has been well established as a valid model to study pro-inflammatory mediators and cytokine generation in paw tissue in inflammatory conditions [11]. The present study showed that *Sanchezia Speciosa* leaves extract, via oral administration, reduced paw volumes in the carrageenan-induced paw edema test. It can explain the anti-inflammatory action of *Sanchezia Speciosa* leaves exttract is related to the effects on mediators and cytokines.

CONCLUSION

From the leaf of *Sanchezia Speciosa* Leonard. we have isolated four compounds: quercitrin (**1**), hyperoside (**2**), daucosterol (**3**), 3-Methyl-1H-benz[f]indole-4,9-dione (**4**). These compounds (1), (2), and (4) were isolated for the first time from leaves of *S.specsiosa* leaves. *S.specsiosa* leaves at the dose 3 and 1.5 g extract/kg b.w reduced significantly ($p<0,01$, $p<0,05$, respectively) at 24 h in Carrageenan induced paw edema.

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