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TRITERPENES AND PHYTOSTEROLS FROM *SCAEVOLA TACCADA* COLLECTED IN LY SON ISLAND, QUANG NGAI PROVINCE, VIETNAM

Pham Thi Huyen[1], Duong Van Anh[2], Do xuan Cam[3], Nguyen The Anh[4], Tran Van Loc[4], Tran Van Sung[4], Tran Thi Phuong Thao[4*]

[1]*Nguyen Binh Khiem High school for the Gifted, Tran Dai Nghia, Tam Ky, Quang Nam, Vietnam*

[2]*Hai Ba Trung High school, 14 Le Loi, Hue, Vietnam*

[3]*College of Agriculture and Forestry, Hue University, Vietnam*

[4]*Institute of Chemistry, Vietnam Academy of Science and Technology*

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Abstract

From the ethyl acetate extract of the leaves of *Scaevola taccada* (Gaertn.) Roxb., two triterpenes (ursolic acid **6**, myricadiol **5**), two sterols (β-sitosterol **3**, β-sitosterol glycoside **4**), together with two long-chain hydrocarbons, tritriacontane **1** and tetracosane **2** have been isolated. Their structures were elucidated by the analysis of the IR, MS and NMR 1D and 2D spectra. Compound **1**, **2**, **3**, **5**, **6** were isolated for the first time from *Scaevola taccada*.

Keywords. *Scaevola taccada*, triterpenes, sterols, long-chain hydrocarbons, ursolic acid, myricadiol, tritriacotane, tetracosane.

1. INTRODUCTION

Scaevola taccada (Gaertn.) Roxb. (Synonym: *Scaevola frutescens*, *Scaevola sericea*) having Vietnamese names: Cây Hếp, Cây Bão táp, belongs to the family Goodeniacea (họ Hếp) [1]. It is a mangrove plant, distributed in the coastal areas, though out the Indian Ocean and tropical islands of Pacific [2]. In Vietnam, *Scaevola taccada* is found along the salt-march and in islands areas [1]. This plant is up to 3 m in height [3]. The crushed fruit of *S. taccada* was used for treatment of tinea [4], while the ripe fruit juice has been used to treat sores and infected eyes, combined with stems was used against bites and stings [5]. The leaves were used for indigestion and headache treatment [6]. In Indonesia, the root is used as an antidote when poinsonous fish and crabs are consumed [6]. The methanol extract of the leaves of this plant showed antibacterial, antifungal [7], antipyretic [8], anti-inflammatory [9], antidiabetic, anti coagulant and skeletal muscle relaxant [10].

There are some reports on chemical constituents from *S. taccada* [11-13], but no reports from this plant in Vienam. This paper describes the isolation and structural education of the constituents of the leaf ethyl acetate extract of *S. taccada* collected in Ly Son Island, Quang Ngai, Vietnam.

2. EXPERIMENTAL

2.1. Equipments and methods

IR: Impact 410, Nicolet, Germany; ESI-MS: LC-MSD-Trap-SL, Varian, USA, NMR: Bruker Avance 500, Germany with TMS as internal reference (for ^{1}H) and solvent signal (for ^{13}C). CC used silicagel 60G, size 0.043-0.063 mm (Merck), TLC: precoated silica gel G60F254 plates (Merck), spots were detected by spayring with vanillin 1 % in conc. H_2SO_4 and heating at 110 ^{o}C.

2.2. Plant material

Fresh leaves of *Scaevola taccada* were collected in Ly Son Island, Quang Ngai province, Vietnam in June 2013. A voucher specimen (ST01) is deposited in Institute of Chemistry, VAST, Hanoi, Vietnam. The leaves were shaded, dried, ground into powder and stored at room temperature.

2.3. Extraction and isolation of the compounds

The dried powder of the leaves of *Scaevola*

taccada (1.2 kg) was extracted successively with *n*-hexane, ethyl acetate and methanol. The organic solvents were evaporated in reduced pressure to furnish the *n*-hexane, EtOAc and MeOH extract (14.0, 20.7 and 159.9 g, respectively). The ethyl acetate extract (ST2, 20 g) were chromatographed on silica gel (450 g), eluated with CH_2Cl_2/MeOH from 0% MeOH gradient to 50 % and 100 % MeOH (v/v) to afford 19 fractions (ST2.1-ST2.19). From fraction ST2.1 a white solid crystallized, which was washed with cold MeOH and then cold *n*-hexane to give 7 mg white solid (compound **1**) (R_f = 0.5, *n*-hexane/CH_2Cl_2 85:15). From fraction ST2.4 a white solid crystallized which was washed with cold *n*-hexane, then with cold MeOH and a mixture of *n*-hexane/CH_2Cl_2 3:1 to afford a pure white solid (compound **2**), (R_f = 0.30, *n*-hexane/ethyl acetate 93:7). From fraction ST2.9 white needles appeared, which were washed with cold MeOH then cold *n*-hexane to give compound **3** (15 mg). From fraction ST2.15, a white solid crystallized, which was washed with cold *n*-hexane, then with CH_2Cl_2 to give 10 mg compound **4**. Fraction ST2.12 and ST2.13 (9 g) were combined and chromatographed on SiO_2 (*n*-hexane/EtOAc 1:1) to give 9 fractions. A white solid (compound **5)** appeared in fraction ST2.13.5. From fraction ST2.13.8 a white solid precipitated to afford compound **6**.

Tritriacontane (1) ($C_{33}H_{68}$): white solid. ESI-MS m/z: 464 (6 %, $[M]^+$).

^{1}H-NMR ($CDCl_3$, 500 MHz), δ_H (ppm), *J* (Hz): 0.81 (6H, t, *J* = 6.7), 1.19 (60H, brs), 1.48 (2H, brs).

^{13}C-NMR ($CDCl_3$, 125 MHz), δ_C (ppm), *J* (Hz): 14.12 (CH_3), 29.37 (CH_2), 22.70 (CH_2), 29.67-29.71 (very strong, CH_2), 31.94 (CH_2).

Tetracosane (2) ($C_{24}H_{50}$): white solid. ESI-MS m/z: 338 (6 %, $[M]^+$).

^{1}H-NMR ($CDCl_3$, 500 MHz), δ_H (ppm), *J* (Hz): 0.87 (6H, t, *J* = 7.1), 1.25 (44H, brs).

^{13}C-NMR ($CDCl_3$, 125 MHz), δ_C (ppm), *J* (Hz): 14.12 (CH_3), 22.7 (CH_2), 29.38 -29.45 (very strong, CH_2), 31.94 (CH_2).

β-sitosterol (3) ($C_{29}H_{50}O$): white solid.

^{1}H-NMR ($CDCl_3$, 500 MHz), δ_H (ppm), *J* (Hz): 0.70 (3H, s), 0.86 (6H, brs), 0.87 (3H, t, *J* = 7.1), 1.00 (3H, d, *J* = 6.7 Hz), 1.07 (3H, s), 1.11-1.54 (24H, m), 1.65-1.70 (2H, m), 1.82-1.89 (3H, m), 1.97-2.05 (2H, m), 2.25-2.33 (2H, m), 3.52-3.56 (1H, m), 5.36-5.38 (1H, m).

^{13}C-NMR: identical with [14].

β-sitosterol 3-O-β-D-glycopyranoside (4) ($C_{35}H_{60}O_6$)**:** white solid.

^{1}H-NMR (DMSO-d_6, 500 MHz), δ_H (ppm), *J* (Hz): 0.65 (3H, s), 0.96 (3H, s), 0.80 (3H, d, *J* = 6.9 Hz), 0.81 (3H, d, *J* = 6.8), 0.90 (3H, *t*, *J* = 6.5), 1.00 (3H, d, *J* = 6.7), 3.42 (1H, m), 5.32 (1H, brs); glucopyranosyl: 3.05-3.08 (1H, m), 3.14-3.20 (3H, m), 3.64 (1H, dd, *J* = 5.5, 10.1), 3.46 (1H, m), 4.22 (1H, d, *J* = 7.8, H-1'), 4.39 (1H, t, *J* = 5.7 Hz), 4.83 (3H, m).

^{13}C-NMR (DMSO-d_6, 125 MHz), δ_C (ppm), *J* (Hz) identical with data in [15]: 35 carbon signals including 121.13 and 140.12 (olefine), 11.6, 11.7, 18.5, 18.9, 19.0, 19.6 (all methyl groups), 70.09 (C-3), glucopyranosyl: 100.7 (C-1'), 73.4, 76.6, 76.7 and 76.9 (oxymethine).

Myricadiol (5) ($C_{30}H_{50}O_2$.): colourless amorphous powder. IR (KBr, cm^{-1}): 3493.99, 3299.9, 3209.79 (OH), 2981 (alkane), 1627.36 (C=C), 1088.85 (C-O). ESI-MS m/z: 443 (15 %, $[M+H]^+$), 413 (100) $[M+2H-CH_2OH]^+$.

^{1}H- and ^{13}C-NMR: see table 1.

Ursolic acid (6) ($C_{30}H_{48}O_3$): white solid. IR (KBr, cm^{-1}): 3414 (OH), 1692 (COOH), 2928, 1454, 1388 (alkane). ESI-MS m/z: 479 $[M+Na]^+$, 455 $[M-H]^-$.

^{1}H- and ^{13}C-NMR, see table 1.

3. RESULTS AND DISCUSSION

Compound **1** (tritriacontane, $C_{33}H_{68}$) and **2** (tetracosane, $C_{24}H_{50}$) have been isolated as amorphous powder. Their structures were determined by the analysis of their ^{1}H-, ^{13}C-NMR and ESI-MS spectra. They are presented in many plant waxes. Tritriacontane is the major constituent of some waxes, e.g. *Agave sisalane* (33 %) and *Calocephalus brownii* (12 %) [17]. Tetracosane (synonym: Lignocerane) was isolated from some plants, e.g. rose and orange oil and in the pheromone of female white marked tussock moth *Orgyia leucostigma* [18].

Compound **3** (ß-sitosterol) was isolated as white needles, its structure was determined by comparison of its ^{1}H- and ^{13}C-NMR spectral data with those in [14].

Compound **4** (β-sitosterol 3-O-β-D-glycopyranoside or daucosterol) was isolated as white needles and determined by comparison of its ^{1}H-, ^{13}C-NMR data with the reported data in [15].

Compound **5** (myricadiol) was isolated as amorph colourless powder. Its ^{1}H-NMR spectrum showed signals of 7 methyl groups, one olephinic proton (δ_H 5.51, 1H, dd), one oxy methylene (δ_H 3.02, 2H, m), one oxymethine (δ_H 2.87, 1H, m). The ^{13}C-NMR spectrum contained 30 carbon signals including 7 methyl, 2 olephinic (δ_C 157.70, 115.85), one oxymethine (δ_C 76.79), one oxymethylene (δ_C 63.20) signal. Further analysis of its NMR spectra

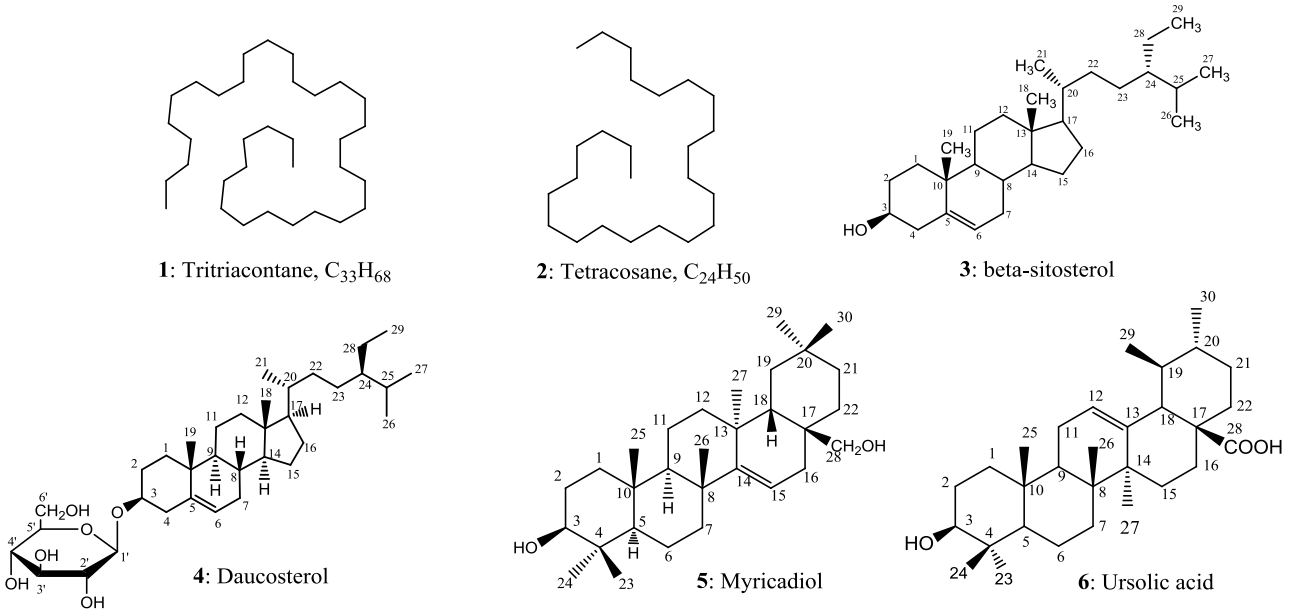

Figure 1: Compounds isolated from the ethyl acetate extract of *Scaevola taccada*

Table 1: ^{1}H- and ^{13}C-NMR data of compound **5**, **6** compared with literature data [19], [16]

Position	**5** (DMSO-d_6)		Myricadiol ($CDCl_3$) [19]		**6** (DMSO-d_6)		Ursolic acid (DMSO-d_6) [16]	
	δ_H (ppm, *J*/Hz)	δ_C/ppm	δ_H (ppm, *J*/Hz)	δ_C/ppm	δ_H (ppm, *J*/Hz)	δ_C/ppm	δ_H (ppm, *J*/Hz)	δ_C/ppm
1	$H_{1\alpha}$ = 1.61 (1H, m); $H_{1\beta}$ = 1.59 (1H, m)	37.32	$H_{1\alpha}$ = 1.61 (1H, m); $H_{1\beta}$ = 1.58 (1H, m)	37.5		38.24		38.22
2	$H_{2\alpha}$ = 1.56 (1H, m); $H_{2\beta}$ = 1.53 (1H, m)	26.85	$H_{2\alpha}$ = 1.6 (1H, m); $H_{2\beta}$ = 1.59 (1H, m)	26.9		26.98		26.79
3	3.01 (1H, *br s*)	76.79	3.16 (1H, dd, *J* = 4.8; 10.8)	78.5	3.01-2.99 (1H, m)	76.85	3.01 (1H, dd, *J* = 9.5, 5.2)	76.82
3-O<u>H</u>	4.25 (1H, *br s*)				4.30-4.29 (1H, m)			
4		38.50		38.5		38.37	5.14 (1H, m)	38.35
5	0.72 (1H, s)	55.09	0.77 (1H, dd, *J* = 2.4; 12.6)	55.3		54.79		54.77
6		18.43		18.5		18.00		17.97
7	H_{α} = 2.17 (1H, m); H_{β} = 1.33 (1H, m)	41.05	H_{α} = 2.01 (1H, dt, *J* = 3.0; 12.6) H_{β} = 1.33 (1H, td, *J* = 3.0; 12.6)	41.1		30.19		30.77
8		39.02		38.8		39.10		39.09
9	1.44 (1H, m)	48.69	1.42 (1H, m)	48.9		47.02		47.00
10		37.49		37.7		36.53	2.12 (1H, d, *J* = 11.1)	36.51

11		16.95		17.1		23.81		23.80
12	H_α = 1.384 (1H, m); H_β = 1.35 (1H, m)	33.13	H_α = 1.66 (1H, m); H_β = 1.62 (1H, m)	33.2	5.12 (1H, br s)	124.58		124.54
13		36.89		37.2		138.19		138.18
14		157.70		158.6		41.64		41.62
15	5.41 (1H, m)	115.85	5.51 (1H, dd, *J* = 3.0; 8.4)	115.6		32.71	0.90 (3H, s)	32.69
16	H_α = 2.12 (1H, m) H_β = 1.96 (1H, m)	30.15	H_α = 2.14 (1H, dd, *J* = 8.4; 15) H_β = 1.7 (1H, dd, *J* = 3.0; 15)	30.4		22.85	0.68 (3H, s)	22.82
17		40.09		40.2		46.83	0.87 (3H, s)	46.81
18	0.47 (1H, m)	44.54	0.57 (1H, dd, *J* = 3.6; 13.8)	44.6	2.10 (1H, d, *J* = 11.5)	52.38	0.69 (3H, s)	52.37
19	H_α = 1.41 (1H, m); H_β = 1.134 (1H, m)	35.51	H_α = 1.41 (1H, m); H_β = 1.0 (1H, dd, *J* = 3.6; 13,2)	35.6		38.44	1.05 (3H, s)	38.42
20		28.19		28.3		38.50		38.48
21	H_α = 1,25 (1H, m); H_β = 1.23 (1H, m)	32.40	H_α = 1.26 (1H, m); H_β = 1.24 (1H, m);	32.4		27.54		27.52
22	H_α = 1.48 (1H, m); H_β = 1.46 (1H, m)	27.32	H_α = 1.5 (1H, m); H_β = 1.47 (1H, m)	27.6		36.32		36.29
23	0.98 (3H, s)	28.06	0.97 (3H, s)	27.8	0.89 (3H, s)	28.26		28.23
24	0.7 (3H, s)	15.84	0.79 (3H, s)	15.3	0.67 (3H, s)	16.91		16.90
25	0.88 (3H, s)	15.15	0.91 (3H, s)	15.2	0.86 (3H, s)	16.07		16.03
26	1.01 (3H, s)	25.77	1.06 (3H, s)	25.8	0.75 (3H, s)	15.22		15.18
27	0.90 (3H, s)	21.42	0.96 (3H, s)	21.3	1.04 (3H, s)	23.27		23.24
28	H_α= 2.98 (1H, m) H_β = 2.87 (1H, m)	63.20	H_α = 3.24 (1H, d, 10.8) H_β = 3.1 (1H, d, 10.8)	64.8		178.27		178.23
28-OH	4.25 (1H, *br* s)							
29	0.93 (3H, s)	33.65	0.96 (3H, s)	33.3	0.81 (3H, d, *J* = 6.5)	17.01		16.97
30	0.86 (3H, s)	29.69	0.89 (3H, s)	29.6	0.90 (3H, d, *J* = 9.5)	21.07		21.10

indicated the identity of these data with the data of myricadiol in [19]. This is the first time myricadiol is isolated from *Scaevola taccada*. The ^{1}H- and ^{13}C-NMR spectral data of **5** are given in table 1.

Compound **6** (ursolic acid): The ESI-MS, ^{1}H- and ^{13}C-NMR spectral data of compound **6** are in full agreement with the reported data for ursolic acid in [16]. The ^{1}H- and ^{13}C- NMR spectral data of **6** and ursolic acid are given in table 2.

4. CONCLUSION

The first time the mangrove plant *Scaevola taccada* collected in Ly Son island, Quang Ngai province, Vietnam has been chemically investigated. Two long-chain hydrocarbons, one phytosterol, one phytosterol glycoside and two triterpenes were isolated and determined the structures. Compound **1**, **2**, **3**, **5**, **6** were isolated for the first time from *Scaevola taccada*.

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REFERENCES

1. Phạm Hoàng Hộ. *An illustrated flora of Vietnam*, page 122, **1**, Nr. III, The Youth Publisher, Ho Chi Minh City (2000).
2. F. R. Fosberg, and M. H. Sachet. *Scaevola sericea Vahl versus S. taccada* (Gaertn.) Roxb., Taxon, **10**, 225-226 (1961).
3. G. C. H. Drury, M. Arup. *Hand book of Indian flora*, Vol II, Bishen singh Mahendra Pal Singh publishers, Dharadun, pp.**99** (1982).
4. F. Bodkin, Encyclopedia Botanica. *The essential reference guide to native and exotic plants in Australia*, Angus and Robertson Publisher (1986).
5. A. Bar, J. Chapman, N. Smith, G. Wightman, T. Knight, L. Mills, M. Andews, V. Alexander. *Traditional and original medicines in the northern territory of Australia by Aboriginal Communities of the Northern Territory*, Conservation Commission of the Northern Territory (1993).
6. YC. Wee. *A guide to medical plants*, Singapore Science Centre Publication (1992).
7. B. Manimegalai, L. Inbathamizh, T. Mekalai Ponnu. *In vitro studies on antimicrobial activity and phytochemical screening of leaf extracts of Scaevola taccada*, International Journal of Pharmacy and Pharmaceutical Sciences, **4(4)**, 367-370 (2012).
8. A. Chandran, G. Arunachalam. *Study of antipyretic activity of Scaevola taccada Roxb. leaf extract*, International Journal of Research in Pharmacology & Pharmacotherapeutics, **2(2)**, 2278-2748 (2013).
9. A. Chandran, G. Arunachalam. *Study of anti-inflammatory activity of Scaevola taccada Roxb. leaf extracts*, International Journal of Phytopharmacology, **4(4)**, 263-265 (2013).
10. S. Umadevi, GP. Mohanta, R. Manavalan. *Screening of folklore claim of Scaevola frutescens Krause*, Indian Journal of traditional knowledge, **5(4)**, 531-536 (2006).
11. S. Yamaguchi, S. Muro, M. Kobayashi, M. Miyazawa, Y. Hirai. *Absolute structures of some naturally occurring isopropenyldihydrobenzofurans, remirol, remiridiol, angenomalin and isoangenomalin*, Journal of Organic Chemistry, **68**, 6274-6278 (2003).
12. T. Nemoto, T. Ohshima, M. Shibasaki. *Enantioselective total syntheses of (+)-decursin and related natural compounds using catalytic asymmetric epoxidation of an enone*, Tetrahedron, **59**, 6889-6897 (2003).
13. K. Wohlrabe & R. Hansel. *Coumarins from Scaevola frutescens*, Arch Pharm, **310**, 972 (1977).
14. V. S. P. Chaturvedula, I. Prakash. *Isolation of stigmasterol and β-sitosterol from the dicholormethane extract of Rubus suavissimus*, International Current Pharmaceutical Journal, **1(9)**, 239-242 (2012).
15. Pham Duc Thang, Ph.D. thesis, Institute of Chemistry, VAST, 86-87 (2012).
16. M. Goretti, V. Silva. *Variation of ursolic acid content in eight Ocimum species from Northeastern Brazil*, Molecules, **13**, 2482-2487 (2008).
17. A. Goswami**,** Y. N. Shukla**,** R. S. Thakur**.** *Aliphatic compounds from Hyoscyamus muticus*, Phytochemistry, **20(6)**, 1315-1317 (1981).
18. G. L. K. Hunter, W. B. Brogden Jr. *Analysis of cold-pressed orange oil paraffin waxes*, Phytochemistry, **5(4)**, 807-809 (1966).
19. Michele Mejin. *Isolation, Structual elucidation and antibacterial activity of the chemical constituents of Scaevola spinescens*, The University of Adelaide, South Australia, Doctor Thesis, 47-59 (2009).

Corresponding author: **Tran Thi Phuong Thao**
Institute of Chemistry, Vietnam Academy of Science and Technology
18 Hoang Quoc Viet, Cau Giay, Hanoi, Vietnam
E-mail: ntuelam2010@gmail.com.