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Research Article

THE CHEMICAL CONSTITUENTS OF LEAVES AND STEMS VINES OF *STRETOCAULON JUVENTAS*

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ABSTRACT

"Ha thu o trang" has the scientific name of ***Streptocaulon juventas****. As a traditional medicine, "Ha thu o trang" has a bittersweet taste, acrid and cool properties, and has the effect of tonic blood, liver, and kidney. In Vietnam, "Ha thu o trang" has been known for a long time and is widely used in folk medicine to cure snakebites, premature graying of hair, clear heat, detoxify, and swelling pain. It is used as a tonic to treat depression and neurological diseases, strengthen bones and bones, live long, and blacken hair and beard. The previous studies only focused on the chemical constituents of the root of S. juventas, proving the cardenolide glycosides were the main of this species. In this study, the dried powder sample of leaves and stems vines of S. juventas was collected in Tinh Bien district, An Giang province, and hot extracted with methanol solvent to obtain the methanol extract. Dispersion of the methanol residue in water and liquid-liquid extraction with n-hexane, ethyl acetate, and n-butanol solvents, respectively, was carried out to obtain n-hexane, ethyl acetate, and n-butanol fractions. After performing column chromatography several times on the ethyl acetate fraction, four pure compounds were isolated: lupeol (**1**), 11-oxo-α-amyrin acetate (**2**), ursolic acid (3), and 3-α-O-acetyl betulinic acid (**4**). Their chemical structures were determined based on the analysis of 1D, 2D-NMR nuclear magnetic resonance spectroscopy data, combined with reference comparison. This is the first time that the isolation and structural determination of these compounds have been published.*

Keywords: Ha thu o trang*;* leaves and stems vines; *Streptocaulon juventas*

1. Introduction

The genus *Streptocaulon* belongs to the family of Asclepiadaceae, consisting of five species, distributed throughout Southeast Asia, including *S. juventas* (Lour.) Merr., *S. griffithii* Hook. f., *S. horsfieldii* Miq., *S. kleinii* W. Arn., and *S. Wallichii* Wight. Vietnam has only three species (Pham, 1999), including *S. juventas* (Lour.) Merr., *S. horsfieldii* Miq. (Vo, 1996), and *S. kleinii* W. Arn (Do, 2004). *S. juventas* is a creeping plant, 2 to 5 m long,

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with a reddish or reddish-brown trunk and branches, with many hairs. The leaves of *S. juventas* are opposite, ovate-shaped, rounded or slightly conical at the base, dark green above, hairless, pale white below, and covered with fine hair. Leaf-blade about 8 to 14 cm long, about 4 to 9 cm wide, petioles short, hairy (Vo, 1996). Flowers are light brown or purplish-yellow, growing in cymes. The fruit is large, split horizontally to look like an ox's horn, globose, gray, about 7 to 11 cm long, about 8 mm wide. Seeds flattened, dorsal, about 5 to 7 mm long, about 2 mm wide, with fine tufts, about 2 cm long (Do et al., 2004).

In this paper, we describe the isolation and structural elucidation of four compounds by 1D, 2D-NMR spectroscopy, compared with data from previous studies.

2. Experiment

2.1. Experimental methods

Column chromatography was carried out using silica gel 60, 0.06-0.2 mm (Scharlau, Barcelona) and LiChroprep® RP-18, 40−63 μm (Merck KGaA, Darmstadt). Analytical and TLC were carried out on precoated Kieselgel $60F_{254}$ or RP_{18} plates (Merck KGaA, Darmstadt). The NMR spectra were measured on a Bruker AM500 FT-NMR (500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR) spectrometer with TMS as the internal standard. Chemical shifts are displayed in units of δ (ppm), and the coupling constants are expressed in hertz.

2.2. Plants

The leaves and stems vines of *S. juventas* (Lour.) Merr. was collected in An Phu commune, Tinh Bien district, An Giang province, Vietnam in April 2018 and identified by traditional medicine physician Nguyen Thien Chung, chairman of the Oriental Medicine Association of Tinh Bien district, An Giang province. A voucher specimen (HAO2018-HTO) was deposited at the Department of Organic Chemistry, Faculty of Chemistry, Ho Chi Minh City University of Education.

2.3. Extraction and isolation

The leaves and stems of *S. juventas* vines were collected, pooled, then washed, dried, and ground into a powder to obtain 20 kg. This powder was extracted with methanol (80 L, reflux, three h × 3) to obtain 950 g of the methanol extract residue. This residue was suspended in H_2O and successively partitioned with *n*-hexane (30.0 L), ethyl acetate (25.0 L), and *n*-butanol saturated with water (20.0 L) to extract residues of 405 g, 105 g, and 150 g, respectively, and the rest is water extract. The water extract was heated to dryness, resulting in a residue weighing 95 g. Using 100 g ethyl acetate residue chromatographed on a silica gel column eluting with gradient solvent of *n*-hexane-EtOAc (49:1, 19:1, 9:1, 8:2, 7:3, 5:5, 3:7, 2:8, EtOAc), then eluting with gradient solvent of EtOAc-MeOH (19:1, 9:1, 8:2) to obtain twelve fractions, named S.J.EA.1-S.J.EA.12. The S.J.EA.2 fraction (2.05 g) was chromatographed on a silica gel column eluting with *n*-hexane-EtOAc (49:1, 19:1, 9:1, 8:2) to obtain four subfractions, S.J.EA.2.1-S.J.EA.2.3. The S.J.EA.2.2 subfraction was

chromatographed on an RP-18 column eluting with H_2O-Me_2CO (1:1, 1:2, 1:4, 1:8, 1:12) to obtain compounds **1** (4.0 mg). The S.J.EA.2.3 subfraction was chromatographed on an RP-18 column eluting with H_2O-Me_2CO (1:1, 1:2, 1:4, 1:8) to obtain compounds **2** (4.5 mg). The S.J.EA.4 fraction (4.25 g) was chromatographed on a silica gel column eluting with *n*-hexane-EtOAc (49:1, 19:1, 9:1, 8:2, 7:3, 5:5) to obtain six subfractions, S.J.EA.3.1-S.J.EA.3.6. The S.J.EA.3.4 subfraction was chromatographed on an RP-18 column eluting with H_2O-Me_2CO (2:1, 1:1, 1:2, 1:4, 1:8) to obtain compound **3** (5.0 mg) and **4** (3.5 mg).

2.4. Spectroscopic data

Lupeol (**1**): White amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 3.20 (dd, 1H, J = 12.0, 4.8 Hz, H-3), 0.97 (s, 3H, H-23), 0.76 (s, 3H, H-24), 0.83 (s, 3H, H-25), 1.03 (s, 3H, H-26), 0.94 (s, 3H, H-27), 0.79 (s, 3H, H-28), 4.69 (d, 1H, J = 2.4, Hz, H-29$_a$), 4.57 (dd, 1H, J = 2.4, 1.8 Hz, H-29$_b$), 1.66 (s, 3H, H-30); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 38.9 (C-1); 27.4 (C-2); 79.0 (C-3); 38.7 (C-4); 55.3 (C-5); 18.0 (C-6); 34.3 (C-7); 40.9 (C-8); 50.5 (C-9); 37.2 (C-10); 21.0 (C-11); 25.2 (C-12); 38.1 (C-13); 42.9 (C-14); 27.4 (C-15); 35.6 (C-16); 48.0 (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); 14.6 (C-27); 18.3 (C-28); 109.3 (C-29); 19.3 (C-30).

11-Oxo-α-amyrin acetate (**2**): White amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 4.52 (dd, 1H, J = 12.0, 4.5 Hz, H-3), 5.54 (s, 1H, H-12); 0.88 (s, 3H, H-23); 0.89 (s, 3H, H-24); 1.19 (s, 3H, H-25); 1.17 (s, 3H, H-26); 1.29 (s, 3H, H-27); 0.82 (s, 3H, H-28); 0.81 (d, J = 2.0, H-29); 0.95 (d, J = 6.5, 3H, H-30); 2.05 (s, $\underline{C}$OOMe); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 38.9 (C-1); 23.6 (C-2); 80.7 (C-3); 38.0 (C-4); 55.0 (C-5); 17.5 (C-6); 32.8 (C-7); 45.1 (C-8); 61.5 (C-9); 36.8 (C-10); 199.7 (C-11); 130.4 (C-12); 165.0 (C-13); 43.7 (C-14); 27.2 (C-15); 40.9 (C-16); 33.9 (C-17); 59.0 (C18-); 39.2 (C-19); 39.3 (C-20); 30.9 (C-21); 27.5 (C-22); 28.1 (C-23); 16.7 (C-24); 18.5 (C-25); 16.6 (C-26); 20.5 (C-27); 28.8 (C-28); 17.5 (C29-); 20.5 (C-30); 171.0 ($\underline{C}OOCH_3$); 21.1 ($COO\underline{C}H_3$).

Ursolic acid (**3**): White amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 3.22 (dd, 1H, J = 11.4, 4.8 Hz, H-3), 5.26 (t, 1H, J = 3.3 Hz, H-12), 2.02 (2H, *m*, H-16), 2.22 (d, 1H, J = 11.4 Hz, H-18), 1.31 (1H, m, H-19), 0.95 (1H, m, H-20), 0.99 (3H, s, H-23), 0.78 (3H, s, H-24), 0.93 (3H, s, H-25), 0.79 (3H, s, H-26), 1.09 (3H, s, H-27), 0.86 (3H, d, J = 6.6 Hz, H-29), 0.94 (d, J = 6.5 3H, s, H-30); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 38.9 (C-1); 28.2 (C-2); 78.0 (C-3); 38.8 (C-4); 55.2 (C-5); 18.3 (C-6); 33.2 (C-7); 39.7 (C-8); 47.9 (C-9); 37.2 (C-10); 23.7 (C-11); 125.9 (C-12); 138.0 (C-13); 42.1 (C-14); 30.8 (C-15); 24.3 (C-16); 48.0 (C-17); 53.0 (C-18); 39.0 (C-19); 39.0 (C-20); 31.1 (C-21); 37.0 (C-22); 28.5 (C-23); 15.8 (C-24); 15.8 (C-25); 17.0 (C-26); 23.7 (C-27); 181.5 (C-28); 17.0 (C-29); 21.2 (C-30).

3-α-O-acetyl betulinic acid (**4**): White amorphous solid; ^{1}H NMR (500 MHz, $CDCl_3$, δ ppm): 4.26 (1H, brs, H-3), 2.2 (td, J=12.0, 3.6 Hz, H-13), 1.65 (t, J= 11.4 Hz H-18), 3.00

(m, H-19), 0.83 (3H, s, H-23), 0.86 (3H, s, H-24), 0.85 (3H, s, H-25), 0.95 (3H, s, H-26), 1.03 (3H, s, H-27), 4.74 (d, 1H, J=1.8 Hz, H-29$_a$), 4.61 (d, 1H, J=2.4 Hz, H-29$_b$), 1.70 (3H, s, H-30); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm): 33.9 (C-1); 22.9 (C-2); 78.4 (C-3); 36.7 (C-4); 50.2 (C-5); 18.1 (C-6); 34.1 (C-7); 40.9 (C-8); 50.3 (C-9); 37.1 (C-10); 20.7 (C-11); 25.5 (C-12); 38.4 (C-13); 42.5 (C-14); 29.7 (C-15); 32.2 (C-16); 56.5 (C-17); 49.3 (C-18); 46.9 (C-19); 150.4 (C-20); 30.6 (C-21); 37.2 (C-22); 27.82 (C-23); 21.7 (C-24); 15.91 (C-25); 16.06 (C-26); 14.94 (C-27); 182.2 (C-28); 109.7 (C-29); 19.4 (C-30); 170.8 (-**C**OOCH$_3$); 21.4 (-COO**C**H$_3$).

3. Results and discussion

From the EtOAc residues of the leaves of *S. Juventas* performed by column chromatography with different stationary phase adsorbents (silica gel, TLC) and using suitable solvent systems, four compounds (**1**–**4**) were isolated. The chemical structures of these compounds were determined by spectroscopic methods.

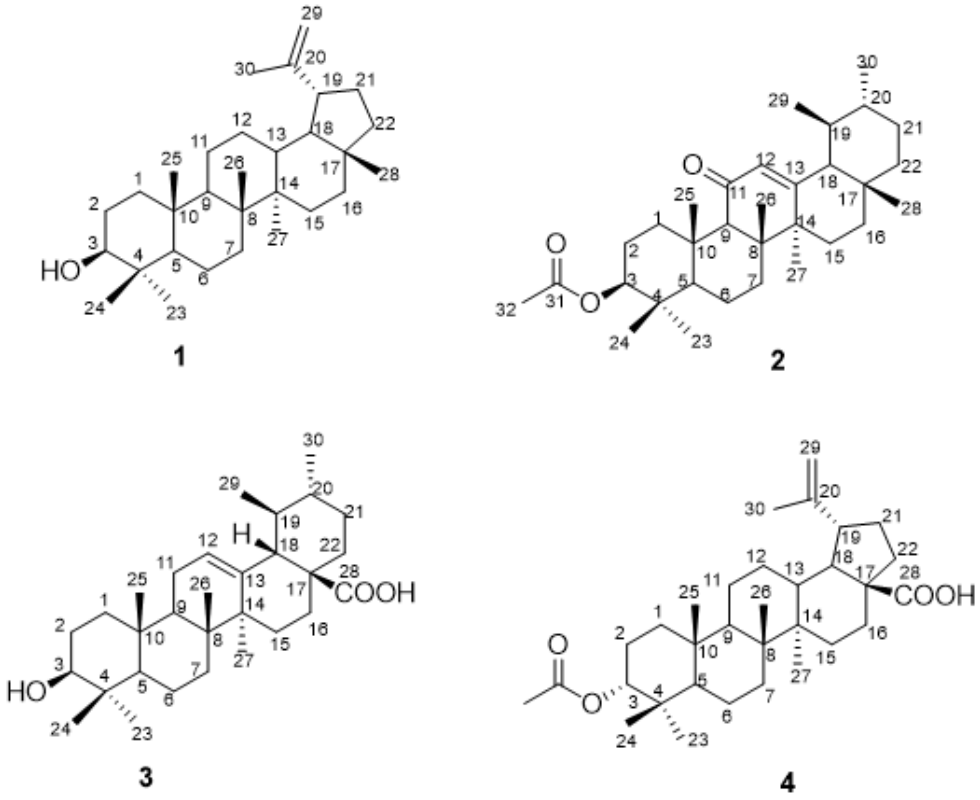

Figure 1. *Chemical structure of isolated compounds*

The ^{1}H-NMR spectrum of compound **1** shows the resonance signal of seven methyl groups at 0.97 (s, 3H, H-23), 0.76 (s, 3H, H-24), 0.83 (s, 3H, H-25), 1.03 (s, 3H, H-26), 0.94 (s, 3H, H-27), 0.79 (s, 3H, H-28), and 1.66 (s, 3H, H-30). The proton carbinol attaches at 3.20 (dd, 1H, J = 12.0, 4.8 Hz, H-3). The coupling constant J =12.0 Hz shows β orientation of hydroxyl at C-3. In addition, there are two protons of olefin at 4.69 (d, 1H, J = 2.4, Hz,

H-29$_a$) and 4.57 (dd, 1H, J = 2.4, 1.8 Hz, H-29$_b$). The other methylene and methine protons resonance signals show between 1.20 and 1.90. The ^{13}C-NMR spectrum of compound **1** showed the presence of 30 carbons in the range 15.0 to 151.0 ppm, including seven methyl groups, ten methylene groups, six methine groups, including one oxygenated methine group at 79.0 (C–3), six quaternary carbons including one carbon olefin at 151.0 (C-20), the other quaternary carbons displaying between 37.0 and 48.0, and a carbon terminal olefin at 109.3 (C-29). From the data of NMR spectra and references (Ragasa et al., 2015), compound **1** is predicted to be a lupane skeleton triterpenoid. The HMBC spectrum shows a correlation between proton 4.69 (d, 1H, J = 2.4, Hz, H-29$_a$) and 4.57 (dd, 1H, J = 2.4, 1.8 Hz, H-29$_b$) with carbon 48.0 (C–19) confirming a propenyl group attaching at C-19. The other HMBC correlations are shown in *Figure 2*. From the above spectral data combined with data from previous studies (Ragasa et al., 2015), the structure of **1** is suggested to be lupeol.

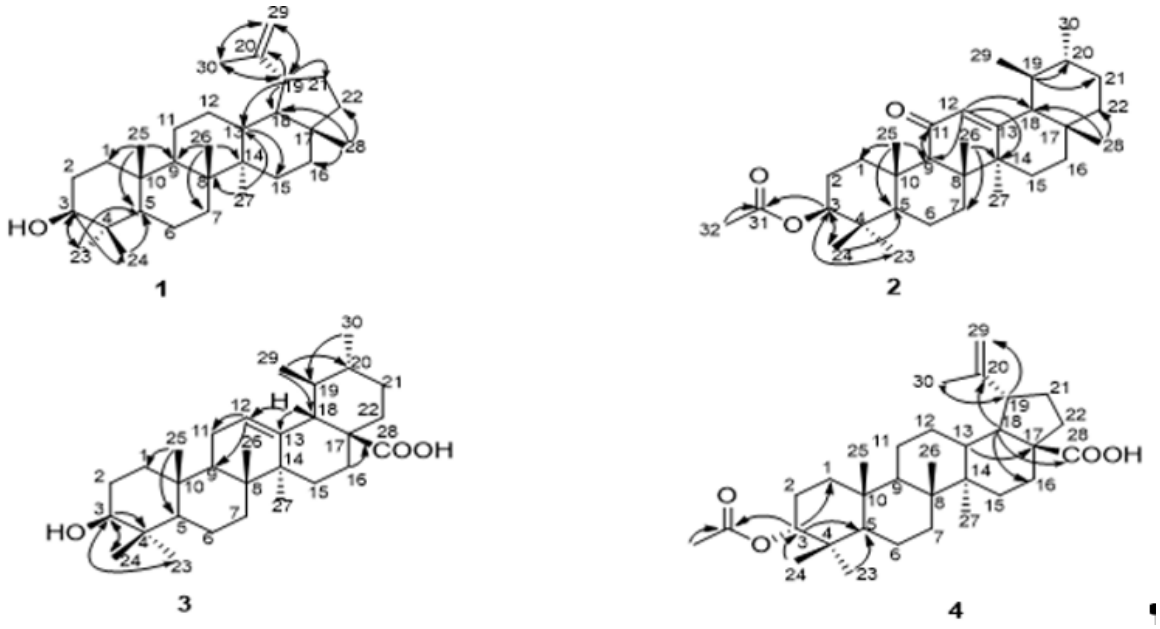

Figure 2. *The HMBC correlations of 1-4*

The ^{1}H-NMR spectrum of compound **2** shows a resonance signal of eight methyl groups at 0.88 (s, 3H, H-23), 0.89 (s, 3H, H-24), 1.19 (s, 3H, H-25), 1.17 (s, 3H, H-26), 1.29 (s, 3H, H-27), 0.82 (s, 3H, H-28), 0.81 (d, J = 2.0, H-29), and 0.95 (d, J = 6.5, 3H, H-30), an acetoxy group at 2.05 (s, 3H, -COOCH$_3$) and a methine olefin at 5.54 (s, 1H, H-12). The proton carbinol attaches at 4.52 (dd, 1H, J = 12.0, 4.5 Hz, H-3), showing β orientation of hydroxyl at C-3. The other methylene and methine protons resonance signals show between 1.10 and 2.80. The ^{13}C-NMR spectrum of compound **2** shows the presence of 32 carbons in the range 16.6 to 171.0 ppm, including eight methyl groups, eight methylene groups, an acetoxy group at 21.1 (-COO**C**H$_3$) and 171.0 (-**C**OOCH$_3$), a carbon carbonyl at 199.7 (C–11), six methine groups, including one oxygenated methine group at 80.7 (C–3), six quaternary carbons, including one methine carbon at 165.0 (C-13). The HMBC spectrum

shows a correlation between proton 4.52 (H–3) and carbon 171.0 (-**C**$OOCH_3$), confirming that the acetoxy group attaches at C-3. At the same time, the HMBC correlation between proton 2.35 (H–9) and carbonyl carbon 199.7 (C-11) shows the presence of a carbonyl carbon at position C-11. The other significant HMBC correlations are shown in Fig. 2. From the above spectral data combined with data from previous studies (Fingolo et al., 2013), the structure of **2** is suggested to be 11-oxo-α-amyrin acetate.

The ^{1}H-NMR spectrum of compound **3** shows the resonance signal of seven methyl groups at 0.99 (3H, s, H-23), 0.78 (3H, s, H-24), 0.93 (3H, s, H-25), 0.79 (3H, s, H-26), 1.09 (3H, s, H-27), 0.86 (3H, d, J = 6.6 Hz, H-29), and 0.94 (d, J = 6.5, 3H, H-30). The proton carbinol attaches at 3.22 (dd, 1H, J = 11.4, 4.8 Hz, H-3), presenting β orientation of hydroxyl at C-3. In addition, there is a methine olefin at 5.26 (t, 1H, J = 3.3 Hz, H-12). The other methylene and methine protons resonance signals are between 1.20 and 2.40. The ^{13}C-NMR spectrum of compound **3** shows the presence of 30 carbons in the range 15.0 to 182.0 ppm, including seven methyl groups, nine methylene groups, six methine groups, including one oxygenated methine group at 78.0 (C–3), six quaternary carbons including one carbon olefin at 138.0 (C-13), a carboxyl carbon at 181.5 (C–28). The HMBC spectrum shows a correlation between the proton at 5.26 (t, 1H, J = 3.3 Hz, H-12) with carbon 47.9 (C–9) and 23.7 (C–11), confirming that proton H-12 attaches at C-12. The position of H-18 is confirmed by attaching C-18 by the correlation between the proton at 2.22 (d, 1H, J = 11.4 Hz, H-18) with carbon 125.9 (C–12) and 138.0 (C–13). The carboxyl group (C-18) is confirmed by attaching C-17 by the correlation between the proton at 2.02 (2H, *m*, H-16) with carbon 181,5 (C–28). The other HMBC correlations are shown in Fig. 2. From the above spectral data combined with data from previous studies (Annan et al., 2011), the structure of **3** is suggested to be ursolic acid.

The ^{1}H-NMR spectrum of compound **4** shows the resonance signal of six methyl groups at 0.83 (3H, s, H-23), 0.86 (3H, s, H-24), 0.85 (3H, s, H-25), 0.95 (3H, s, H-26), 1.03 (3H, s, H-27), 1.70 (3H, s, H-30). The proton carbinol attaches at 4.26 (1H, brs, H-3). In addition, there are two protons of olefin at 4.74 (d, 1H, J=1.8 Hz, H-29$_a$) and 4.61 (d, 1H, J=2.4 Hz, H-29$_b$). The other methylene and methine protons resonance signals are between 1.10 and 2.20. Besides, there is an acetoxy group at 2.07 (s, 3H, -$COOCH_3$). The ^{13}C-NMR spectrum of compound **4** shows the presence of 32 carbons in the range 15.0 to 183.0 ppm, including six methyl groups, ten methylene groups, six methine groups, including one oxygenated methine group at 78.4 (C–3), six quaternary carbons including one carbon olefin at 150.4 (C-20), a carbon terminal olefin at 109.7 (C-29). In addition, there is an acetoxy group at 21.4 (-COO**C**H_3) and 170.8 (-**C**$OOCH_3$) and a carbon carboxyl at 182.8 (C–18). The HMBC spectrum shows a correlation between the proton at 1.70 (3H, s, H-30) with carbon at 46.9 (C–19), the correlation between the proton at 3.00 (m, H-19) with carbon at 109.7 (C-29) confirms that a propenyl group attaches at C-19. The HMBC spectrum shows

a correlation between proton 4.62 (H–3) with carbon 170.8 (-**C**OOCH$_3$), confirming that the acetoxy group attaches at C-3. The α-orientation of hydroxyl at C-3 is confirmed by chemical shifts at C-3. According to Kitajima et al. (1989) and Wang et al., (2014), the configuration at 3β-OH of 3β-acetoxy betulinic acid if chemical shifts at C-3 is 81.2 ppm (solvent $CDCl_3$). In contrast, the configuration at 3α-OH of 3α-acetoxy betulinic acid is 78.6 ppm (solvent $CDCl_3$) (Wang et al., 2014). Therefore, the structure of **4** is suggested to be 3α-acetoxy betulinic acid.

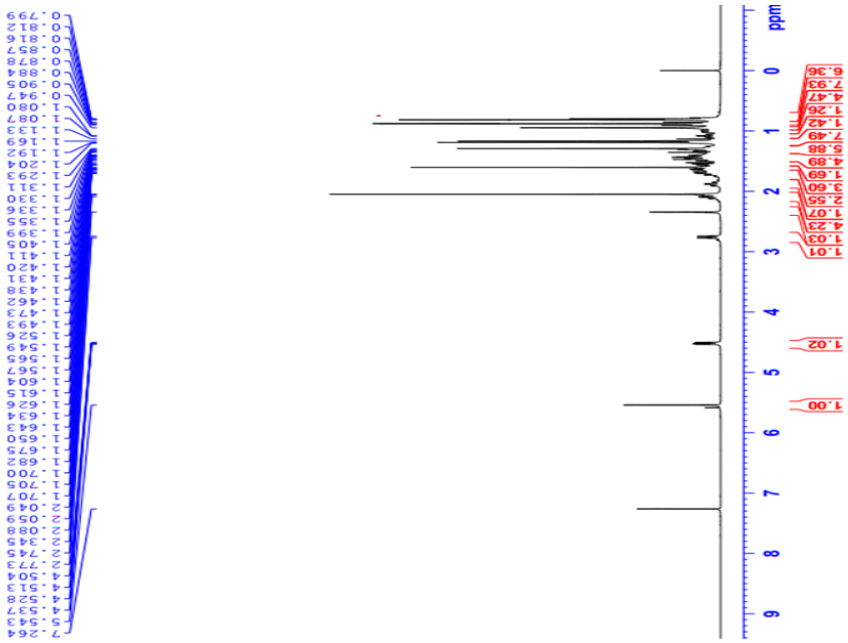

Figure 3. *^{1}H NMR spectra of* ***2***

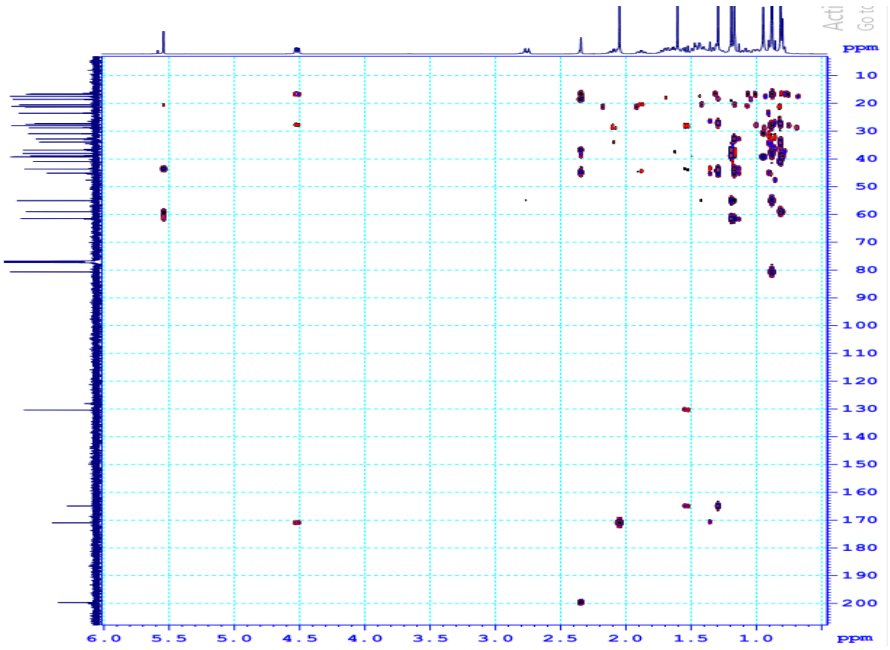

Figure 4. *^{13}C NMR spectra of* ***2***

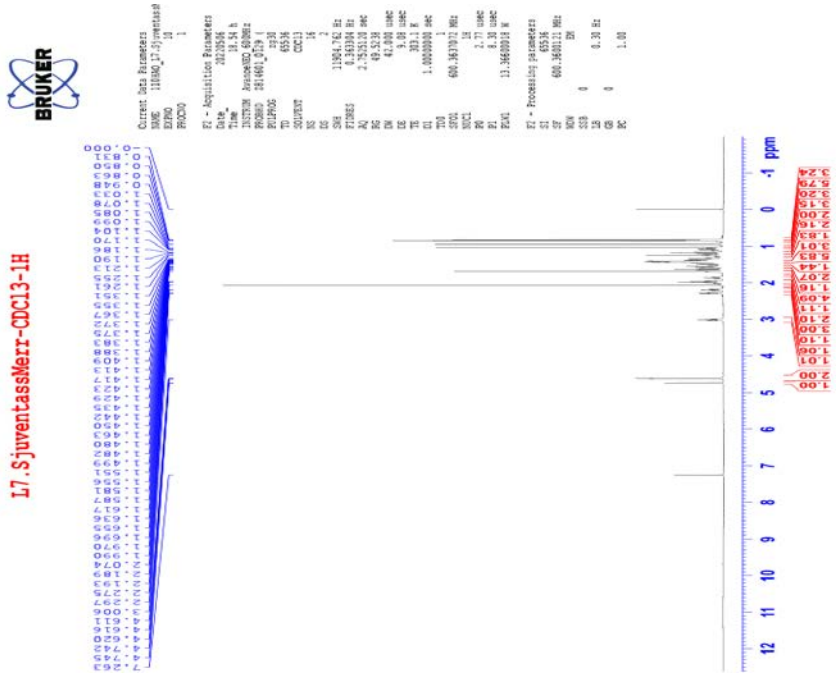

Figure 5. *^{1}H NMR spectra of* ***4***

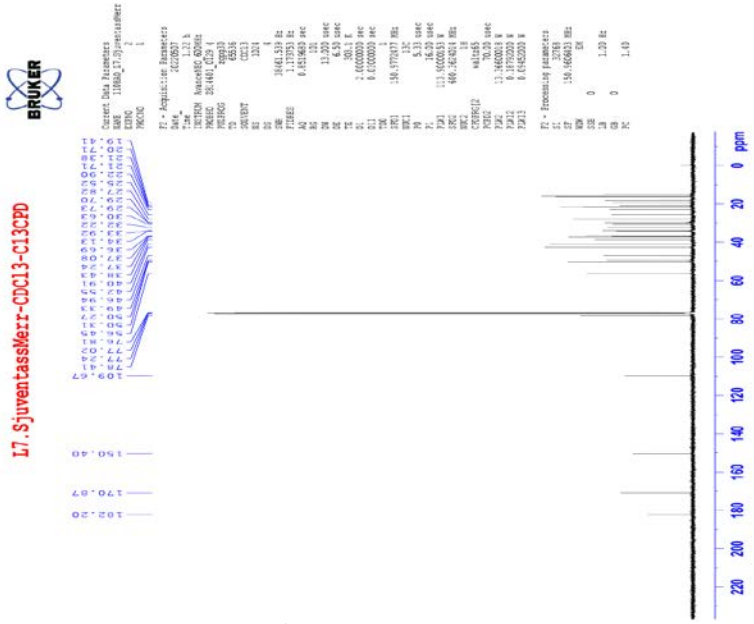

Figure 6. *^{13}C NMR spectra of* ***4***

4. Conclusion

From the ethyl acetate extract of the leaves and stems of *S. juventas* vines collected at An Phu commune, Tinh Bien District, An Giang Province, Vietnam, four pure compounds were isolated. This is the first time that the structures of these compounds were reported.

❖ ***Conflict of Interest: Authors have no conflict of interest to declare.***

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NGHIÊN CỨU THÀNH PHẦN HÓA HỌC CỦA LÁ VÀ THÂN DÂY LEO CỦA *STREPTOCAULON JUVENTAS*

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TÓM TẮT

*Hà thủ ô trắng có tên khoa học là **Streptocaulon juventas**. Theo y học cổ truyền, Hà thủ ô trắng có vị đắng, chát, mát, có tác dụng bổ máu, gan, thận. Tại Việt Nam, Hà thủ ô trắng đã được biết đến từ lâu và được sử dụng rộng rãi trong y học dân gian để chữa rắn cắn, tóc bạc sớm, thanh nhiệt, giải độc, sưng đau. Nó được sử dụng như một loại thuốc bổ để điều trị trầm cảm, và các bệnh thần kinh, tăng cường xương, sống lâu, và làm đen tóc và râu. Các nghiên cứu trước đây chỉ tập trung vào thành phần hóa học của rễ cây Hà thủ ô trắng, cho thấy glycoside cardenolide là thành phần chính. Trong nghiên cứu này, mẫu bột khô của lá, thân dây leo của S. Juventas được thu hái và gộp chung tại huyện Tịnh Biên, tỉnh An Giang và chiết xuất nóng bằng dung môi methanol thu được dịch chiết methanol. Tiến hành phân tán hoàn toàn cao metanol vào nước và chiết lỏng với dung môi n-hexane, ethyl acetate và n-butanol, thu được các phân đoạn n-hexane, ethyl acetate và n-butanol tương ứng. Thực hiện sắc kí cột nhiều lần trên phần ethyl acetate, bốn hợp chất tinh khiết đã được phân lập, tên là lupeol (**1**), 11-oxo-α-amyrin acetate (**2**), ursolic acid (3), 3-α-O-acetyl betulinic acid (**4**). Cấu trúc hóa học của chúng được xác định dựa trên phân tích dữ liệu phổ cộng hưởng từ hạt nhân 1D, 2D-NMR, kết hợp với so sánh tham khảo. Đây là lần đầu tiên sự cô lập và xác định cấu trúc của các hợp chất này được công bố.*

Từ khóa: Hà thủ ô trắng; lá và thân dây leo; *Streptocaulon juventas*