ISOLATED COMPOUNDS FROM AERIAL PARTS OF *ABELMOSCHUS SAGITTIFOLIUS*

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Abstract: *Abelmoschus sagittifolius is a species belonging to the Malvaceae family. According to traditional medicine, the root tuber of Abelmoschus sagittifolius was used to treat diseases such as cough, constipation, neurasthenia, malnutrition, boils, back pain, and stomach pain. In this study, from Abelmoschus sagittifolius aerial parts collected in Vinh Hung commune, Vinh Loc district, Thanh Hoa province, three compounds were isolated including sitostenone (1), tiliroside (2) and kaempferin (3). The structures of these compounds were identified by NMR, MS spectroscopic data and comparison with the reported literatures.*

Keywords: *Abelmoschus sagittifolius, isolation, sitostenone, tiliroside, kaempferin.*

1. Introduction

Abelmoschus sagittifolius (*A. sagittifolius*) belongs to the *Abelmoschus* genus, Malvaceae family. There are about 7 species of this genus in the world in which *A. sagittifolius* is found in Hainan island and some countries in Southeast Asia [1]. According to folk experience, this plant was used as a healthy food to treat diseases such as cough, constipation, neurasthenia, malnutrition, boils, back pain, dizziness and stomach pain [2,3]. Despite being used for a long time, there have been few studies on the phytochemistry of *A. sagittifolius*. Some studies show that the roots of *A. sagittifolius* contain a lot of mucilage and starch [4,5]. The qualitative study of the roots of *A. sagittifolius* in Bac Lieu province showed phytosterols, coumarins, fatty acids, organic acids, amino acids, reducing sugars, and uronic compounds [6]. A study about *A. sagittifolius* plants collected in Ha Trung, Thanh Hoa obtained 5 substances including ventricosin A, 4(15)-eudesmene-11-on, tagitinin A, *β*-sitosterol and daucosterol [7]. Recently, Chinese scientists isolated a number of compounds from the roots of *A. sagittifolius* in Hainan island, including cadinan sesquiterpenes, lignans and amide derivatives [8] [9]. In this study, we report our isolation of 3 compounds including sitostenone (**1**), tiliroside (**2**) and kaempferin (**3**) from aerial parts of *A. sagittifolius* collected at Vinh Loc district, Thanh Hoa province.

2. Materials and Methods

2.1. Plant materials

The plant samples were collected in Bao mountain area, Vinh Hung commune, Vinh Loc district, Thanh Hoa province in October 2020 and were identified by Prof. Dr.

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Tran The Bach (Institute of Ecology and Biological Resources) with the scientific name as *Abelmoschus sagittifolius* (Kurz) Merr., belonging to the Malvaceae family. Plant voucher (SB-2020) was deposited at the Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology.

2.2. General Experimental Procedures

The electrospray ionization mass spectra (ESI-MS) were obtained on an Agilent 1260 series single quadrupole LC/MS system. Column chromatography (CC) was performed on silica gel (Merck, 230-400 mesh) or Sephadex LH-20. Thin layer chromatography used precoated silica gel plates (Merck 60 F_{254}). Compounds were visualized by spraying with Ce-Mo stain. The ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were recorded by a Bruker AM500 FT-NMR spectrometer using TMS as an internal standard.

2.3. Extraction and isolation

The aerial parts (stems and leaves) of *A. sagittifolius* were dried and ground to obtain 4 kg of dried material. The plant sample was extracted with MeOH (20 L x 4 times) at room temperature for 24 h. The extracts were combined and MeOH solvent was removed under reduced pressure to obtain MeOH residue. The MeOH residue was added with water (1 L) and extracted consecutivly with n-hexane and EtOAc, to obtain the *n*-hexane extract (48 g) and the EtOAc extract (8 g).

The *n*-hexane extract residue (48 g) was separated by column chromatography on normal phase silica gel with a gradient elution system of *n*-hexane/EtOAc (20/1), n-hexane/EtOAc (15/1), *n*-hexane/EtOAc (10/1), *n*-hexane/EtOAc (5/1) and EtOAc to obtained 5 fractions (H1-H5). The H2 fraction (6.8 g) was subjected to column chromatography on normal-phase silica gel with the eluent system *n*-hexane/EtOAc (10/1) to obtain compound **1** (13.6 mg).

The EtOAc extract (8 g) was submitted to a normal phase silica gel chromatography column and eluted with *n*-hexane/EtOAc gradient solvent system (from 30% to 100% ethyl acetate), the fractions were checked by thin layer chromatography to obtain 7 fractions (E1- E7).

The E4 fraction (0.96 g) was purified by normal phase silica gel column chromatography with the eluent system CH_2Cl_2/MeOH (10/1) to obtain three sub-fractions labeled E4.1 to E4.3. The fraction E4.2 (0.52 g) was purified through reverse phase silica gel column with eluent system MeOH/H_2O (1/1.5) to obtain compound **2** (6.4 mg).

The E6 fraction (1.68 g) was purified by normal phase silica gel column chromatography with the eluent system CH_2Cl_2/EtOAc (4/1) to get two sub-fractions E6.1 and E6.2. compound **3** (12 mg) was obtained from the sub-fraction E6.2 (0.92 g) by recrystallization.

2.4. Properties and spectral values of the isolated compounds

Sitostenone (**1**): White solid, ESI-MS *m/z*: 413 $[M+H]^+$. Molecular formula $C_{29}H_{48}O$ (M = 412). ^{1}H-NMR (500 MHz, $CDCl_3$): δ (ppm) 5.71 (1H, s, H-4), 1.17 (3H, s, H-19), 0.91 (3H, d, *J* = 6.5 Hz, H-21), 0.84 (3H, t, *J* = 7.5 Hz, H-29), 0.83 (3H, d, *J* = 7.0 Hz, H-27), 0.81 (3H, d, *J* = 7.0 Hz, H-26), 0.70 (3H, s, H-18). ^{13}C-NMR (125 MHz, $CDCl_3$): δ (ppm) 199.6 (C-3), 171.6 (C-5), 123.7 (C-4), 56.0 (C-14), 55.9 (C-17), 53.8 (C-9), 45.8 (C-24), 42.4 (C-13), 39.6 (C-12), 38.6 (C-10), 36.1 (C-20), 35.7 (C-1), 35.6 (C-8), 33.9 (C-2), 33.9 (C-22), 32.9 (C-6), 32.0 (C-7), 29.1 (C-25), 28.1 (C-16), 26.1 (C-23), 24.1 (C-15), 23.0 (C-28), 21.0 (C-11), 19.8 (C-26), 19.0 (C-27), 18.7 (C-21), 17.3 (C-19), 11.9 (C-29), 11.9 (C-18).

Tiliroside (**2**): Light brown solid, ESI-MS *m/z*: 595 $[M+H]^+$. Molecular formula $C_{30}H_{26}O_{13}$ (M = 594). ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) 7.99 (2H, d, *J* = 8.5 Hz, H-2',6'), 7.41 (1H, d, *J* = 16.0 Hz, H-7'''), 7.32 (2H, d, *J* = 8.5 Hz, H-2''',6'''), 6.83 (2H, d, *J* = 8.5 Hz, H-3',5'), 6.81 (2H, d, *J* = 8.5 Hz, H-3''',5'''), 6.29 (1H, s, H-8), 6.14 (1H, s, H-6), 6.10 (1H, d, *J* = 16.0 Hz, H-8'''), 5.23 (1H, d, *J* = 7.0 Hz, H-1''), 4.32 (1H, dd, *J* = 12.0, 2.0 Hz, H-6''), 4.20 (1H, dd, *J* = 12.0, 6.5 Hz, H-6''), 3.51~3.47 (3H, m), 3.36 (1H, m). ^{13}C-NMR (125 MHz, CD_3OD): δ (ppm) 179.2 (C-4), 168.8 (C-9'''), 166.9 (C-7), 163.0 (C-5), 161.5 (C-4'), 161.2 (C-4'''), 159.2 (C-2), 158.4 (C-9), 146.6 (C-7'''), 135.4 (C-3), 132.2 (C-2',6'), 131.2 (C-2''',6'''), 127.7 (C-1'''), 122.8 (C-1'), 116.8 (C-3''',5'''), 116.0 (C-3',5'), 114.7 (C-8'''), 105.4 (C-10), 104.1 (C-1''), 99.1 (C-8), 94.9 (C-6), 78.0 (C-2''), 75.8 (C-5''), 75.7 (C-3''), 71.7 (C-4''), 64.3 (C-6'').

Kaempferin (**3**): pale yellow solid, ESI-MS *m/z*: 433 $[M+H]^+$. Molecular formula $C_{21}H_{20}O_{10}$ (M = 432). ^{1}H-NMR (500 MHz, CD_3OD): δ (ppm) 7.78 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.95 (2H, d, *J* = 8.5 Hz, H-3', H-5'), 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.23 (1H, d, *J* = 2.0 Hz, H-6), 5.39 (1H, d, *J* = 2.0 Hz, H-1''), 4.24 (1H, m, H-2''), 3.73 (1H, m, H-3''), 3.36-3.33 (2H, m, H-4'', H-5''), 0.94 (1H, d, J = 5.5 Hz, H-6''). ^{13}C-NMR (125 MHz, CD_3OD): δ (ppm) 179.6 (C-4), 165.9 (C-7), 163.1 (C-4'), 161.6 (C-5), 159.3 (C-9), 158.6 (C-2), 136.2 (C-3), 131.9 (C-2', C-6'), 122.7 (C-1'), 116.5 (C-3', C-5'), 105.9 (C-10), 103.5 (C-1''), 99.8 (C-6), 94.7 (C-8), 73.2 (C-2''), 72.1 (C-3''), 72.0 (C-4''), 71.9 (C-5''), 17.6 (C-6'').

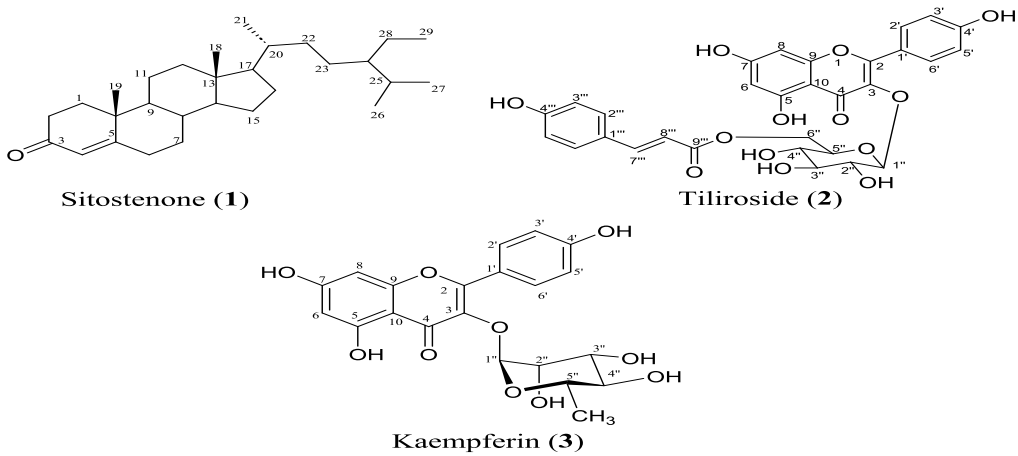

Fig.1**. Chemical structures of isolated compounds **1-3

3. Results and Discussion

3.1. Determination of the structure of sitostenone (1)

Compound **1** was obtained as a white solid. The ESI-MS spectrum of compound **1** showed a protonated molecular ion peak at *m/z* 413 $[M+H]^+$, corresponding to the molecular formula $C_{29}H_{48}O$ (M = 412). The ^{1}H-NMR spectrum shows the characteristic of sterol compounds with signals of 6 methyl groups at different position δ_H 1.17 (3H, s, H-19); 0.91 (3H, d, *J*=6.5 Hz, H-21); 0.84 (3H, t, *J*=7.5 Hz, H-29); 0.83 (3H, d, *J*=7.0 Hz, H-27); 0.81 (3H, d, *J*=7.0 Hz, H-26) and 0.70 (3H, s, H-18). In addition, there was a proton signal of a double bond at δ_H 5.71 (1H, s, H-4). The ^{13}C-NMR, DEPT spectrum show a signal of 29 carbons, including 6 CH_3 group signals, 11 CH_2 group signals, and 8 CH group signals (in which the CH=C signal is at position 123.7 (C-4)), four quaternary C signals including a ketone group at 199.6 (C-3). The NMR spectral data suggest that the structure of this compound was sitostenone. Comparison of spectral data of this compound with published reference [10] confirmed that compound **1** was sitostenone.

3.2. Determination of the structure of tiliroside (2)

Compound **2** was obtained from the EtOAc extract as a light brown solid. The ESI-MS spectrum of compound **2** exhibited a protonated molecular ion peak at *m/z* 595 $[M+H]^+$, corresponding to the molecular formula $C_{30}H_{26}O_{13}$ (M = 594). In the ^{1}H-NMR spectrum, there are signals of flavonoid compound glysoside with signal of 2 aromatic ring protons at δ_H 6.29 (1H, s, H-8), 6.14 (1H, s, H-6), signal of 4 protons of an A_2B_2 system at δ_H 7.99 (2H, d, J = 8.5 Hz, H-2',6') and 6.83 (2H, d, J = 8.5 Hz, H-3',5'). There was also a coumaroyl moiety signal with 4 protons of an A_2B_2 system at δ_H 7.32 (2H, d, *J* = 8.5 Hz, H-2''',6''') and 6.81 (2H, d, *J* = 8.5 Hz , H-3''',5'''), 2 *trans* protons at δ_H 7.41 (1H, d, *J* = 16.0 Hz, H-7''') and 6.10 (1H, d, *J* = 16.0 Hz, H-8''). The signals of the sugar part were detected at δ_H 5.23 (1H, d, J = 7.0 Hz, H-1''), 4.32 (1H, dd, J = 12.0, 2.0 Hz, H-6''a), 4.20 (1H, dd, J = 12.0, 6.5 Hz, H-6''b), 3.51~3.47 (3H, m), 3.36 (1H, m). The ^{13}C and HSQC spectra give a signal of 30 carbons in which the signal of 9 carbons belongs to the coumaroyl group at δ_C 168.8 (C-9'''), 161.2 (C-4'''), 146.6 (C-7'''), 131.2 (C-2''',6'''), 127.7 (C-1'''), 116.8 (C-3''',5'''), 6 signals of Glc part at 104.1 (C-1''), 78.0 (C-2''), 75.8 (C-5''), 75.7 (C-3''), 71.7 (C-4'') and 64.3 (C-6''), the remaining 15 signals belong to the flavonone ring in which group C=O at 179.2 (C-4). The HMBC spectra allowed identification of the coumaroyl group bound to the sugar at the C-6 site. The Glc sugar linked to the flavononol skeleton at the C-3 site by the HMBC correlation of H-1'' (δ_H 5.23) to C-3 (δ_C 135.4). Comparison with reference [11], compound **2** was identified as tiliroside.

3.3. Determination of the structure of kaempferin (3)

Compound **3** was obtained as a pale yellow solid. The ESI-MS spectrum of **3** showed a protonated molecular ion peak at *m/z* 433 $[M+H]^+$, corresponding to the molecular formula $C_{21}H_{20}O_{10}$ (M = 433). In the ^{1}H-NMR spectrum, aromatic proton signals of an A_2B_2 system

appeared at δ_H 7.78 (2H, d, *J* = 8.5 Hz, H-2', H-6'), 6.95 (2H, d, *J* = 8.5 Hz, H-3', H-5'); 2 meta protons at δ_H 6.40 (1H, d, *J* = 2.0 Hz, H-8), 6.23 (1H, d, J = 2.0 Hz, H-6), and Rhamnose sugar signal with proton anomer at δ_H 5.39 (1H, d, *J* = 2.0 Hz, H-1"), 4.24 (1H, m, H-2"), 3.73 (1H, m, H-3"), 3.36-3.33 (2H, m , H-4", H-5"), 0.94 (1H, d, *J* = 5.5 Hz, H-6"). The ^{13}C-NMR spectrum showed signals of 21 carbons, including 15 signals belonging to the flavonoid framework and 6 signals of rhamnose at δ_C 103.5 (C-1"), 73.2 (C-2"), 72.1 (C-3"), 72.0 (C-4"), 71.9 (C-5"), 17.6 (C-6"). From the above analyzed MS and NMR data and comparison with reference [12], compound **3** was identified as kaempferin.

4. Conclusion

A phytochemical investigation of aerial parts of *A. sagittifolius* led to the isolation of three known compounds including sitostenone (**1**), tiliroside (**2**) and kaempferin (**3**). The chemical composition as well as biological activities of this species will be further studied in the near future.

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