TRITERPENOIDS ISOLATED FROM HELICTERES HIRSUTA

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Received 27/12/2019, Peer reviewed 06/01/2020, Accepted for publication 10/02/2020

ABSTRACT

Helicteres family widely distributed from southern China to India, Southeast Asia. There are seven species in Vietnam and described as traditional medicinal plants. The information about the chemical constituents of Helicteres hirsuta in Vietnam has not been fully reported. In present work, we presented the isolation and structural elucidation of four terpenoids (1-4) isolated from Helicteres hirsuta L. which collected from Binh Phuoc province. The powder of the leaves and stem were separately macerated in ethanol at room temperature. The ethanol solutions were removed solvent under reduced pressure to obtain the ethanolic extracts. The ethanolic extract was applied on the silica gel dried column and sequentially eluted with n-hexane, chloroform, ethyl acetate (EtOAc) and methanol. Basing on TLC results, the sub-extract residues were selected to isolate the pure compounds by using CC technique in combination with recrystallization technique. (1) was isolated from the chloroform residue of the stem ethanolic extract while (2) obtained from a sub-fraction by recrystallizing in a mixture of n-hexane/EtOAc (8:2). (3) was a white precipitate which obtained while eluting with EtOAc to the stem extract. The crude extract n-hexane from the leaf was separated by CC using n-hexane/EtOAc gradient elution to afford (4). Chemical structures of isolated compounds were elucidated by NMR and MS spectroscopies in comparison with the literature.

Keywords: *Helicteres; helicteres hirsuta; terpenoid; NMR and MS; chemical constitutent***.**

1. INTRODUCTION

Helicteres hirsuta L. belongs to *Helicteres* family which was used as Chinese traditional medicinal components [1]. Phenolic, triterpenoid and glucoside compounds have been isolated from *Helicteres* family, interestingly, these compounds showed cytotoxicity on cancer cell lines [2-20]. In Viet Nam, *Helicteres hirsuta* L. has been used to treat some skin diseases such as dysentery, measles. Furthermore, this plant is also used as medicine to support the treatment of liver diseases including hepatitis, cirrhosis. The extracts of *Helicteres hirsuta* L. have been prepared to evaluate the cytotoxicity on human liver cancer cell line, Hep G2. The results demonstrated that some extracts gave promising *in vitro* assays [2]. In this work, we reported the isolation and structural determination of four terpenoids from *Helicteres hirsuta* L. collected from Binh Phuoc province. The results would contribute on the elucidation of chemical constituents of this plant and give a potential development in drug for the future in Vietnam.

2. EXPERIMENT

2.1. Chemicals and analytical methods

All solvents were purchased from China, stored in 500 mL glass bottles and used without further purification. The pure compounds were purified by using thin layer chromatography-TLC (silica gel 60 F254, Merck) and column chromatography-CC (silica gel 200-400 mesh, India). The UV–detection at λ = 254 and 365 nm or H_2SO_4 (40% in EtOH) was carried out to visualize spots on TLC. Nuclear magnetic resonance (^{1}H-NMR, 500 MHz; ^{13}C-NMR, 125 MHz) spectra of isolated compounds were measured on a Bruker Avance III Spectrometer. The samples were disolved in $CDCl_3$ or DMSO-d_6 for NMR measurements. Chemical shifts are given on the δ scale with solvent peak as reference, and coupling constants (J) are expressed in Hz. Mass spectroscopy (MS) was performed on HPLC-MS Agilent 1100 Spectrometer. Melting points are measured on Kruss M5000.

2.2. Plant material

Helicteres hirsuta L. was collected from Bu Nho village, Phuoc Long district, Binh Phuoc province. The plant was confirmed by Dr. Nguyen Huu Tri, Department of Biology, Nong Lam University. Extraction and Isolation: The leaves and stem of *Helicteres hirsuta* L. were separated and washed. Materials was then dried in desiccator at 60 ^{o}C. The powder of leaves and stem were exhaustively extracted in ethanol (EtOH) by maceration at room temparature for one week. The ethanol was removed under reduced pressure to obtain the ethanolic extracts. The leaf (87.8 g) and stem (58.1 g) extracts were separately applied on the silica gel dried columns and sequentially eluted with *n*-hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc) and methanol (MeOH). The residue $CHCl_3$ (8.39 g) of the stem extract was then subjected to a silica gel column using *n*-hexane/EtOAc mixtures of increasing polarity (100:0 to 80:20) to yield Fr. HS1− HS6. Fraction HS2 (0.75 g) was further subjected to column chromatography on silica gel eluting with *n*-hexane/EtOAc (95:5) to give **1** (21.2 mg). Fraction HS5 (0.5 g) was chromatographed eluting with *n*-hexane/EtOAc (95:5→90:10) to obtain Fr. HS5.1-Fr. HS5.5. Compound **2** (17.5 mg) was purified from subfraction HS5.5 by recrystallization in a mixture of *n*-hexane/EtOH (98:2). The white precipitate was obtained by eluting with EtOAc to the stem extraction. The solid was washed several times with EtOAc (5×10 mL) to afford **3** (14.1 mg). The crude residue *n*-hexane from the leaf (10.0 g) was separated by silica gel column chromatography using *n*-hexane/EtOAc gradient elution to afford six fractions (Fr. HL1- HL6). The subfraction HL5 (0.55 g) was processed under the chromatographic conditions using a binary solvent of *n*-hexane/EtOAc (95:5) as the mobile phase to obtain **4** (5.0 mg).

3. RESULTS AND DISCUSSION

Stigmast-5-en-3β-ol and stigmast-5,22-dien-3β-ol (**1**): white needle crystal (crystallized in $CHCl_3$); R_f = 0.4 (*n*-hexane/EtOAc = 8/2, UV); mp 134-136 ^{o}C; The signals at δ 5.35; 5.16; 5.02 indicated the presence of olefinic protons. In addition, the integrals of these signals (1.0; 0.34; 0.32) indicated that **1** is a mixture including two compounds with a ratio of 2:1. The ^{13}C-NMR spectrum gives four peaks at δ 140.78; 121.68; 138.28; 129.33 which specified the chemical shifts of olefinic carbons. In comparison with the literature, **1** is assigned a mixture of stigmast-5-en-3β-ol and stigmast-5,22-dien-3β-ol with the ratio of 1:2 (Figure 1) [21]. ^{1}H-NMR (500 MHz, $CDCl_3$) δ ppm: 5.35 (2H, d, *J* = 5.0 Hz, -C**H**=), 5.16 (1H, dd, *J* = 8.5 Hz & *J* = 15.5 Hz, -C**H**=), 5.02 (1H, dd, *J* = 8.5 Hz & *J* = 15.0 Hz, -C**H**=), 3.52 (2H, m, >C**H**-OH). ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm: 140.78 (>**C**=), 138.28 (-**C**H=), 129.33 (-**C**H=), 121.68 (-**C**H=), 71.81 (>**C**H-OH).

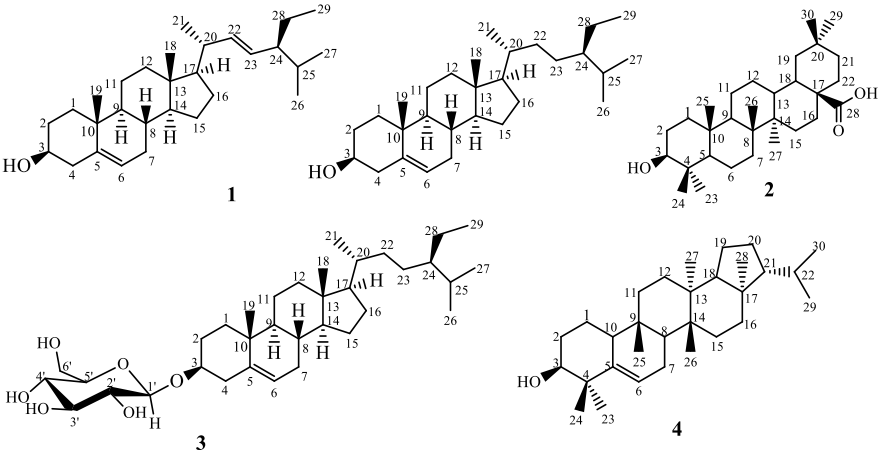

Fig. 1. *Chemical structures of (**1**-**4**) isolated from the leaves and stem of Helicteres hirsuta L.*

Oleanolic acid (**2**): white powder (crystallized in $CDCl_3$); R_f = 0.26 (*n*-hexane/EtOAc = 8/2, H_2SO_4 40% in EtOH); mp 308-310 ^oC; $C_{30}H_{48}O_3$; ESI-MS *m/z* $[M+H]^+$ 457.3, calcd for $C_{30}H_{48}O_3$, 456.7. The ^{13}C-NMR and DEPT spectra showed signals for 30 carbons, including a carbonyl group (δ 182.87), two olefinic carbons (δ 143.67, 122.67), a hydroxylated methine (δ 79.07) and seven methyl groups. The ^1H-NMR spectrum gave a signal of the olefinic proton at δ 5.22. The pseudomolecular ion peak $[M+H]^+$ was observed in positive ESI-MS, which was in agreement with the reported results on Oleanolic acid (Figure 1) [22]. ^1H-NMR (500 MHz, $CDCl_3$) δ ppm: 5.28 (1H, t, *J* = 3.5 Hz, >C=C**H**-), 3.22 (1H, dd, *J* = 4.0 Hz & *J* = 11 Hz, HO-C**H**<), 2.82 (1H, dd, *J* = 4.0 Hz & *J* = 14.0 Hz, >C=C-C**H**-), 1.13, 0.99, 0.93, 0.92, 0.90, 0.78, 0.76 (21H, s, 7-C$\mathbf{H_3}$). ^{13}C-NMR (125 MHz, $CDCl_3$) δ ppm: 182.87 (>**C**=O), 143.62 (>**C**=C), 122.67 (-**C**H=), 79.07 (>**C**-OH).

Daucosterol (**3**): white powder (crystallized in MeOH); R_f = 0.46 ($CHCl_3$/MeOH= 9/1, H_2SO_4 40% in EtOH). mp 274-277°C; $C_{35}H_{60}O_6$; ESI-MS *m/z* $[M]^+$ 576.2, calcd for $C_{35}H_{60}O_6$, 576.8. The ^1H-NMR spectra gave the signal of an olefinic proton at δ 5.32. The signal of the anomeric proton of β-D-glucopyranose was observed at δ 4.22. The C_{sp2} peaks at δ 140.45, 121.17 were found in the ^{13}C-NMR spectrum. Furthermore, the signal at δ 100.78 was identified as an anomeric carbon of β-D-glucopyranose. The positive mode ESI-MS exhibited a molecular ion at *m/z* 576.2 $[M]^+$ (calcd 576.8), corresponding to a molecular formula of $C_{35}H_{60}O_6$. These data are compatible with daucosterol, a glucoside of β-sitosterol (Figure 1) [23, 24]. ^1H-NMR (500 MHz, DMSO-d_6) δ ppm: 5.32 (1H, br, >C=C**H**-), 3.47 (1H, m, >C**H**-OH), 4.22 (1H, d, *J* = 7.5 Hz, H_{anomer}). ^{13}C-NMR (125 MHz, DMSO-d_6) δ ppm: 140.45 (>**C**=C), 121.17 (>**C**H=C), 100.78 (C_{anomer}), 71.70 (>**C**-OH).

Simiarenol (**4**): white needle crystal (crystallized in *n*-hexane). R_f = 0.40 (*n*-hexane/EtOAc = 9/1, H_2SO_4 40% in EtOH). mp 202–204 ^oC; $C_{30}H_{50}O$; ESI-MS *m/z* $[M+H]^+$ 427.1, calcd for $C_{30}H_{50}O$, 426.7. The ^1H, ^{13}C-NMR, HSQC and HMBC data of **4** were presented in Table 1. By comparison of all spectral data with the corresponding reported compound in the literature, **4** was elucidated as simiarenol (Figure 1) [19, 25].

Table 1. *^1H, ^{13}C-NMR and HMBC for **4**.*

Position	Type of Carbon	δ_C (ppm)	δ_C (ppm)*	δ_H (ppm) (*J* in Hz)	HMBC (C→H)	
					^2J	^3J
1	>CH_2	18.07	22.81			
2	>CH_2	27.79	30.07			
3	>CH-	76.35	76.93	3.47 brs		
4	>C<	40.83	41.53		H-23, 24	H-6
5	>C=	142.02	144.73			H-23, 24
6	>CH=	121.99	119.87	5.62 m	H-23	H-10
7	>CH_2	24.08	23.84		H-6	
8	>CH-	44.29	44.09			H-6, 25
9	>C<	34.85	34.65			
10	>CH-	50.28	50.69			H-6, 9
11	>CH_2	34.16	34.21			
12	>CH_2	29.02	29.01			H-27
13	>C<	38.64	38.63		H-27	
14	>C<	39.34	39.29			
15	>CH_2	29.14	29.12			
16	>CH_2	35.44	35.43			H-28

Position	Type of Carbon	δ_C (ppm)	δ_C (ppm)*	δ_H (ppm) (*J* in Hz)	HMBC (C→H)	
					^2J	^3J
17	>C<	42.82	42.81		H-28	
18	>CH-	51.76	51.75			H-27, 28
19	$>CH_2$	19.93	19.92			
20	$>CH_2$	29.31	28.32			
21	>CH-	60.07	60.06			H-28, 29, 30
22	>CH-	30.77	30.78		H29, 30	
23	$-CH_3$	25.45	22.10	1.14 s		
24	$-CH_3$	29.06	24.33	1.05 s		H-23
25	$-CH_3$	17.85	18.01	0.90 s		
26	$-CH_3$	15.40	15.77	1.01 s		
27	$-CH_3$	15.00	15.01	0.93 s		H-27
28	$-CH_3$	16.07	16.08	0.78 s		
29	-CH3	21.95	21.96	0.89 (d, *J* = 6.5)		H-30
30	$-CH_3$	22.89	22.93	0.83 (d, *J* = 6.5)		

(*) Reported data from [24]. Abbreviations: brs: broad singlet, s: singlet; d: doublet; m: multiplet

4. CONCLUSION

In conclusion, four compounds (**1**-**4**) were isolated from the leaf and stem of *Helicteres hirsuta* L. collected from Binh Phuoc province. The compounds were purified by using column chromatography. Their chemical structures were elucidated by NMR and MS techniques in comparision with the data reported in the literatures. The outcomes would elucidate of chemical constituents of this plant and enrich potentially natural candidates for drug development in Vietnam.

ACKNOWLEDGEMENTS

The authors are thankful to HCMC University of Technology and Education for supporting websites to download the scientific articles and additional equipment used in experiments.

REFERENCES

[1] Vo Van Chi, *Dictionary of Vietnamese medicinal plants*, pp. 1011-1012, Medical Publising House, 2013.
[2] Nguyen Huu Duyen, Le Thanh Phuoc, Survey of chemical composition and toxicity activity in Hep-G2 cell of *Helicteres hirsuta* L, *Can Tho University Science Journal*, 47(A), pp. 93-97, (2016).
[3] C. M. Chen, Z. T. Chen, and Y. L. Hong, A mansonone from *Helicteres angustifolia*, *Phytochemistry*, 29(3), pp. 980-982, (1990).
[4] X. D. Gou *et al*, New Triterpenoids from *Helicteres angustifolia*, *Chemical Journal of Chinese Univ1ersities*, 24(11), pp. 2022-2023, (2003).
[5] Z. T. Chen, S. W. Lee, and C. M. Chen, Cucurbitacin B 2-sulfate and cucurbitacin glucosides from the root bark of *Helicteres angustifolia*, *Chemical pharmaceutical bulletin*, 54(11), pp. 1605-1607, (2006).
[6] W. Chen *et al*, Pregnane, coumarin and lupane derivatives and cytotoxic constituents from *Helicteres angustifolia*, *Phytochemistry*, 67(10), pp. 1041-1047, (2006).
[7] M. H. Pan *et al*, Cytotoxic Triterpenoids from the Root Bark of *Helicteres angustifolia*," *Chemistry & Biodiversity*, 5(4), pp. 565-574, (2008).

[8] G. C. Wang *et al.*, Two pregnane derivatives and a quinolone alkaloid from *Helicteres angustifolia*, *Fitoterapia*, 83(8), pp. 1643-1647, (2012).
[9] X. Yin *et al*, Anti-complementary components of *Helicteres angustifolia*, *Molecules*, 21(11), 9 pages, (2016).
[10] M. F. Bean *et al*, Cucurbitacin B and isocucurbitacin B: cytotoxic components of *Helicteres isora*, *Journal of natural products*, 48(3), pp. 500-500, (1985).
[11] T. Satake *et al.*, Studies on the Constituents of Fruits of *Helicteres isora* L, *Chemical Pharmaceutical Bulletin*, vol. 47(10), pp. 1444-1447, (1999).
[12] K. Kamiya *et al.*, Flavonoid glucuronides from *Helicteres isora*, *Phytochemistry*, 57(2), pp. 297-301, (2001).
[13] X. Hu, D. Cheng, and Z. Zhang, Antidiabetic activity of *Helicteres angustifolia* root, *Pharmaceutical Biology*, 54(6), pp. 938-944, (2016).
[14] S. Sun *et al*, Characterization of polysaccharide from Helicteres angustifolia L. and its immunomodulatory activities on macrophages RAW264.7, *Biomedicine & Pharmacotherapy*, 109, pp. 262-270, (2019).
[15] D. Su *et al*, Helicteric acid, oleanic acid, and betulinic acid, three triterpenes from *Helicteres angustifolia* L., inhibit proliferation and induce apoptosis in HT-29 colorectal cancer cells via suppressing NF-κB and STAT3 Signaling, *Evidence-Based Complementary Alternative Medicine*, 2017, pp. 1-8, (2017).
[16] N. Loganayaki, P. Siddhuraju, and S. Manian, Antioxidant activity and free radical scavenging capacity of phenolic extracts from Helicteres isora L. and Ceiba pentandra L., *J Food Sci Technol*, 50(4), pp. 687-695, (2013).
[17] V. Shriram *et al.*, Antibacterial & antiplasmid activities of *Helicteres isora* L., *Indian J Med Res*, 132, pp. 94-99, (2010).
[18] D. N. Quang *et al.*, Cytotoxic constituents from *Helicteres hirsuta* collected in Vietnam, *Natural product research*, pp. 1-5, (2018).
[19] T. T. Nguyen, N. Kretschmer, E. M. Pferschy Wenzig, O. Kunert, and R. Bauer, Triterpenoidal and Phenolic Compounds Isolated from the Aerial Parts of *Helicteres hirsuta* and their Cytotoxicity on Several Cancer Cell Lines, *Natural Product Communications*, 14(1), pp. 4-10, (2019).
[20] Nguyen Van Thuy, Reseach antioxidant activity of ethanolic extractions of stem of *Helicteres hisuta* Lour., Department of Pharmaceutical Chemistry, Nguyen Tat Thanh University, 2018.
[21] Y. W. Chin *et al.*, Cytotoxic lignans from the stems of *Helicteres hirsuta* collected in Indonesia, *Phytother. Res.*, 20(1), pp. 62-65, (2006).
[22] L. L. Pierre and M. N. Moses, Isolation and Characterisation of Stigmasterol and B - Sitosterol from Odontonema Strictum (Acanthaceae), *Journal of Innovations in Pharmaceuticals and Biological Sciences*, 2(1), pp. 88-95, (2015).
[23] P. Dais *et al*, Complete ^{1}H and ^{13}C NMR assignment and ^{31}P NMR determination of pentacyclic triterpenic acids, *Analytical Methods*, 9, pp. 1-25, (2017).
[24] H. Kojima *et al*, Sterol Glucosides from *Prunella vulgaris*, *Phytochemistry*, 29(7), pp. 2351-2355, (1990).
[25] A. K. Chakravarty *et al*, Unambiguous Assignment of 13C Chemical Shifts of Some Hopane and Migrated Hopane Derivatives by 2D NMR, Temhedron, 50(9), pp. 2865-2876, (1994).

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