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Chemical constituents of the fruits from *Fissistigma oldhamii* (Hemsl.) Merr. growing in Vietnam

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Abstract

Phytochemical studied on methanol extract of fruits *Fissistigma oldhami* (Hemsl.) Merr. (Annonaceae) led to the isolation of four flavonoids: 6-hydroxy-5,7,8-trimethoxy flavanone, 2',5'-dihydroxy-3',4',6'-trimethoxy chalcone, quercetin, rutin and two steroids: β-sitosterol **(5)** and β-sitosterol-3-O-β-D-glucopyranoside (**6**). The structures of the compounds were established on the basic of spectroscopic methods (UV, IR, MS, 1D-NMR and 2D-NMR) and comparison with literature data. This is first time the 6 compounds isolated from the plant.

Keywords. *Fisistigma oldhamii*, Annanoceae, flavonoids, steroids.

1. INTRODUCTION

The genus *Fissistigma* (Annonaceae) consists of about 80 species, widely distributed in Australia and Asia [1]. This plant has been used as a folklore medicine for liver protection, anti-inflammatory and anti-arthritic effects, and anti-tumor action [1], [2]. *F. oldhamii* (Hemsl.) Merr., a member of the genus *Fissistigma*, is perennial shrub, 4-6 m tall or climbing shrub, 10-12 m tall [3]. In Vietnam, it is mainly distributed in Laocai, Hagiang, Caobang, Hatinh, Quangtri, Kontum, Gialai and Dongnai [3]. In the previous studies, a new aporphin alkaloid: fissoldin [4]; two cyclopentenon: stigmahamon I and stigmahamon II [5]; a new (*R*)-4,5-dimethoxy-3-(4′-phenyl-2′-oxobutyl)-*5H*-furan-2-on: fissohamion [6]; a new alkaloid type of hydro-oxadiazine: fissoldhimin [7]; 11 aristolactams such as: stigmalactam, aristolactam AII, aristolactam BIII, aristolactam FII; two dioxoaporphins: enterocarpam I and velutinam; two new stigmalactams: noraristolodion and norcepharadion B [8]; a morphinandienon alkaloid: N-methyl-2,3,6-trimethoxy morphinandien-7-on [9] and 4 alkaloid: xyclopin, fissistigma A, B, and C [10] were isolated from *F. oldhami* (Hemsl.) Merr. However, it has not seen any documents about phytochemiscal research of fruits *F. oldhami* (Hemsl.) Merr. in Vietnam.

This paper describes the study on flavonoid and steroid constituents of fruits *F. oldhami* (Hemsl.) Merr. in Vietnam.

2. MATERIAL AND METHODS

2.1. Plant materials

The fruits of *Fissistigma oldhami* (Hemsl.) Merr. (Annonaceae) were collected in Hatinh in April 2011 and identified by Assoc. Dr. Tran Huy Thai (Institute of Ecology and Biological Resources-Vietnam Academy of Science and Technology). A voucher specimen has been deposited in the Faculty of Chemistry, Vinh University.

2.2. General experimental procedures

The melting points were determined on Boetius microscope. UV spectra were measured on HP Packard 8452A. IR spectra were measured on a Nicolet Impact-410 with KBr tablets. ESI-MS spectra were measured on the Agilent 1200 Series LC-MSD Trap. EI-MS spectra were measured on LC-MS-Trap-00127. NMR spectra were measured on a Bruker AM 500 FT-NMR spectrometer at the Institute of Chemistry, Vietnam Academy of Science and Technology. Column chromatography was carried out on Kieselgel 60 (0.063-0.2 and 0.040-0.063) mm (Merck, Germany). Thin-layer chromatography was performed on precoated plates Kieselgel 60 F_{254}, the traces was detected by UV light at two wavelengths 254 and 365 nm or iodine

reagent.

2.3. Extraction and isolation

The dried powdered fruits of *F. oldhami* (2.0 kg) were extracted with methanol at room temperature for one week. The methanol extract was concentrated under vacuum at 55°C. The obtained residue (90 g) was suspended in water and successively partitioned with ethyl acetate and *n*-butanol to afford 26 g ethyl acetate and 15 g butanol residues, respectively.

The ethyl acetate fraction was subjected to silica gel column chromatography with a gradient of chloroform:metanol (100:0, 40:1: 30:1; 20:1; 10:1: 4:1; 2:1) to yield 10 fractions. The fraction 1 was subjected to repeated silica gel chromatography eluted with *n*-hexane:acetone (15:1) to give compound **5** (172 mg). The fraction 3 was subjected to repeated silica gel chromatography eluted with *n*-hexane:acetone (9:1, 4:1) to yield compound **1** (35 mg) and compound **2** (29 mg). The fraction 4 was subjected to repeated silica gel chromatography eluted with chloroform:metanol (20:1) to afford compound **3** (83 mg).

The butanol fraction was chromatographed on silica gel column using a gradient of chloroform:metanol (30:1, 20:1, 10:1, 5:1) to give 10 fractions. The fraction 5 was subjected to repeated silica gel chromatography eluted with cloroform:metanol (10:1; 6:1) to give compound **4** (34 mg). The fraction 6 was subjected to repeated silica gel chromatography eluting with CH_3Cl:CH_3OH (7:1) to give compound **6** (112 mg).

Compound **1**: yellow needle crystals; m.p. 162-163ºC; UV (MeOH) λ_{max}: 203, 248, 283, 316. ESI-MS *m/z*: 331 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$) (δ ppm): 7.45 (5H, *m*, H-2', 3', 4', 5', 6'), 5.43 (1H, *dd*, *J* = 3.0, 13.5 Hz, H-2), 4.08 (3H, *s*, 7-OCH_3), 3.88 (6H, *s*, 5-OCH_3; 8-OCH_3), 3.06 (1H, *dd*, *J* = 13.5, 17.0 Hz, H-3b), 2.86 (1H, *dd*, *J* = 3.0, 17.0 Hz, H-3a); ^{13}C-NMR (125 MHz, $CDCl_3$) (δ ppm): 198.5 (C-4), 146.8 (C-9), 145.7 (C-7), 146.5 (C-5), 141.3 (C-1'), 138.3 (C-8), 128.9 (C-2'), 128.9 (C-6'), 134.2 (C-6), 129.0 (C-3'), 126.3 (C-4'), 129.0 (C-5'), 111.1 (C-10), 80.2 (C-2), 61.8 (7-OCH_3), 61.6 (8-OCH_3), 61.2 (5-OCH_3), 46.1 (C-3).

Compound **2**: yellow power, m.p. 195-196 ºC; UV (MeOH) λ_{max}: 216, 278, 322. ESI-MS *m/z*: 331 $[M+H]^+$; ^{1}H-NMR (500 MHz, $CDCl_3$) (δ ppm): 12.89 (1H, *s*, 2'-OH), 7.93 (1H, *d*, *J* = 15.5 Hz, H-8), 7.88 (1H, *d*, *J* = 15.0 Hz, H-7), 7.64 (2H, *dd*, *J* = 2.0, 9.5 Hz, H-2,6), 7.41-7.45 (3H, *m*, H-3,4,5), 5.35 (1H, *s*, 5'-OH), 4.15 (3H, *s*, 4'-OCH_3), 3.91 (3H, *s*, 3'-OCH_3), 3.85 (3H, *s*, 6'-OCH_3); ^{13}C-NMR (125 MHz, $CDCl_3$) (δ ppm): 193.6 (C-9), 152.2 (C-2'), 143.9 (C-7), 147.1 (C-4'), 143.0 (C-6'), 136.6 (C-3'), 135.2 (C-1), 134.3 (C-5'), 130.5 (C-4), 128.6 (C-3), 128.6 (C-5), 129.0 (C-2), 129.0 (C-6), 126.3 C-8), 110.9 C-1'), 62.6 (6'-OCH_3), 61.4 (4'-OCH_3), 61.0 (3'-OCH_3).

Compound **3**: yellow needle crystal, m.p. 313-314 ºC; UV (MeOH) λ_{max} nm (logε): 213 (4.59), 254 (4.75), 271 (4.47), 311 (4.00), 349 (4.04), 400 (3.97); ESI-MS *m/z*: 303 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) (δ ppm): 12.59 (1H, *s*, 5-OH), 7.55 (1H, *d*, *J* = 2.0 Hz, H-2'), 7.53 (1H, *dd*, *J* = 2.0, 8.5 Hz, H-6'), 6.85 (1H, *d*, *J* = 8.8 Hz, H-5'), 6.38 (1H, *d*, *J* = 1.8 Hz, H-8), 6.19 (1H, *d*, *J* = 1.8 Hz, H-6); ^{13}C-NMR (125 MHz, DMSO-d_6) (δ ppm): 175.7 (C-4), 163.8 (C-7), 160.7 (C-5), 156.2 (C-9), 147.6 (C-4'), 146.8 (C-2), 145.0 (C-3'), 135.7 (C-3), 122.0 (C-1'), 120.1 (C-6'), 115.5 (C-5'), 115.1 (C-2'), 103.1 (C-10), 98.2 (C-6), 93.4 (C-8).

Compound **4**: yellow crystal, m.p. 214-215 ºC; UV (EtOH) λ_{max} nm (logε): 256 (4.14), 267 (4.11), 293 (4.02), 346 (3.83); IR ν_{max}^{KBr} cm^{-1}: 3415 (OH), 1660 (C=O), 1500, 1490 (C=C), 1060 (C-O), 1015; ESI-MS *m/z*: 611 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6) (δ ppm): 12.59 (1H, *s*, 5-OH), 7.55 (1H, *d*, *J* = 2.5 Hz, H-2'), 7.54 (1H, *dd*, *J* = 2.0, 12.5 Hz, H-6'), 6.85 (1H, *d*, *J* = 8.8 Hz, H-5'), 6.38 (1H, *d*, *J* = 1.8 Hz, H-8), 6.19 (1H, *d*, *J* = 1.8 Hz, H-6), 5.34 (1H, *d*, *J* = 7.0 Hz, glc H-1), 4.38 (1H, *brs*, rham-1), 3.71-3.05 (*m*,12H of sugar moieties) and 1.00 (3H, *d*, J = 6.0, rham-CH_3). ^{13}C-NMR (125 MHz, DMSO-d_6) (δ ppm): 177.5 (C-4), 164.2 (C-7), 161.3 (C-5), 156.5 (C-2), 156.7 (C-9), 148.5 (C-4'), 144.8 (C-3'), 133.4 (C-3), 121.7 (C-6'), 121.3 (C-1'), 115.3 (C-2'), 116.4 (C-5'), 104.1 (C-10), 98.8 (C-6), 93.7 (C-8), Rham: 101.3 (C-1''), 76.5 (C-3''), 76.0 (C-5''), 74.2 (C-2''), 67.1 (C-6''), Glu: 100.8 (C-1'''), 71.9 (C-4'''), 70.7 (C-3'''), 70.5 (C-2'''), 70.1 (C-4''), 68.3 (C-5'''), 17.8 (C-6''').

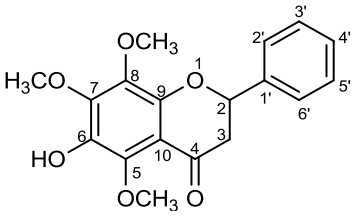
Compound **5**: needle crystals, m.p. 135-136 ºC; IR ν_{max}^{KBr} cm^{-1}: 3400, 3025, 1410, 1250, 690 and 820; ESI-MS *m/z* (%): 414 (M^+, $C_{29}H_{50}O$, 20), 413(41), 398 (28), 397 (100), 395 (32), 383 (11), 361 (11), 257 (3), 255 (6.3), 151 (5.6), 139 (11);

Compound **6**: White powder, m.p. 283-285 ºC; IRν_{max}^{KBr} cm^{-1}: 3400, 3050, 815; ESI-MS *m/z* (%): 396 [M^+-$C_6H_{12}O_6$] (9), 273 (2), 255 (9), 185 (5), 161(15), 145 (25), 133 (21), 105 (42), 91 (46), 81 (51), 69 (100).

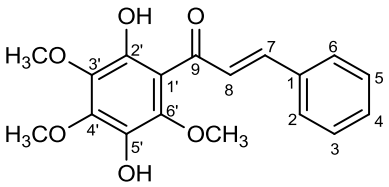
3. RESULTS AND DISCUSSION

The methanol extract of the fruits of *F. oldhami*

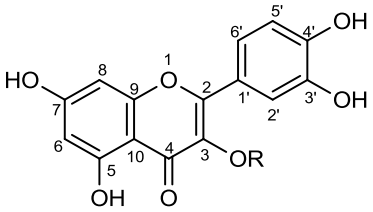
was re-extracted with EtOAc and BuOH, successively. The resulting EtOAc and BuOH extract was subjected to repeated column chromatography on silica gel to give compounds **1-6**



1 6-hydroxy-5,7,8-trimetoxy flavanon



2 2',5'-dihydroxy-3',4',6'-trimethoxychalcone



3 R = H
4 R = α-L-rhamnopyranosyl-(1→ 6))-β-D-glucopyranose

Compound **1** was obtained as yellow powder (m.p. 162-163 ºC). The ESI-MS of **1** showed a pseudomolecular ion peak $[M+H]^+$ at *m/z*: 331, corresponding to the formula of $C_{18}H_{18}O_6$. The UV spectra of **1** characterized flavanone compound. The ^{1}H-NMR spectrum of **1** showed two signals of methylene protons at 2.86 (1H, *dd*, *J* = 3.0, 17.0 Hz, H-3a) and 3.06 (1H, *dd*, *J* = 13.5, 17.0 Hz, H-3b); three methoxy groups at 3.88 (6H, *s*, 5-OCH_3, 8-OCH_3) and 4.08 (3H, *s*, 7-OCH_3 and a methine proton at 5.43 (1H, *dd*, *J* = 3.0, 6.0 Hz, H-2). Moreover, it showed 5 signal of 5 protons of aromatic ring at 7.45 (5H, *m*, H-2', 3', 4', 5', 6'). The ^{13}C-NMR and DEPT spectra indicated the presence of 18 carbons: a carbonyl carbon at 198.5 (C-4); an oxygenated carbon at 80.2 (C-2); three methoxy groups at 61.8 (7-OCH_3), 61.6 (8-OCH_3), and 61.2 (5-OCH_3) and a methylene carbon at 46.1 (C-3). It also showed 12 signals of aromatic carbons at 110-160 ppm. The data demonstrated that compound **1** is a flavanoid skeleton. The position of three methoxy groups (C-5, C-7 and C-8) was established on the basis of HMBC spectra. The ^{13}C-^{1}H long-range couplings of ^2J and ^3J showed correlations between 5-OCH_3 (3.88)/C-5, 8-O-CH_3 (3.88)/C-8, and 7-OCH_3 (4.08)/C-7. The comparison with literature data [12] confirmed that **1** to be 6-hydroxy-5,7,8-trimetoxy flavanone, a known compound from *Fissistigma polyanthides* [12].

Compound **2** was obtained as yellow powder (m.p. 195-196 ºC). The ESI-MS of **2** showed a pseudomolecular ion peak $[M+H]^+$ at *m/z*: 331; corresponding to the formula of $C_{18}H_{18}O_6$. The ^{1}H-NMR spectrum of **2** indicated the presence of one mono-substituted aromatic ring signal at 7.64 (2H, *dd*, *J* = 2.0, 9.5 Hz, H-2,6) and 7.41-7.45 (3H, *m*, H-3,4,5), three methoxyl groups at 4.15 (3H, *s*, 4'-OCH_3), 3.91 (3H, s, 3'-OCH_3) and 3.85 (3H, s, 6'-OCH_3)], one olefinic at 7.93 (1H, *d*, *J* = 15.5 Hz, H-8) and 7.88 (1H, *d*, *J* = 15.0 Hz, H-7)], two hydroxyl groups at 12.89 (1H, *s*, 2'-OH) and 5.35 (1H, *s*, 5'-OH). The ^{13}C-NMR and DEPT confirmed the presence of six aromatic carbons, seven quaternary carbons (including a carbonyl at δ C 193.6), two methine and three methoxyl signals. The data confirmed that compound **2** is chalcone skeleton. The comparison of spectral data of **2** with the literature data [13] showed that **2** is 2',5'-dihydroxy-3',4',6'-trimetoxychalcone. It was found in *Fissistigma lanuginosum* [13].

Compound **3**: The ESI-MS of **3** showed a pseudomolecular ion peak $[M+H]^+$ at *m/z* 303, corresponding to the molecular formula $C_{15}H_{10}O_7$. ^{1}H-NMR spectrum showed signals 6.19 and 6.38 ppm as doublets δ (1H, *J* = 1.8 Hz) which are specific for the C-6 proton and two C-8 protons of the ring. There are three signal protons of ABX system at 7.55 (2H, *dd*, *J* = 8.5, 2.0 Hz) and 6.85 ppm (1H, *d*, *J* = 8.8 Hz). The ^{13}C-NMR and DEPT spectra of compounds **3** showed signals of 15 carbons of the flavone skeleton. From these data and comparison with literature data [14], it can be determined that compound **3** is quercetin. This compound has been isolated from *Ruta graveolens* and found in many plant families, has antioxidant activity and blood vascular resistance [16].

Compound **4**, m.p. 214-215 ºC. The IR spectrum of **4** showed absorption bands at 3415 (O-H stretching), 1660 (C=O), 1500, 1490 (C=C), 1060 (C-OH vibrations). The ESI-MS of **4** showed a pseudomolecular ion peak $[M+H]^+$ at *m/z*: 611; corresponding to the formula of $C_{27}H_{30}O_{16}$. The ^{1}H-NMR spectrum of **4** indicated the presence of signals corresponding to one tri-substituted aromatic ring at 7.55 (1H, *d*, *J* = 2.5 Hz, H-2'), 7.54 (1H, *dd*, *J* = 2.0, 12.5 Hz, H-6') and 6.85 (1H, *d*, *J* = 8.8 Hz, H-5'); two hydroxyl protons at 12.59 (1H, *s*, 5-OH) and 5.35 (1H, *s*, 5''-OH); two anomeric protons at 5.34 (1H, *d*, *J* = 7.0 Hz, glc H-1'') and 4.38 (1H, *brs*, rham H-1'''). Moreover, it showed the signals of two glycosides at 3.71-3.05 (*m*, 12H of sugar moieties) and a methyl group at 1.00 (3H, *d*, J

= 6.0, rham-CH_3). The ^{13}C-NMR and DEPT spectra of **4** showed signals of 27 carbons, including 15 carbons of flavone skeleton and 12 carbons of the two sugar moieties of rutin (quercetin-3-O-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside).

This compound has been isolated from *Ruta graveolens* trees and found in many plant families. It has antioxidant activity and blood vascular resistance [16].

Compounds **5** and **6** were determined by the analysis of spectral data combined with literature data [15, 16].

4. CONCLUSION

The column chromatography isolation and spectroscopic analysis methods led to the isolation and identification of 4 flavonoids (6-hydroxy-5,7,8-trimetoxy flavanon, 2',5'-dihydroxy-3',4',6'-trimethoxychalcone, quercetin, rutin) and 2 steroids (*β*-sitosterol, *β*-sitosterol-3-O-*β*-D-glucopyranoside) from the fruits of *Fissistigma oldhami* (Hemsl.) Merr. growing in Vietnam. This is the first time these compounds were isolated from this plant.

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