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Research Article

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Coumarins from the stem bark of *Feronia limonia*

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ABSTRACT

*Three coumarins were isolated from the stem bark of Feronia limonia namely as auraptene (**1**), osthol (**2**), and xhantotoxin (**3**). Their structures were elucidated by spectroscopic methods including UV, IR, HRESIMS, 1D and 2D NMR analysis. Compounds **1**–**3** were evaluated for their cytotoxic properties against HeLa cells, showing their IC_{50} were 65.09, 7.62, and 21.51 ppm, respectively.*

Keywords*:* Auraptene, Osthol, Xhantotoxin, Coumarin, *Feronia limonia*, Cytotoxic

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INTRODUCTION

Feronia limonia belongs to the family Rutaceae, commonly known as Kawista in Indonesia. The phytochemical investigations on *Feronia limonia* from different parts of this plant, have isolated various compounds, including alkaloids [1,2], coumarins [3,4,5], flavonoids [6,7], and tyramine derivatives [8]. In continuation of our phytochemical work of Indonesian *Feronia* plants aiming to find coumarin compounds from *Feronia limonia*, we report the isolation of coumarin compounds, auraptene (**1**), osthol (**2**), and xhantotoxin (**3**) from the methanol extract of the stem bark of *Feronia limonia*. The cytotoxic activity of compounds **1**– **3** against HeLa is also briefly described.

EXPERIMENTAL SECTION

The stem bark of *Feronia limonia* was collected in July 2014 from Panjunan Village, Tuban District, East Java, Indonesia. The plant was identified at Herbarium Bogoriense, Center of Biological Research and Development, National Institute of Science, Bogor, Indonesia, and the voucher specimen was deposited in the herbarium. The dried and powdered stem bark of *Feronia limonia* (4.0 kg) were macerated in methanol at room temperature two times, and the methanol extract was evaporated under reduced pressure to give a dark brown residue (170 g). Furthermore, the methanol extract was partitioned with n-hexane and ethyl acetate. The ethyl acetate extract (47 g) was separated by vacuum liquid chromatography on silica gel eluted with n-hexane–ethyl acetate mixture with gradient amount of ethyl acetate (90:10, 80:20; 50:50 and 30:70) to give four major fractions A-D. The separation of fraction C (1.2 g) by flash chromatography with n-hexane-ethyl acetate (from 8:1 and 7:3) to give three subfractions C_1-C_3. Further purification of subfraction C_2 (150 mg) by radial chromatography with n-hexane-diisopropylether (from 9:1; 8:2, and 7:3) to give compound **1** (18 mg) and **2** (12 mg). The purification of subfraction C_3 (100 mg) by radial chromatography with n-hexane- chloroform (from 3:7; 1:1, and 7:3) to give compound **3** (10 mg).

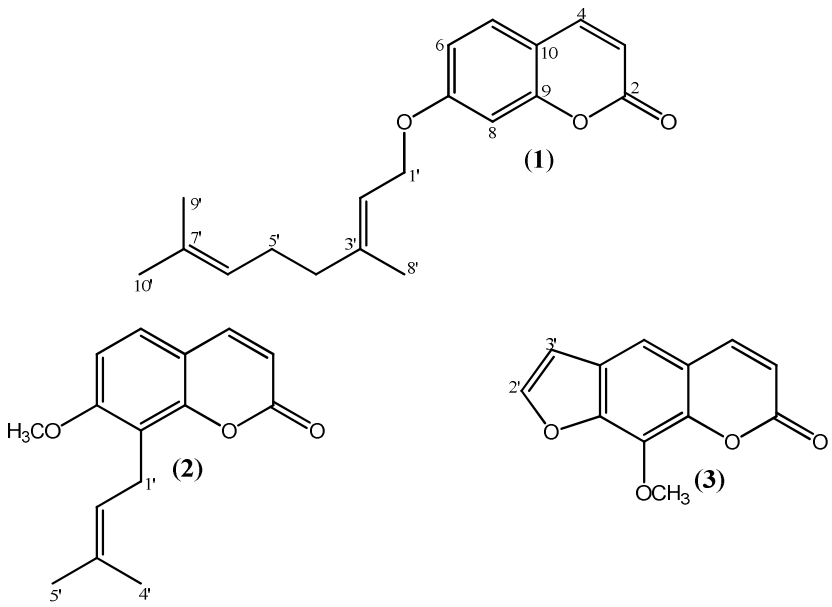

Fig. 1. Structures of isolated coumarin

Auraptene (**1**), pale yellow solid, UV (MeOH) λ_{maks} nm (log ε): 224 (3.21), 289 (3.08), and 324 (3.43) nm. HRESIMS *m/z* $[M+H]^+$ 299.1650 (calcd for $C_{19}H_{23}O_3$, 299.1647). ^{1}H NMR (400 MHz, $CDCl_3$): see Table 1. ^{13}C NMR (100 MHz, $CDCl_3$): see Table 1.

Osthol (**2**), pale yellow solid, UV (MeOH) λ_{maks} nm (log ε): 235 (3.15), 255 (2.61), and 322 (3.01) nm. HRESIMS *m/z* $[M-H]^-$ 229.0861 (calcd for $C_{14}H_{13}O_3$, 229.0865). ^{1}H NMR (400 MHz, $CDCl_3$): see Table 2. ^{13}C NMR (100 MHz, $CDCl_3$): see Table 2.

Xhantotoxin (**3**), yellow solid, UV (MeOH) λ_{maks} nm (log ε): 248 (3.19), 264 (2.94), and 303 (2.90) nm. ^{1}H-NMR NMR (400 MHz, $CDCl_3$): see Table 3. ^{13}C NMR (100 MHz, $CDCl_3$): see Table 3.

Cytotoxicity assay**:** Cytotoxic properties of the isolated compounds **1**–**3** on human cervical cancer (HeLa) were evaluated according to the method of MTT assay as described previously [9,10]. The cytotoxicity assay was performed against HeLa cells grown in RPMI 1640 medium containing 10% fetal bovine serum, 2 mg mL^{-1} sodium carbonate, 100 μg mL^{-1} penicillin sodium salt, and 100 μg mL^{-1} penicillin streptomycin sulfate. The cells were harvested at the log phase of growth, and then seeded into 96-well plates (1×10^4 cells/well). After 24 h incubation at 37 ºC and 5% CO_2 to allow cell attachment, the cultures were exposed to the test compounds **1**-**3** in DMSO at various concentrations and incubated for 48 h followed by MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay at 570 nm

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RESULTS AND DISCUSSION

Three coumarins, auraptene (**1**), osthol (**2**), and xhantotoxin (**3**) were isolated from the stem bark of *Feronia limonia*. Their structures were elucidated with extensive by UV, IR, HRESIMS, 1D and 2D NMR spectra.

Auraptene (**1**) was obtained as pale yellow solid, showed a quasimolecular ion $[M+H]^+$ at *m/z* 299.1650 consistent to the molecular formula of $C_{19}H_{23}O_3$. The UV spectrum of **1** exhibited absorption maximum for a coumarin structure at λ_{max} 224, 289, and 324 nm. The ^{1}H-NMR spectrum of compound **1** showed three aromatic proton signals for ABX system at δ_H 7.34 (1H, *d*, *J* = 8.6 Hz, H-5); 6.83 (1H, *dd*, *J* = 8.6, 2.4 Hz, H-6); and 6.80 ppm (1H, *d*, *J* = 2.4 Hz, H-8). The ^{1}H NMR spectrum of **1** also showed a pair doublets (*J* = 9.6 Hz) *cis* vinylic signal at δ_H 7.61, and 6.23 corresponding to the coumarin with substitutent at C-7. Based on HRESIMS of **1** is a coumarin derivative containing one geranyl group at C-7 [3]. The existence of geranyl chain of compound **1** showed by the presence of three methyl groups (δ_H 1.75, 1.65, and 1.59 ppm), three methylene groups (δ_H 4.58, 2.12, and 2.10 ppm), and two

methine vinyl groups (δ_H 5.45, and 5.06 ppm). The ^{13}C NMR spectrum of **1** (APT experiment, Table 1) showed the presence of 19 carbon atom signals. Two carbon signals (δ_C 162.2 and 155.9 ppm) characteristic for the region oxyaryl signals which indicate that the structure is a derivative of 7-hydroxycoumarin. The correlation of the one bond and the two/three bond ^1H-^{13}C compound **1** can be seen in the HMQC and HMBC spectra (Table-1). The presence of a geranyl group at C-7 showed in the HMBC spectrum, the long-range correlation between a proton signal methylene at δ_H 4.58 ppm with two quaternary atoms at δ_C (162.2 (C-7), 142.5 (C-3') and one methine carbon at δ_C 118.5 (C-2'). Based on data from 1D and 2D NMR of compound **1** is 7-*O*-geranylumbeliferon or known as auraptene [3]. Other HMBC correlations consistent with the structure **1** are shown in Table 1.

Table 1. NMR spectroscopic data of auraptene (1)

No.C	δ_H (mult, *J* Hz)	δ_C	HMBC
1	-	-	-
2	-	161.4	-
3	6.23 (*d*, 9.6)	113.0	C-2; C-10
4	7.61 (*d*, 9.6)	143.6	C-2; C-5; C-6; C-8; C-9
5	7.34 (*d*, 8.6)	128.8	C-4; C-6; C-7; C-9
6	6.83 (*dd*,8.6; 2.4)	113.3	C-7; C-8; C-10
7	-	162.2	-
8	6.80 (*d*, 2.4)	101.7	C-6; C-7; C-9
9	-	155.9	-
10	-	112.5	-
1'	4.58 (*d*, 6.5)	65.6	C-7; C-2'; C-3'
2'	5.45 (*t*, 6.6)	118.5	C-8; C-1'; C-4'
3'	-	142.5	-
4'	2.10 (*m*)	39.6	C-2'; C-6'; C-8'
5'	2.12 (*m*)	26.3	C-3'; C-4'
6'	5.06 (*t*, 6.6)	123.7	C-9', C-10'
7'	-	132.1	-
8'	1.75 (*s*)	16.9	C-2'; C-3', C-4'
9'	1.65 (*s*)	25.8	C-6'; C-7'; C-10'
10'	1.59 (*s*)	17.8	C-6'; C-7'; C-9'

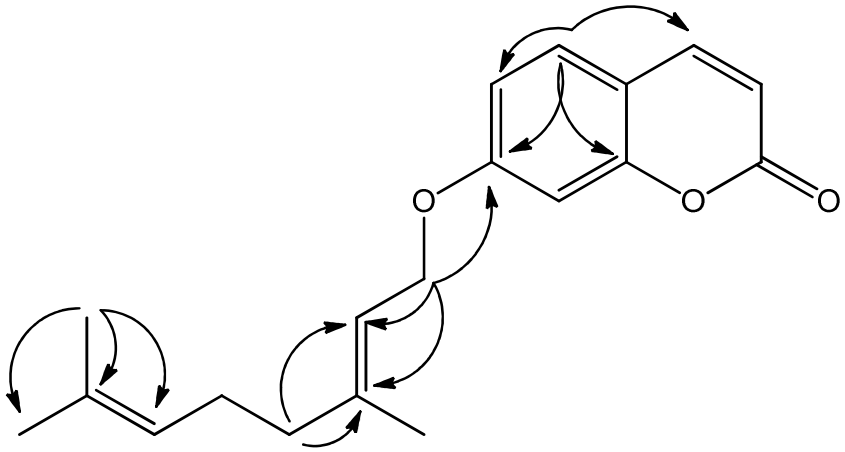

Fig. 2. Significant HMBC correlation for 1

Osthol (**2**) was also obtained as pale yellow solid. The ion peak at *m/z* 229.0861 $[M-H]^-$ in the HRESIMS spectrum gave the molecular $C_{14}H_{13}O_3$. Based on UV, HRESIMS, ^1H and ^{13}C NMR spectrum suggesting that **2** is a coumarin derivative containing one methoxyl group and one isoprenyl group. Analysis ^1H-NMR spectrum of compound **2** showed a pair doublets (J = 8.6 Hz) signals for aromatic proton region at δ_H 7.29 and 6.82 ppm. The ^1H NMR spectrum of **2** also showed a pair doublets (J = 9.6 Hz) *cis* vinylic signal at δ_H 7.62, and 6.24 corresponding to the coumarin with substitutent at C-7 and C-8. The existence of isoprenyl chain of compound **2** showed the presence of two methyl groups (δ_H 1.84, and 1.67 ppm), one methylene group (δ_H 3.53 ppm), and one vinyl group (δ_H 5.23 ppm). The methoxyl group showed singlet proton signal at δ_H 3.92 ppm. The placement of isoprenyl and methoxyl shown in HMBC spectrum (Table 2). The presence of long-range correlations in the HMBC spectrum of **2** between the singlet proton signal of a methoxyl group at δ_H 3.92 with one oxyaryl carbon at δ_C 160.2 (C-7), and the doublet

proton of aromatic region at δ_H 7.29 with two oxyaryl carbon signals at δ_C [160.2 (C-7), and 153.0 (C-9)], and two methine carbon signals at δ_C [143.9 (C-4), and 107.4 (C-6)] showed methoxyl at C-7. Thus the placement of isoprenyl chain located at C-8. Based on data from 1D and 2D NMR, compound **2** is 8-isoprenyl-7-methoxycoumarin or known as osthol [3]. Other HMBC correlations consistent with the structure **2** are shown in Table 2.

Table 2. NMR spectroscopic data of osthol (2)

No.C	δ_H (mult, *J* Hz)	δ_C	HMBC
1	-	-	-
2	-	161.2	-
3	6,24 (*d*, 9,6)	113.1	C-2; C-10
4	7,62 (*d*, 9,6)	143.9	C-2; C-5; C-6; C-8; C-9
5	7,29 (*d*, 8,6)	126.3	C-4; C-6; C-7; C-9
6	6,82 (*d*, 8,6)	107.4	C-7; C-8; C-10
7	-	160.2	-
8	-	118.1	-
9	-	153.0	-
10	-	115.7	-
1'	3.53 (*d*, 7.3)	22.0	C-7; C-2'; C-3'
2'	5,23 (*t*, 7,2)	121.2	C-8; C-1'; C-4'
3'	-	132.8	-
4'	1,84 (*s*)	18.0	C-2'; C-6'; C-8'
5'	1,67 (*s*)	25.9	
7-OCH_3	3,92 (*s*)	56.1	C-7

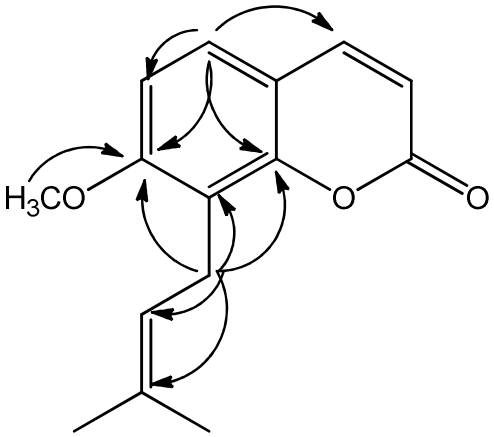

Fig. 3. Significant HMBC correlation for 2

Xhantotoxin (**3**) was obtained as yellow solid, and its UV spectrum exhibited absorption maximum of 248, 264, and 303 nm, typical for furanocoumarin derivative [3]. The ^{1}H-NMR spectrum of compound **3** showed a pair of doublets (J = 9.6 Hz) at δ_H [7.75 (H-4); 6.36 (H-3)], a singlet aromatic region at δ_H 7.34 (H-5), and a pair of doublets (J = 2.2 Hz) at δ_H [7.67 (H-2'); 6.80 (H-3')] suggested the signal of a furanocoumarin with substitutent at C-5 or C-8. The methoxyl group showed singlet proton signal at δ_H 3.92 ppm suggested that the methoxyl group is either at C-5 or C-8 of the furanocoumarin structure. The one bond and two/three bonds ^{1}H-^{13}C correlations found in the HMQC and HMBC spectra of compound **3** (Table 3) unambiguously placed the methoxyl group at C-8 by the following observations. The presence of long-range correlations in the HMBC spectrum of **3** between the doublet proton signals (J = 2.2 Hz) of a furano group at δ_H 7.67 (H-2'), and 6.80 (H-3') with one oxyaryl carbon signal at δ_C 147.8 (C-7), and the correlation between a proton aromatic signal at δ_H 7.34 with two oxyaryl carbon signals δ_C 147.8 (C-7), 143.1 (C-9) and one methine carbon signal δ_C 106.8 (C-3') showed methoxyl at C-8. Based on data from 1D and 2D NMR, compound **3** is 8-methoxy-psoralen or known as xhantotoxin [3]. Other HMBC correlations consistent with the structure **3** are shown in Table 3.

Table 3. NMR spectroscopic data of xhantotoxin (3)

No.C	δ_H (mult, *J* Hz)	δ_C	HMBC
1	-	-	-
2	-	160.6	-
3	6.36 (*d*, 9.6)	113.0	C-2; C-10
4	7.75 (*d*, 9.6)	144.4	C-2; C-3; C-9; C-10
5	7.34 (*s*)	146.7	C-7; C-9; C-10; C-3'
6	-	105.4	-
7	-	147.8	-
8	-	132.9	-
9	-	143.1	-
10	-	116.6	-
1'	-	-	-
2'	7.67 (*d*, 2.2)	114.9	C-7; C-3'
3'	6.80 (*d*, 2.2)	106.8	C-7; C-2'
8-OCH_3	4.29 (*s*)	61.4	C-8

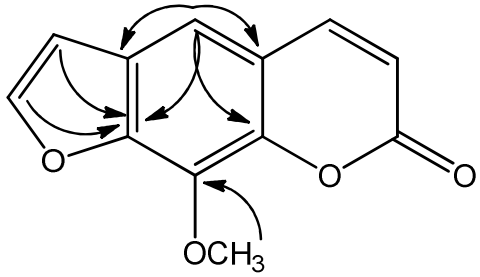

Fig. 3. Significant HMBC correlation for 3

On cytotoxic evaluation against HeLa cells, compounds **1 - 3** exhibited IC_{50} values of 65.09 ± 0.017, 7.62 ± 0.004, and 21,51 ± 0,003 ppm, respectively. The results of cytotoxic activity showed osthol (**2**), xhantotoxin (**3**) have moderate activity, and auraptene (**1**) was inactive [11]. These cytotoxic data suggested that the presence of methoxyl substituent at C-7 and isoprenyl at C-8 of the coumarin structure increases cytotoxic activity.

CONCLUSION

Three coumarins, auraptene (**1**), osthol (**2**), and xhantotoxin (**3**). have been isolated from the stem bark of of *Feronia limonia*. The cytotoxic activity of compounds **1**-**3** against HeLa cells showed osthol>xhantotoxin>auraptene. The structure-activity relationship of compounds **1**–**3** against HeLa cells suggested that the presence of isoprenyl group at C-8 and methoxyl group at C-7 on osthol (**2**) increases cytotoxic activity.

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