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Der Pharmacia Lettre, 2016, 8 (20):33-36
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-5071
USA CODEN: DPLEB4

Oxygeranylated Coumarins from The Root of *Limonia accidisima* L. and Their DPPH Radical Scavenging Activity

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ABSTRACT

*Four oxygeranylated coumarins namely as auraptene (**1**), 6-methoxy-auraptene (**2**), 7-(2'E,5'E)-7'-hydroxy-3',7'-dimethylocta-2',5'-dienyloxy) coumarin (**3**) and 7-((2'E,6'E)-5'-hydroxy-3',7'-dimethylocta-2',6'-dienyloxy) coumarin (**4**) were isolated from the roots of Limonia accidissima L. Their structures were determined based on UV, IR, HRESIMS, 1D and 2D NMR analysis.. The ethyl acetate extract as well as compounds **1-4** were evaluated for their antioxidant activity against DPPH radical scavenging. Compound **3** showed very high activity against DPPH radical*

Keywords: *Limonia accidisima* L., oxygeranylated coumarins, DPPH radical

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INTRODUCTION

Limonia accidisima L. (synonim *Feronia limonia*) belongs to the family Rutaceae, commonly known as Kawista in Indonesia. The plants used in traditional medicine, such as diarrhea, dysentery, tumor, asthma, and hepatitis. The utilization of this plant in traditional medicine is certainly related to secondary metabolites contained in this plant. The phytochemical investigations on *Limonia accidisima* L 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]. Secondary metabolites *Limonia acidissima* L. have a variety of activities, such as, antitumor, antimicrobial, anti-inflammatory, antipyretic, and analgesic. In continuation of our phytochemical work of Indonesian *Limonia* plants aiming to find coumarin compounds from *Limonia accidisima* L., we report the isolation of coumarin compounds, auraptene (**1**), 6-methoxy-auraptene (**2**), 7-((2'*E*,5'*E*)-7'-hydroxy-3',7'-dimethylocta-2',5'-dienyloxy)coumarin (**3**) and 7-((2'*E*,6'*E*)-5'-hydroxy-3',7'-dimethylocta-2',6'-dienyloxy) coumarin **(4)** from the methanol extract of the root of *Limonia accidisima L*. Compounds **1-4** (Fig.1) which were evaluated for their radical scavenging against 2,2-diphenyl-1-picrylhydrazyl (DPPH) also briefly described.

MATERIALS AND METHODS

General

UV and IR spectra were measured with a Shimadzu 1800 (Kyoto, Japan) and Perkin Elmer Spectrum One FTIR spectrometer, respectively. ^{1}H and ^{13}C NMR spectra were recorded with a JEOL ECA 400 spectrometer operating at 400 (^{1}H) and 100 (^{13}C) MHz in $CDCl_3$ using TMS as the internal standard. Mass spectra were recorded using a Waters LCT Premier XE (Santa Clara, CA, USA). Coloumn chromatography and radial chromatography were

carried out using Si gel 60 GF_{254} and Si gel 60 PF_{254}, for TLC analysis, pre-coated silica gel plates (Merck, Kieselgel 60 GF $_{254}$, 0,25 mm thickness) were used. DPPH (2,2-diphenyl-1-picrylhydrazyl) (Merck, Darmstadt, Germany). Solvents used for extraction and preparative chromatography were of technical grade and distilled before use.

Plant Material

The roots of *Limonia accidisima L* was collected from Panjunan Village, Tuban District, East Java, Indonesia on Feb 2015. The plant was identified at Herbarium Bogoriense, Center of Biological Research and Development, Indonesia, and the voucher specimen was deposited in the herbarium. The roots was cleaned, air dried under the shade, cut into small pieces and milled.

Extraction and Isolation

The dried and powdered roots of *Limonia accidisima L* (4.0 kg) was 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 (9:1, 4:1; 1:1 and 3:7) to give four major fractions A-D. The separation of fraction C (1.2 g) by flash chromatography with *n*-hexane-ethyl acetate (from 9:1 to 7:3) to give three subfractions C_1-C_3. Further purification of subfraction C_2 (150 mg) by radial chromatography with *n*-hexane-diisopropyl ether (from 9:1 to 7:3) to give compound **1** (18 mg). The separation of fraction D (3 g) by flash chromatography with *n*-hexane-ethyl acetate (from 8:1 and 7:3) to give four subfractions D_1-D_4. The purification of subfraction D_2 (100 mg) by radial chromatography with *n*-hexane- chloroform (from 9:1 to 3:7) to give compound **2** (10 mg). Further purification of subfraction D_3 (80 mg) and D_4 (150 mg) by radial chromatography with *n*-hexane-acetone (from 9:1 to 7:3) to give compound **3** (6.1 mg) and **4** (26.9 mg).

Structure Elucidation

Auraptene (**1**), pale yellow solid, UV (MeOH) λ_{maks} nm (log ε): 224 (3.21), 289 (3.08), and 324 (3.43). HRESIMS *m/z* $[M+H]^+$ 299.1650 (calcd for $C_{19}H_{23}O_3$, 299.1647). ^{1}H-NMR ($CDCl_3$) δ_H ppm: 7.61 (1H, d, *J* = 9.6 Hz, H-4), 7.34 (1H, d, *J* = 8.6 Hz, H-5), 6.83 (1H, dd, *J* = 8.6; 2.4 Hz, H-6), 6.80 (1H, d, *J* = 2.4 Hz, H-8), 6.23 (1H, d, *J* = 9.6 Hz, H-3), 5.45 (1H, t, *J* = 6.6 Hz, H-2'), 5.06 (1H, t, *J* = 6.6 Hz, H-6'), 4.58 (2H, d, *J* = 6.5 Hz, H-1'), 2.10 (4H, m, H-5'/6'), 1.75 (3H, s, H-10'), 1.65 (3H, s, H-8'), 1.59 (3H, s, H-9'). ^{13}C-NMR ($CDCl_3$) δ_C ppm: 162.2 (C-7), 161.4 (C-2), 155.9 (C-9), 143.6 (C-4), 142.5 (C-3'), 132.1 (C-7'), 128.8 (C-5), 123.7 (C-6'), 118.5 (C-2'), 113.3 (C-6), 113.0 (C-3), 112.5 (C-10), 101.7 (C-8), 65.6 (C-1'), 39.6 (C-4'), 26.3 (C-5'), 25.8 (C-9'), 17.8 (C-8'), 16.9 (C-10').

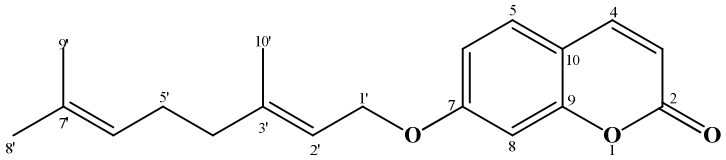
6-Methoxy-auraptene (**2**), white solid, UV (MeOH) λ_{maks} nm (log ε) : 248 (2.81), 264 (2.94), and 303 (2.70). ^{1}H-NMR ($CDCl_3$) δ_H ppm: 7.62 (1H, d, *J* = 9.5 Hz, H-4), 6.85 (1H, s, H-5), 6.83 (1H, s, H-8), 6.27 (1H, d, *J* = 9.5 Hz, H-3), 5.48 (1H, t, *J* = 5.3 Hz, H-2'), 5.06 (1H, t, *J* = 5.3 Hz, H-6'), 4.69 (2H, d, *J* = 6.5 Hz, H-1'), 2.08 (4H, m, H-4'/5'), 1.77 (3H, s, H-10'), 1.65 (3H, s, H-8'),1.59 (3H, s, H-9'), 3.91 (3H, s, 6-OCH_3). ^{13}C-NMR ($CDCl_3$) δ_C ppm: 161.6 (C-2), 152.0 (C-7), 149.8 (C-9), 146.6 (C-6), 143.4 (C-4), 142.1 (C-3'), 131.9 (C-7'), 123.6 (C-6'), 118.3 (C-2'), 113.3 (C-3), 111.2 (C-10), 107.9 (C-5), 101.1 (C-8), 66.3 (C-1'), 56.3 (6-OCH_3), 39.5 (C-4'), 26.1 (C-5'), 25.6 (C-9'), 17.7 (C-8'), 16.8 (C-10').

7-((2'*E*,5'*E*)-7'-Hydroxy-3',7'-dimethylocta-2',5'-dienyloxy)coumarin (**3**), pale yellow solid, UV (MeOH) λ_{maks} nm (log ε): 219 (4.31), 252 (3.52), 295 (4.06) and 323 (4.32). ^{1}H-NMR ($CDCl_3$) δ_H ppm: 7.63 (1H, d, *J* = 9.4 Hz, H-4), 7.36 (1H, d, *J* = 8.6 Hz, H-5), 6.85 (1H, dd, *J* = 8.6; 2.4 Hz, H-6), 6.81 (1H, d, *J* = 2.4 Hz, H-8), 6.24 (1H, d, *J* = 9.4 Hz, H-3), 5.68 (1H, d, *J* = 15.5 Hz, H-6'), 5.61 (1H, m, H-5'), 5.49 (1H, t, *J* = 6.6 Hz, H-2'), 4.59 (2H, d, *J* = 6.6 Hz, H-1'), 2.78 (2H, d, *J* = 6.1 Hz, H-4'), 1.74 (3H, s, H-10'), 1.32 (6H, s, H-9'/10'). ^{13}C-NMR ($CDCl_3$) δ_C ppm: 162.0 (C-7), 161.3 (C-2), 155.8 (C-9), 143.5 (C-4), 141.1 (C-3'), 140.4 (C-6'), 128.7 (C-5), 123.8 (C-5'), 119.2 (C-2'), 113.2 (C-6), 113.0 (C-3), 112.4 (C-10), 101.5 (C-8), 70.7 (C-7'), 65.4 (C-1'), 42.1 (C-4'), 29.8 (C-8'/9'), 16.8 (C-10').

7-((2'*E*,6'*E*)-5'-Hydroxy-3',7'-dimethylocta-2',6'-dienyloxy)coumarin (**4**), pale yellow solid, UV (MeOH) λ_{maks} nm (log ε): 220 (4.28), 252 (3.53), 296 (4.04) and 324 (4.29). ^{1}H-NMR ($CDCl_3$) δ_H ppm: 7.62 (1H, d, *J* = 9.5 Hz, H-4), 7.35 (1H, d, *J* = 8.6 Hz, H-5), 6.82 (1H, dd, *J* = 8.6; 2.4 Hz, H-6), 6.79 (1H, d, *J* = 2.4 Hz, H-8), 6.23 (1H, d, *J* = 9.4 Hz, H-3), 5.55 (1H, t, *J* = 6.5 Hz, H-2'), 5.16 (1H, d, *J* = 8.6 Hz, H-6'), 4.59 (2H, d, *J* = 6.5 Hz, H-1'), 4.51 (1H, m, H-5'), 2.25 (2H, m, H-4'), 1.80 (3H, s, H-10'), 1.69 (6H, s, H-8'), 1.67 (6H, s, H-9'). ^{13}C-NMR ($CDCl_3$) δ_C ppm: 161.9 (C-7), 161.3 (C-2), 155.8 (C-9), 143.4 (C-4), 138.9 (C-3'), 135.5 (C-7'), 128.7 (C-5), 127.2 (C-6'), 121.7 (C-

2'), 113.0 (C-3), 113.1 (C-6), 112.5 (C-10), 101.4 (C-8), 66.3 (C-5'), 65.1 (C-1'), 47.7 (C-4'), 25.7 (C-8'), 18.2 (C-9'), 17.0 (C-10').

Figure. 1. The structures of oxygeranylated coumarins *Limonia accidisima* L



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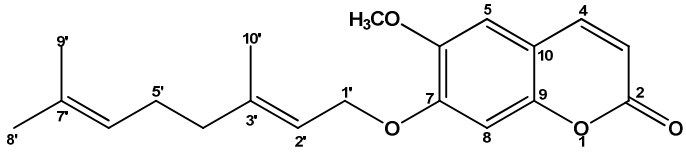

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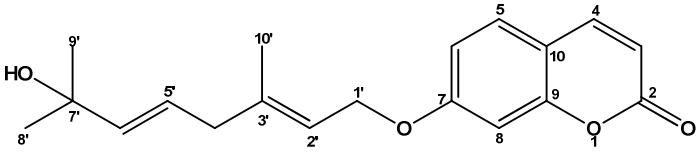

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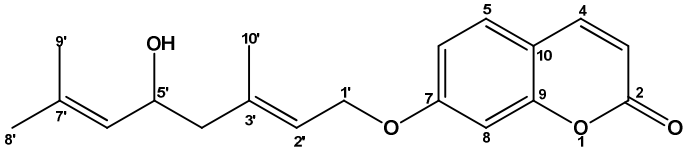

(4)

Determination of DPPH Radical Scavenging

Determination of the antioxidant activity of ethyl acetate extract, as well as compounds **1**-**4** performed using reagent DPPH (2,2-diphenyl-1-picrylhydrazyl) based on the methods of reduction of free radicals as measured by UV spectrometer at λ 517 nm [9,10,11]. Determination of antioxidant activity was done by dissolving a compound assay with methanol, then added solution of 0.1 M buffer acetate (pH 5.5) and added DPPH radical solution of 5.10^{-4} M. Determination of the inhibition of isolated compounds against DPPH radical was observed using a spectrometer at λ 517 nm after incubation for 30 min at 20°C. Samples were dissolved in ethanol at various concentrations (2000, 1000, 500, 100, 10 and 1 µg/mL). The inhibition percentage (%) of radical scavenging activity was calculated using the following equation: Inhibition (%) = $(A_o - A_s/A_{o)} \times 100$. Where A_o is the absorbance of the control reaction (containing all reagents except the test compound), and A_s is the absorbance of the test compound. The inhibitory concentration (IC_{50}) of the samples was calculated using a regression linear from the graph plotting scavenging activity against concentration. Assays were carried out in triplicate.

RESULTS AND DISCUSSION

Isolation geranylated coumarine of the ethyl acetate extract from *Limonia accidissima* L. was monitored by DPPH reagent using TLC bioauthography. Activity as DPPH radical scavenging showed by the yellow spot in the ethyl acetate extract. Based on the TLC bioauthography results showed that fraction C and fraction D had activity as DPPH radical scavenging. Separation and purification by radial chromatography of fractions C and D produced four compounds oxygeranylated coumarins, compounds **1**-**4**, auraptene (**1**), 6-methoxy-auraptene (**2**), 7-((2'*E*,5'*E*)-7'-hydroxy-3',7'-dimethylocta-2',5'-dienyloxy)coumarin (**3**) and 7-((2'*E*,6'*E*)-5'-hydroxy-3',7'-dimethylocta-2',6'-dienyloxy)coumarin (**4**).

DPPH radical is a paramagnetic and unstable radical that able to accept an electron or hydrogen radical to become a stable diamagnetic molecule. Classification of a highly active compound if in small concentrations can change the color DPPH from purple to yellow. The scavenging capacity of ethyl acetate extract, and compounds **1-4** were evaluated for their antioxidant properties against DPPH radical by using a spectrophotometric assay based on the ability of extract or pure compound to decrease DPPH oxidation. The data on antioxidant activity presented in Table 1, the ethyl acetate extract on evaluation for antioxidant activity against DPPH radical scavenging showing their IC_{50} of 174.7 µg/mL. The DPPH radical scavenging of ethyl acetate extract showed very high activity.

Table 1. Antioxidant activities of extracts and compounds 1–4

Compound	DPPH (µg/mL)
Ethyl acetate extract	174.7
Auraptene (**1**)	177.9
6-Methoxy-auraptene (**2**)	210.0
7-((2'*E*,5'*E*)-7'-Hydroxy-3',7'-dimethylocta-2',5'-dienyloxy)coumarin (**3**)	13.3
7-(2'*E*,5'*E*)-7'-Hydroxy-3'5'-hydroxy-3',7'-dimethylocta-2',5'-dienyloxy)coumarin (**4**)	281.0
Ascorbic acid	5.8

The ethyl acetate extract showed more active than auraptene (**1**), 6-methoxy-auraptene (**2**), and 7-((2'*E*,6'*E*)-5'-hydroxy-3',7'-dimethylocta-2',6'-dienyloxy)coumarin (**4**) however 7-((2'*E*,5'*E*)-7'-hydroxy-3',7'-dimethylocta-2',5'-dienyloxy)coumarin (**3**) more active than ethyl acetate extract and ascorbic acid. The study on the structure of the isolated compounds showed that addition of a methoxy group on compounds **2** and oxidation of the double bond of compound **1** yielding compounds **3** and **4** increases antioxidant activity. The relationship structure of compounds **1-4** based on the functional group was not significant to the antioxidant activity. However, polarity of compound seems contribute to the antioxidant activity, where the compound 3>4>2>1. Compounds 7-((2'E,5'E)-7'-hydroxy-3',7'-dimethylocta-2',5'-dienyloxy) coumarin (**3**) more active than ascorbic acid (positive control).

CONCLUSION

Four geranylated coumarin derivatives, auraptene (**1**), 6-methoxy-auraptene (**2**), 7-((2'*E*,5'*E*)-7'-hydroxy-3',7'-dimethylocta-2',5'-dienyloxy)coumarin (**3**) and 7-((2'*E*,6'*E*)-5'-hydroxy-3',7'-dimethylocta-2',6'-dienyloxy) coumarin (**4**) were isolated from the root of *Limonia accidissima* L. Compound 7-((2'*E*,5'*E*)-7'-hydroxy-3',7'-dimethylocta-2',5'-dienyloxy)coumarin **(3)** is very high activity against DPPH radical.

Acknowledgements
This research was supported by The Directorate General of Strengthening Research and Development, Ministry of Research, Technology and Higher Education, Republic of Indonesia (PUPT, Universitas Airlangga, 2016).

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