SHORT REPORT

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Chemical Constituents of *Canarium subulatum* and Their Anti-herpetic and DPPH Free Radical Scavenging Properties

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Abstract: A methanol extract prepared from the bark of *Canarium subulatum* Guillaumin (Burseraceae) showed significant anti-herpetic activity. Chemical investigation of this plant extract resulted in the isolation of nine compounds which included *β*-amyrin (**1**), (-)-cubebin (**2**), scopoletin (**3**), 3,4-dihydroxybenzoic acid (**4**), 3,3′-di-*O*-methylellagic acid-4′-*O*-*α*-L-rhamnopyranoside (**5**), 3,3′-di-*O*-methylellagic acid-4′-*O*-*β*-D-glucopyranoside (**6**), 3-*O*-methylellagic acid-4′-*O*-*α*-L-arabinofuranoside (**7**), scopolin (**8**) and 3-*O*-methylellagic acid-4′-*O*-*β*-D-xylopyranoside (**9**). The structures of these compounds were determined mainly through analysis of ^{1}H and ^{13}C NMR and MS data. All of the isolates were evaluated for anti-herpetic and DPPH free radical scavenging properties. *β*-Amyrin (**1**) and (–)-cubebin (**2**) showed moderate anti-herpetic activity, whereas 3,4-dihydroxybenzoic acid (**4**), 3,3′-di-*O*-methylellagic acid-4′-*O*-*α*-L-rhamnopyranoside (**5**) exhibited recognizable DPPH free radical scavenging potential. This study is the first report on the chemical and biological properties of *C. subulatum*.

Keywords: *Canarium subulatum*; Ellagic acid glycosides; Burseraceae; Anti-herpetic activity; Free radical scavenging activity.

1. Plant Source

In Thailand, about twelve species of *Canarium* have been recognized [1]. *Canarium subulatum* Guillaumin, locally known as “Makok kluean”, is a 10 – 15 m high tree growing throughout Thailand. The fruits of this plant are edible, and have been used by the local people as expectorant. Its white aromatic latex, which exudes from the stem bark when cut or bruised, is used for treating skin itching [2].

The stem bark of *C. subulatum* Guillaumin was collected from Rong Kwang district, Phrae Province, Thailand, in March 2008 and identified by Prof. N. Boonplod. A voucher specimen (BS-CS-032551) is on deposit at the Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

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2. Previous Studies

Plants of the genus *Canarium* (Burseraceae) have been known to produce a wide variety of chemical compounds, including terpenoids, tannins, and biflavonoids [3-5]. Recent biological studies have shown that some *Canarium* species possess hepatoprotective, anti-diabetic and antioxidant activities [4-7]. Prior to this investigation, there were no reports on the chemical and biological properties of this plant.

3. Present Study

As a part of our continuing studies on bioactive compounds from Thai medicinal plants [8, 9], an extract prepared from the bark of this plant was evaluated and found to have inhibitory activity against the *Herpes simplex* virus type 1 (HSV-1), a virus that causes itchy skin and burning blisters. This initial bioassay result was consistent with the traditional use of *C. subulatum* bark as an anti-skin itching medicine. Moreover, the extract was also found to possess scavenging activity against the DPPH free radical. These preliminary observations prompted us to conduct an investigation on the extract in order to find the compounds responsible for these bioactivities. In this communication, we report the chemical components of the bark of this plant, as well as their antiherpetic and DPPH free radical properties.

Dried and powdered stem bark of *C. subulatum* (2.5 kg) was successively extracted with CH_2Cl_2, and MeOH, at room temperature to yield a CH_2Cl_2 extract (17 g) and a MeOH extract (185 g), respectively. A portion of the CH_2Cl_2 extract (3.5 g) was initially subjected to column chromatography (CC) on silica gel (EtOAc-hexane, gradient) to give 41 fractions. Compound **1** (68 mg) was obtained from fraction 8. Fractions 21-24 (330 mg) were combined and dried, and then further separated on Sephadex LH-20 ($CHCl_3$) to give **2** (4 mg). The MeOH extract (150 g) was fractionated by vacuum-liquid chromatography (VLC) on silica gel (acetone-EtOAc-H_2O polarity gradient and then acetone-MeOH-H_2O polarity gradient) to afford 9 fractions (A-I). Fraction A (1.7 g) was resubjected to repeated CC (silica gel; EtOAc-hexane gradient) to give 9 fractions (A1-A9). Fraction A5 (258 mg) was separated on Sephadex LH-20 (acetone) to yield **3** (5 mg). Fractions A6 (135 mg) was purified on Sephadex LH-20 column (acetone) to give **4** (70 mg). Fraction E (6.5 g) was subjected to CC over silica gel (EtOAc-acetone gradient with 1% H_2O) to afford 8 fractions (E1-E8). Compound **5** (58 mg) was obtained from fraction E2 after recrystallization from MeOH. Fraction E3 (661 mg) was separated by CC (silica gel; 10-15% MeOH in CH_2Cl_2) to give 44 fractions. Fractions 29-30 (302 mg) were combined and further purified on Sephadex LH-20 (MeOH) to give **6** (5 mg). Fraction E4 (609 mg) was separated by CC (silica gel; 10-15% MeOH in CH_2Cl_2) and then on a Sephadex LH-20 (MeOH) column to yield **7** (5 mg). Separation of fraction E5 (387 mg) on silica gel (5-10% MeOH in CH_2Cl_2) and then on Sephadex LH-20 (MeOH) afforded **8** (18 mg). Fraction E6 (526 mg), after purification on a Sephadex LH-20 (MeOH) column, gave **9** (3 mg).

The structures of the isolates were determined through analysis of their spectroscopic data in comparison with reported values, and they were identified as *β*-amyrin (**1**) [10], (–)-cubebin (**2**) [11], scopoletin (**3**) [12], 3,4-dihydroxybenzoic acid (**4**) [13], 3,3′-di-*O*-methylellagic acid-4′-*O*-*α*-L-rhamnopyranoside (**5**) [14], 3,3′-di-*O*-methylellagic acid-4′-*O*-*β*-D-glucopyranoside (**6**) [15], 3-*O*-methylellagic acid-4′-*O*-*α*-L-arabinofuranoside (**7**) [16,17], scopolin (**8**) [18] and 3-*O*-methylellagic acid-4′-*O*-*β*-D-xylopyranoside (**9**) [19].

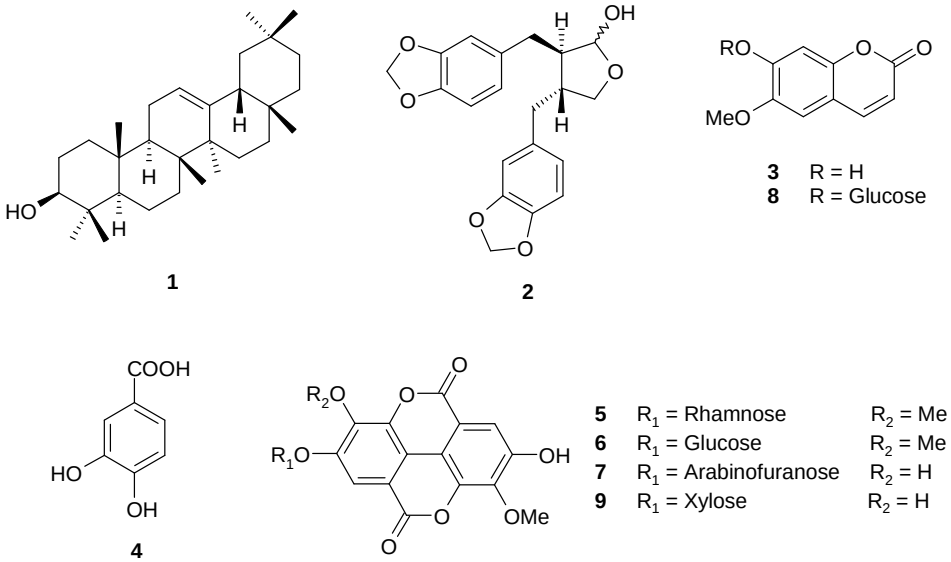

Figure 1. Compounds **1**-**9** isolated from *C. subulatum*.

Table 1. IC_{50} values for inhibitory activities against HSV-1 and DPPH free radical.

Compound	IC_{50} (μM)		Compound	IC_{50} (μM)	
	HSV-1	DPPH		HSV-1	DPPH
1	234	NA	**7**	NA	NA
2	280	NA	**8**	NA	NA
3	NA	NA	**9**	NA	NA
4	NA	10.9	Acyclovir*	2.9	NA
5	NA	32.9	quercetin	NA	4.3
6	NA	NA			

*positive control; NA = less than 50 % inhibition at 100 μg/mL.

As summarized in Table 1, these isolates (**1** – **9**) were evaluated for anti-herpetic and DPPH free radical scavenging activities, using established protocols [9, 20]. The details of these assays are described in the supporting information. The triterpene *β*-amyrin (**1**) and the lignan (–)-cubebin (**2**) were found to possess anti-herpetic activity, although with lower inhibitory effects (IC_{50} values of 234 and 280 μM, respectively) as compared with the positive control acyclovir (IC_{50} 2.9 μM). The presence of these two compounds might be taken as supporting evidence for the traditional use of the bark of this plant as anti-skin itching medication. As for the DPPH free radical scavenging activity, only 3,4-dihydroxybenzoic acid (**4**) and 3,3′-di-*O*-methylellagic acid-4′-*O*-*α*-L-rhamnopyranoside (**5**) demonstrated recognizable activity, with IC_{50} values of 10.9 and 32.9 μM, respectively (quercetin IC_{50} 4.3 μM). The relatively higher activity of **4**, in comparison with **5**, was probably due to the presence of the *ortho*-diphenolic functionality in its structure.

The coumarins **3** and **8** are most likely the chemicals responsible for the pleasant aroma that is generated when the bark of the plant is cut or bruised. Finally, to the best of our knowledge, this study is the first report on the chemical constituents and biological activities of *C. subulatum*.

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Supporting Information

Supporting information accompanies with this paper on http://www.acgpubs.org/RNP

References

[1] T. Smitinand (2001). Thai plant names revised edition. Bangkok: The Forest Herbarium, Royal Forest Department, 102-103.

[2] A. Petelot (1954). Les Plantes Medicinales du Cambodge, du Laos et du Viet-Nam 3. Saigon, 263.

[3] W. M. Bandaranayake (1980). Terpenoids of *Canarium zeylanicum*, *Phytochemistry.* **19**, 255-257.

[4] D. Billet, S. Heitz, D. Raulais and A. Matschenko (1971). Constituents terpeniques de *Canarium boivinii* ENGL, *Phytochemistry.* **10**, 1681-1683.

[5] L. L. Zhang and Y. M. Lin (2008). Tannins from *Canarium album* with potent antioxidant activity, *J. Zhejiang Univ. Sci. B.* **9**, 407-415.

[6] K. K. Anand, V. N. Gupta, V. Rangari, B. Singh and B. K. Chandan (1992). Structure and hepatoprotective activity of a biflavonoid from *Canarium manii*, *Planta Med.* **58**, 493-495.

[7] M Tamai, N. Watanabe, M. Someya, H. Kondoh, S. Omura, Z. P. Ling, R. Chang and C. W. Ming (1989). New hepatoprotective triterpenes from *Canarium album*, *Planta Med*. **55**, 44-47.

[8] K. Likhitwitayawuid, B. Sritularak, K. Benchanak, V. Lipipun, J. Mathew and R. F. Schinazi (2005). Phenolics with antiviral activity from *Millettia erythrocalyx* and *Artocarpus lakoocha*, *Nat. Prod. Res.* **19**, 177-182.

[9] B. Sritularak, K. Tantrakarnsakul, K. Likhitwitayawuid and V. Lipipun (2010). New 2-arylbenzofurans from the root bark of *Artocarpus lakoocha*, *Molecules.* **15**, 6548-6558.

[10] S. A. Knight (1974). Carbon-13 NMR spectra of some tetra- and pentacyclic triterpenoids, *Org. Mag. Res.* **6**, 603-611.

[11] H. Matsuda, Y. Kawaguchi, M. Yamazaki, N. Hirata, S. Naruto, Y. Asanuma, T. Kaihatsu and M. Kubo (2004). Melanogenesis stimulation in murine B16 melanoma cells by *Piper nigrum* leaf extract and its lignan constituents, *Biol. Pharm. Bull.* **27**, 1611-1616.

[12] J. M. J. Vasconcelos, A. M. S. Silva and J. A. S. Cavaleiro (1998). Chromones and flavanones from *Artemisia campestris* subsp. *maritime*, *Phytochemistry.* **49**, 1421-1424.

[13] G. Flamini, E. Antognoli and I. Morelli (2001). Two flavonoids and other compounds from the aerial parts of *Centaurea bracteata* from Italy, *Phytochemistry.* **57**, 559-564.

[14] S. Malhotra and K. Misra (1981). 3,3′-Di-*O*-methylellagic acid 4-*O*-rhamnoside from the roots of *Prosopis juliflora*, *Phytochemistry.* **20**, 2043-2044.

[15] G. Ye, H. Peng, M. Fan and C. G. Huang (2007). Ellagic acid derivatives from the stem bark of *Dipentodon sinicus*, *Chem. Nat. Compd.* **43**, 125-127.

[16] L. Zhou, D. Li, W. Jiang, Z. Qin, S. Zhao, M. Qiu and J. Wu (2007). Two ellagic acid glycosides from *Gleditsia sinensis* Lam. with antifungal activity on *Magnaporthe grisea*, *Nat. Prod. Res.* **21**, 303-309.

[17] L. Ye and J. S. Yang (1996). New ellagic glycosides and known triterpenoids from *Duchesnea indica* Focke, *Yao Xue Xue Bao.* **31**, 844-848.

[18] H. Sibanda and B. Ndengu (1989). A coumarin glucoside from *Xeromphis obovata*, *Phytochemistry.* **28**, 1550-1552.

[19] X. H. Yan and Y. W. Guo (2004). Two new ellagic acid glycosides from leaves of *Diplopanax stachyanthus*, *J. Asian Nat. Prod. Res.* **6**, 271-276.

[20] B. Sritularak, M. Anuwat and K. Likhitwitayawuid (2011). A new phenenthrenequinone from *Dendrobium draconis*, *J. Asian Nat. Prod. Res.* **13**, 251-255.

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