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radical

*by* Nanik Siti Aminah

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## A new pyrano coumarin from *Clausena excavata* roots displaying dual inhibition against $\alpha$ -glucosidase and free radical

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### ABSTRACT

A new pyrano coumarin, identified as excavatin A (**1**) together with two known compounds nordentatin (**2**) and binorpocitrin (**3**) was isolated from the 95% EtOH extract of *Clausena excavata*. All structures were elucidated by using spectroscopy methods such as extensive NMR and HR-FAB-MS spectrometry. All the isolated compounds were tested on antidiabetes activity by using  $\alpha$ -glucosidase inhibition assay and the antioxidant activity by DPPH assay. Compounds **1-3** showed antioxidant activity with IC<sub>50</sub> values 0.286, 0.02, 0.278 mM. Among them, **2** exhibited inhibition activity against maltase (IC<sub>50</sub> 5.45  $\mu$ M) and sucrase (IC<sub>50</sub> 43.57  $\mu$ M). However, compounds (**1**) and (**3**) displayed inhibition on yeast  $\alpha$ -glucosidase with IC<sub>50</sub> values 1.92 and 5.58 mM.

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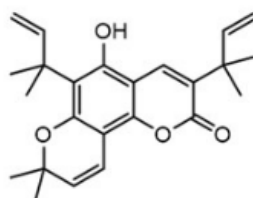
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*Clausena excavata*; pyrano coumarin; inhibition;  $\alpha$ -glycosidase; antioxidant; IC<sub>50</sub>




*Clausena excavata*




excavatin A (**1**)

## 1. Introduction

Diabetes mellitus (DM) is the common endocrinal disorder and rapidly increasing disease in the human population all over the world. The numbers concerning the

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9 prevalence of DM are alarming; about 415 million people worldwide are estimated to have diabetes, expected that the numbers will rise to 642 million or more diabetic patients in 2040 (Gothai et al. 2016). Diabetes Mellitus can be classified into clinical categories as: Type 1 Diabetes (T1DM), caused by  $\beta$ -cell destruction and usually leading to absolute insulin deficiency, Type 2 Diabetes (T2DM), due to a defect on the background of insulin resistance, and others such as gestational diabetes and specific types such as monogenic diabetes syndromes, exocrine pancreas diseases, and drug or chemical-induced diabetes (Deepak et al. 2014; Munhoz and Frode 2018). Although obesity and physical inactivity are known to be major risk factors for type 2 diabetes (T2DM), recent evidence suggests that oxidative stress may contribute to the pathogenesis of T2DM by increasing insulin resistance or impairing insulin secretion (Bajaj and Khan 2012). Various studies have shown that diabetes mellitus is associated with increased formation of free radicals and decreases antioxidant potential which, leads to disturbances in the balance between radical formation and protection against which ultimately results in oxidative damage of cell components such as proteins, lipids, and nucleic acids. An increased oxidative stress can be observed in both insulin-dependent (type 1) and non-insulin-dependent diabetes (type 2) (Sindhi et al. 2013). Type 2 diabetes is the predominant form of diabetes and accounts for at least 90% of all cases of diabetes. One of the established therapeutics to treat type 2 diabetes is to control blood glucose levels after eating. Decreased blood glucose levels after eating can be done with delaying the absorption of glucose by inhibiting the enzyme  $\alpha$ -glucosidase activity (Cahyani and Purwaningsih 2015). In recent years, some of the standard synthetic drugs used for the treatment of diabetes lead to cause side effects like nausea, vomiting, abdominal pain, diarrhoea, head ache, abnormal weight gain, allergic reaction, low blood glucose, dark urine, fluid retention or swelling. Moreover, they are not safe for use during pregnancy. Active research has been performed on traditional available medicinal plants for discovery of new antidiabetic drug as an alternative for synthetic drugs (Abirami et al. 2014).

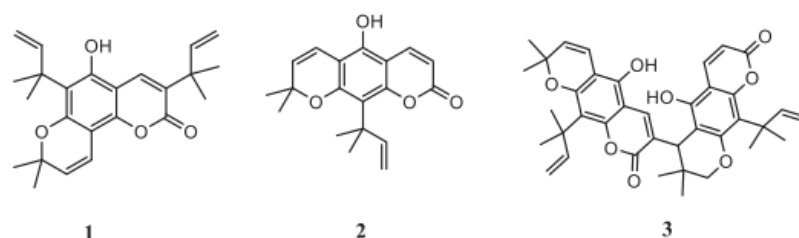
10 The genus *Clausena* belongs to the Rutaceae family, comprises of about 14 species of evergreen trees (Arbab et al. 2013). *C. excavata* is one the most well-known species in genus *Clausena*, is a shrub with strong and rather objectionable smell, found from the Himalayas and China to and throughout Southeast Asia (Taufiq-Yap et al. 2007). In Myanmar, it is locally known as 'Sat pu Kharyar', 'Taw Pyin Daw Thein'. People in Myanmar usually used this plant to treat headache, itching, flu, snake-bite detoxification. *Clausena spp.* is an abundant source of secondary metabolites, especially carbazole alkaloids, coumarins (furano and pyrano) and few lemonoids (Wu et al. 1999; Ito et al. 2000, Kumar et al. 2012; Liu et al. 2018). Many pharmacologically active compounds isolated from *Clausena* species have been used for the treatment of human diseases such as cardiovascular disease, anti-inflammatory, antioxidants, anti-snake venom, anticancer, anti-HIV, and antiplatelet (Auranwiwat et al. 2014; Arbab et al. 2015; Chakthong et al. 2016; Ma et al. 2017). In addition, *Clausena* species has been found to possess anti-diabetic activity (Damsud et al. 2017). The current research is conducted to isolate bioactive antidiabetic and antioxidant compounds from the roots of *C. excavata*. A new pyrano coumarin (**1**), excavatin A along with two pyrano coumarin, nordentatin (**2**), binorpocitrin (**3**) was isolated. Based on our knowledge, there was

not yet reported on  $\alpha$ -glucosidase inhibitory activity and antioxidant activity of these isolated compounds. So we conducted to these bioactive compounds on  $\alpha$ -glucosidase inhibition activity by using yeast and rat intestinal (maltase, sucrose) enzymes and antioxidant activity was performed by DPPH assay.

## 2. Results and discussion

The excavatin-A (**1**) was obtained as a colorless crystal with melting point 261–262 °C and it was assigned the molecular formula,  $C_{24}H_{28}O_4$ , as deduced from the positive HR-FAB-MS at  $m/z$  381.2058  $[M + H]^+$  (calcd for  $C_{24}H_{28}O_4$ , 381.2066). The IR spectrum indicated the presence of hydroxyl ( $3174\text{ cm}^{-1}$ ), conjugated lactone ( $1670\text{ cm}^{-1}$ ) and aromatic ( $1608$ ,  $1591$ ,  $1469\text{ cm}^{-1}$ ) groups. The UV spectrum showed absorption maxima at 335, 278 and 227 nm due to 7-oxygenated coumarin.

The  $^1\text{H}$ -NMR spectrum (Table S1) displayed the presence of pyrone substituted coumarin was revealed by the aromatic singlet proton at  $\delta_{\text{H}}$  7.90 (1H, s, H-4). The existence of olefinic protons of chromene ring was indicated by two peaks at  $\delta_{\text{H}}$  6.56 (1H, d,  $J = 9.9\text{ Hz}$ , H-9) and  $\delta_{\text{H}}$  5.66 (1H, d,  $J = 9.9\text{ Hz}$ , H-10) respectively. The existence of two pairs of exomethylene protons was displayed at  $\delta_{\text{H}}$  6.18 (1H, dd,  $J = 16.5$ ,  $10.6\text{ Hz}$ , H-2') and 5.08 (1H, dd,  $J = 16.5$ ,  $1.2\text{ Hz}$ , H-3'a), 5.06 (1H, dd,  $J = 1.2$ ,  $10.6\text{ Hz}$ , H-3'a) and another attached to C-6 was revealed 1,1-dimethyl group at  $\delta_{\text{H}}$  6.29 (1H, dd,  $J = 17.4$ ,  $10.6\text{ Hz}$ , H-2''), 4.92 (1H, dd,  $J = 17.4$ ,  $1.1\text{ Hz}$ , H-3a'') 4.85 (1H, dd,  $J = 10.6$ ,  $1.2\text{ Hz}$ , H-3b''). At the aliphatic regions 3 pairs of 1,1 dimethyl group showed three singlets at  $\delta_{\text{H}}$  1.42 (s, 6H, H-11a, H-11b), 1.47 (s, 6H, H- 1a' and 1b'), 1.63 (s, 6H, H- 1a'' and 1b'') (Table S1). The  $^{13}\text{C}$ -NMR spectrum of compound (**1**) indicated the presence of one cyclic lactone carbonyl carbon, five  $\text{sp}^2$  and three  $\text{sp}^3$  quaternary carbons, five methane carbons, two exomethylene carbons and six methyl carbons (Table S1). The 2D NMR, DQF-COSY spectrum displayed the adjacent proton-proton correlation of three sets of proton pairs  $\delta_{\text{H}}$  6.56 (H-9) and  $\delta_{\text{H}}$  5.66 (H-10),  $\delta_{\text{H}}$  6.18 (1H, dd,  $J = 16.5$ ,  $10.6\text{ Hz}$ , H-2') and 5.08 (H-3'a), 5.06 (H-3'a),  $\delta_{\text{H}}$  6.29 (H-2'') and 4.92 (H-3a'') 4.85 (H-3b'') (figure S1). The inter-correlation of basic coumarin with chromene ring and two prenyl groups were confirmed by  $^1\text{H}$ - $^{13}\text{C}$  long range coupling of HMBC spectrum (Figure S6). The HMBC spectrum of compound (**1**) showed some correlation between H-4/C-1', C-2, C-3, C-5, C-8a. It was revealed that the pyrone substituted coumarin with the position of H-4 proton and the prenyl group that attached to C-3 carbon of pyrone ring. Another prenyl group proton H-1a'', 1b''/C-1'', C-2'', C-6 revealed the attachment of prenyl to C-6 position of core coumarin.  $^1\text{H}$ -NMR and  $^{13}\text{C}$ -NMR of compound (**1**) is similar with clausarin that chromene ring was attached to core coumarin linearly. But in the HMBC spectrum of compound (**1**) strong correlation between H-9 ( $\delta$  6.56) to C-8 ( $\delta$  106.0), C-8a ( $\delta$  146.5) and C-7 ( $\delta$  154.9) by HMBC spectrum (Figure S6) (Figure S1) showed that chromene ring was angularly connected to core coumarin (Takemura et al. 1996). Moreover, clausarin has to showed correlation between H-11 ( $\delta$  6.56 Hz) with C-6 (115.4 Hz). The correlation was not found in compound (**1**) (Table S1, figure S6). So it is showed that the structure is more reliable to compound (**1**). The NOESY spectrum of (**1**) showed the cross-peak of H-4 proton with 1,1dimethyl protons H-1a' and 1b' and exomethylene protons H-1'. Another cross peak displayed H-10 to H-9,



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Figure 1. Structures of isolated compounds from *C. excavata*.

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Table 1.  $\alpha$ -glucosidase inhibitory activities of isolated compounds (1-3).

Compound	$\alpha$ -Glucosidase			DPPH IC <sub>50</sub> (mM)
	Baker's yeast IC <sub>50</sub> (mM)	Maltase IC <sub>50</sub> ( $\mu$ M)*	Sucrase IC <sub>50</sub> ( $\mu$ M)*	
1	1.92	NI	NI	0.286
2	37.62	5.45	43.57	0.02
3	5.58	NI	NI	0.278
Acarbose	0.1030	2.35	15.48	—
Ascorbic acid	—	—	—	0.0118

\*Nonlinear regression analyzes were evaluated by SigmaPlot 12.5.

H-11a, H-11b and H-2'' to H-1a'', H-1b'', H-3''. Hence the structure of compound (1) was unambiguously elucidated to be 5-hydroxy-8,8-dimethyl-3,6-bis(2-methylbut-3-en-2-yl)-2H,8H-pyrano[2,3-f]chromen-2-one (Figure 1).

By analysis of the physicochemical properties, NMR, and MS data, and comparison with those reported in the literature, the two known compounds were identified as pyrano coumarin, namely, nordentatin (2) and binorpocitrin (3) (Sripisut et al. 2012). All compounds were further evaluated for their inhibitory effects against  $\alpha$ -glucosidase such as baker's yeast and rat intestinal (maltase and sucrase)  $\alpha$ -glucosidase and antioxidant activity were measured by DPPH assay (Table 1). All compounds 1-3 exhibited radical scavenging activity with IC<sub>50</sub> values 0.286, 0.02, 0.278 mM and displayed against sucrase with IC<sub>50</sub> values 1.92, 37.62 and 5.58 mM respectively. However, in maltase  $\alpha$ -glucosidase assay nordentatin (2) showed inhibition 5.45  $\mu$ M but compound 1 and 3 showed no inhibition.

## 12 3. Experimental

### 3.1. Plant material

The roots of *C. excavata* were collected from Pyin Ma Nar Township, Mandalay Division, Myanmar in October 2016. The plant materials were authenticated by Prof. Soe Myint Aye, botanist from Department of Botany, Mandalay University, Myanmar, where the voucher specimen (UM-22032018) was deposited.

### 14 3.2. Extraction and isolation

The dried roots (3.6 kg) were extracted successively with 95% EtOH (12.0L) over a period of two weeks at room temperature. Removal of the solvent under reduce pressure gave 156 g of dark gummy extracts. The extract (100g) was partitioned three times using solvents; *n*-hex: methanol (1:1, v/v) successfully. Then methanol portion (80.4 g) was fractionated by vacuum liquid chromatography over silica gel eluted

with different mixtures of *n*-hex: EtOAc by stepwise increasing gradient polarity gave a total of 7 combined fractions (MF-1 to -7) were obtained. Among them the pale yellow crystals were come out from the combine fraction MF-5. After washing with ethyl acetate the pure compound nordentatin (**2**, 2.1 g) was afforded. The sub fraction MF-2 was fractionated by silica gel column eluting with gradient polarity *n*-hex: EtOAc, (0-10% EtOAc) afforded new compound, excavatin A (**1**, 68 mg). The fraction MF-6 (25.6 g) was subjected to VLC chromatography with *n*-hex:EtOAc (EtOAc, 10-100%) with gradient polarity and afforded 23 subfraction and after combining same component fractions gave (MF-6.1 to 6.7). Fraction MF-6.2 (3.4 g) was subjected to silica gel column chromatography with three solvent system (*n*-hex:CHCl<sub>3</sub>:EtOAc/100- 70: 5-20:5-20) afforded a total of 300 fractions. White amorphous solid was come out from 201-215 and yielded pure compound, binorponcitrin (**3**, 138 mg).

Excavatin A, colorless crystal, mp. 261-262 °C; UV (MeOH),  $\lambda_{\max}$  (log $\epsilon$ ): 335 (1.23), 279 (2.08), 228 (1.53). FT-IR (KBr) cm<sup>-1</sup>: 3174, 2968, 1670, 1608, 1591, 1570, 1465, 1379, 1340, 1186, 1147, 1026, 908, 891. <sup>1</sup>H NMR and <sup>13</sup>C NMR data see (Table S1), HR-FAB-MS (*m/z* [M<sup>+</sup>] H<sup>+</sup>) 381.2058, (calcd for C<sub>24</sub>H<sub>28</sub>O<sub>4</sub>, 381.2066).

#### 4. Conclusion

In summary, three compounds were isolated from Myanmar medicinal plant *C. excavata* including a new pyrone substituted coumarin (**1**) together with two known bioactive compounds (**2-3**). The examination of all isolated compounds was done on antidiabetes activity by using  $\alpha$ -glucosidase inhibition assay and the antioxidant activity was conducted by DPPH assay. Of isolated compounds, **2** exhibited inhibition activity against maltase (IC<sub>50</sub> 5.45  $\mu$ M) and sucrase (IC<sub>50</sub> 43.57  $\mu$ M) respectively. However, compounds (**1**) and (**3**) displayed inhibition on yeast  $\alpha$ -glucosidase with IC<sub>50</sub> values 1.92, and 5.58 mM. Moreover, all isolated compounds showed high antioxidant activity with IC<sub>50</sub> values values 0.286, 0.02, 0.278 mM respectively. According recent study, isolated components from root of *C. excavata* can be candidate of natural antidiabetes and antioxidant.

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#### Disclosure statement

No potential conflict of interest was reported by the authors.

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