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Abstract

A phytochemical investigation of the fruit and root extracts of Micromelum integerrimum resulted in the isolation and identification of a new compound, integerravone (1), together with 23 known compounds (2-24). Their structures were characterized by spectroscopic methods as well as comparisons made from the literature. Compounds 2, 3-15, 17-18 and 20-23 were evaluated for their cytotoxicities against the colon cancer cell line, HCT116. All of them were inactive at 50 μM. Most of the phenolic compounds were evaluated for their antioxidant activity using the DPPH assay. Compounds 14 and 22-24 showed antioxidant activity with IC50 values ranging from 24.83-135.05 μM.

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Coumarins and flavones from the fruit and root extracts of *Micromelum integerrimum*

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Abstract

A phytochemical investigation of the fruit and root extracts of *Micromelum integerrimum* resulted in the isolation and identification of a new compound, integerravone (**1**), together with 23 known compounds (**2-24**). Their structures were characterized by spectroscopic methods as well as comparisons made from the literature. Compounds **2**, **3-15**, **17-18** and **20-23** were evaluated for their cytotoxicities against the colon cancer cell line, HCT116. All of them were inactive at 50 μM. Most of the phenolic compounds were evaluated for their antioxidant activity using the DPPH assay. Compounds **14** and **22-24** showed antioxidant activity with IC_{50} values ranging from 24.83-135.05 μM.

Keywords: *Micromelum integerrimum*; Rutaceae; Coumarin; Flavone

1. Introduction

Micromelum integerrimum belongs to the Rutaceae family which is widely distributed throughout South and Southeast Asia. Decoctions made from the fruit of this plant have been

used in the treatment of jaundice, while those made from the twigs, have been used to treat dysentery by the indigenous community of Tripura, India (Deb et al. 2016). Decoctions of the roots, bark, and leaves have been used to treat malaria, rheumatic, arthralgia and traumatic injuries in traditional Chinese medicine (Fang et al. 2003). In previous phytochemical investigations of the *Micromelum* genus, coumarins (Cassady et al. 1979; Das et al. 1994; Kamperdick et al. 1999; Ito et al. 2000; Rahmani et al. 2003; Lekphrom et al. 2011; Siridachakorn et al. 2012; Phakhodee et al. 2013; Phakhodee et al. 2014; Lekphrom et al. 2016), quinolone alkaloids (Tantivatana et al. 1983), polyoxygenated flavonoids (Phakhodee et al. 2013; Yan et al. 2015), phenylpropanoids (Wang et al. 2014), triterpenoids (Susidarti et al. 2006; Susidarti et al. 2009), benzene derivatives (Yan et al. 2015), norsesquiterpenoid (Yan et al. 2015), triterpenoids (Susidarti et al. 2006; Susidarti et al. 2009), and acridone (Yang et al. 2009), indole (Kong et al. 1988), and carbazole alkaloids (Siridachakorn et al. 2012; Sakunpak et al. 2013) have been isolated and identified. Some of these compounds exhibited a wide range of biological and pharmacological activities, including cytotoxicity (Tantivatana et al. 1983; Susidarti et al. 2009; Lekphrom et al. 2011; Sakunpak et al. 2013; Lekphrom et al. 2016), and anti-mycobacterial (Lekphrom et al. 2011), anti-mutagenicity (Nakahara et al. 2002), anti-corpulence (Hitara et al. 2009), and anti-platelet (Chen et al. 2003) activities. Herein, we report the isolation and structure elucidation of a new compound (**1**) together with 23 known compounds (**2**-**24**) from the root and fruit extracts of *M. integerrimum*. Some of these compounds were tested for their cytotoxicities against a colon cancer cell line and for their antioxidant activities.

2. Results and discussion

The individual EtOAc extracts of the roots and fruit of *M. integerrimum* were separated by various chromatographic techniques to obtain a new compound (**1**) together with 23 known compounds (**2**-**24**), 6-(3-methyl-2-oxobutyroyl)-7-methoxycoumarin (**2**) (Li et al. 2016), phebalosin (**3**) (Ito et al. 1987), murralongin (**4**) (Talapatra et al. 1973), osthenon (**5**) (Ito et al. 1987), microminutin (**6**) (Suthiwong et al. 2014), minutuminolate (**7**) (Lekphrom et al. 2016), murrangatin acetate (**8**) (Ito et al. 1987), (-)-murrangatin (**9**) (Ito et al. 1990), 2′-*O*-ethylmurrangatin (**10**) (Choudhary et al. 2002), minumicrolin (**11**) (Ito et al. 1990), hopeyhopin (**12**) (Dominguez et al. 1977), dehydrogeijerin (**13**) (Dominguez et al. 1977), isoscopoletin (**14**) (Shafizadeh et al. 1970), scopoletin (**15**) (Cassady et al. 1979), citropten (**16**) (Gray et al. 1978), micromelin (**17**) (Cassady et al. 1979), dihydromicromelin B (**18**) (Das et al. 1994), acetyldihydromicromelin A (**19**) (Das et al. 1994), flindulatin (**20**) (Collins

et al. 2004), gossypetin 3,7,8,4′-tetramethylether (**21**) (Wollenber et al. 2008), 5,7-dihydroxy-3,4′,6,8-tetramethoxyflavone (**22**) (Silva et al. 2005), 5,7-dihydroxy-3,8,4′-trimethoxyflavone (**23**) (Tandon et al. 1977) and acerosin (**24**) (Greenham et al. 2001).

Compound **1** was isolated as a yellow solid with mp. 128-130 ^{o}C. This compound showed a $[M+Na]^+$ ion peak at *m/z* 437.1217 (calcd 437.1212) in the ESITOFMS spectrum indicating the molecular formula of $C_{22}H_{22}O_8$. The ^{1}H NMR and ^{13}C NMR spectra of **1** (Table S1, Supplementary material) displayed resonances for a hydrogen-bonded hydroxy proton [δ_H 12.37 (1H, s, OH-5)], a 1,4-disubstituted aromatic ring [δ_H 8.19 (2H, d, J = 9.0 Hz, H-2′/H-6′)/δ_C 130.4 and 7.07 (2H, d, J = 9.0 Hz, H-3′/H-5′)/δ_C 114.3], a pentasubstituted aromatic ring [δ_H 6.23 (1H, s, H-6)/δ_C 105.0] and three methoxy groups [δ_H 3.90 (3H, *s*, H-7)/δ_C 60.1 and 3.93 (6H, s, H-8/H-4′)/δ_C 55.7]. The locations of these methoxy groups at C-7 (139.4), C-8 (132.5) and C-4′ (162.1) were indicated from the HMBC correlations shown in Figure S10 and Table S1 (Supplementary material). The isobutyroyl substituent was evident from the NMR spectroscopic data by the resonances at δ_H 2.93 (1H, sept, J = 7.0 Hz, H-2″)/δ_C 33.9, δ_H 1.40 (6H, *d*, J = 7.0 Hz, H-3″/H-4″)/δ_C 19.0 and the resonance for the ester carbon at δ_C 174.8 (C-1″). The unusual lower field chemical shift of the sp^2 carbon C-3 (δ_C 148.3) suggested the flavone core structure and the isobutyroyl unit was linked via the ester linkage at C-3 of the flavone skeleton. The fragment ion at *m/z* 344 (68%) $[M–C_4H_7O^{\bullet} + H]^+$, in the EIMS spectrum of compound **1** also supported this ester linkage. Therefore, compound **1** was named as integerravone.

A proposed biogenetic pathway of the prenylated coumarins is shown in Scheme S1 (Supplementary material). Coumarin **25** (Lekphrom et al. 2016) is proposed as a precursor of all prenylated coumarins via various reactions, including oxidation, reduction, elimination, cylization, alkylation and acylation pathways. It should be note that the biogenetic pathway of intermediates **25.1** and **25.4,** obtained from compound **25,** has already been determined (Bourgaud et al. 2006). From Pathway A, a major route, the coumarins **2**, **12**, **13** and **17**-**19** would be produced. While Pathway B would produce coumarins **3**, **5** and **8**-**10**, and Pathway C would result in the coumarins, **4** and **6**.

Compounds **2**, **3**-**15**, **17**-**18** and **20**-**23** were evaluated for their cytotoxicities against the colon cancer cell line HCT116. Unfortunately, all compounds were inactive at 50 μM. Most of the phenolic compounds were evaluated for their antioxidant activities using the DPPH assay. However, only compounds **14** and **22**-**24** showed antioxidant activities with

IC_{50} values ranging from 24.83-135.05 μM (IC_{50} of standard control (ascorbic acid) 18.51±0.08 μM).

3. Experimental

For the details of all experimental parts see the Supplementary material.

Integerravone (**1**). Yellow solid, mp. 128-130 ^{o}C; UV (MeOH) λ_{max} (log ε) 327 (3.70) and 291 (3.58) nm; IR (neat) υ_{max} 3340, 2923, 1762, 1652, 1491, 1434, 1093, 808 cm^{-1}; ^{1}H NMR ($CDCl_3$, 600 MHz) δ_H 12.37 (1H, s, 5-OH), 8.19 (2H, d, *J* = 9.0 Hz, H-2′ and H-6′), 7.07 (2H, d, *J* = 9.0 Hz, H-3′ and H-5′), 6.23 (1H, s, H-6), 3.93 (6H, s, 8-OMe and 4′-OMe), 3.90 (3H, s, 7-OMe), 2.93 (1H, sept, *J* = 7.0 Hz, H-2″) and 1.40 (6H, d, *J* = 7.0 Hz, H-3″ and H-4″); ^{13}C NMR ($CDCl_3$, 150 MHz):δ_C 179.3 (C-4), 174.8 (C-1″), 162.1 (C-4′), 156.6 (C-9), 156.5 (C-2), 149.4 (C-5), 148.3 (C-3), 139.4 (C-7), 132.5 (C-8), 130.4 (C-2′ and C-6′), 122.8 (C-1′), 114.3 (C-3′ and C-5′), 110.1 (C-10), 105.0 (C-6), 60.1 (7-OMe), 55.7 (8-OMe and 4′-OMe), 33.9 (C-2″) and 19.0 (C-3″ and C-4″); EIMS *m/z* $[M]^+$ 414 (34), 344 (68), 329 (100), 315 (8), 311 (4), 301 (7), 286 (7), 148 (7), 135 (14), 119 (7), 71 (10) and 69 (6); ESITOFMS *m/z* 437.1217 $[M + Na]^+$ (calcd for $C_{22}H_{22}O_8Na$, 437.1212).

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Supplementary material

The NMR and HR-ESI-MS spectra of compound **1** are available in supporting information.

Disclosure statement

The authors declare no conflicts of interest.

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