ฤทธิ์ยับยั้งเอนไซม์อะเซทิลโคลีนเอสเทอเรสของสารสกัดจากรางแดง
และองค์ประกอบทางเคมี

**Antiacetylcholinesterase Activity of Ventilago denticulata Extracts and its Chemical Constituents**

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บทคัดย่อ

ได้ทำการศึกษาฤทธิ์ยับยั้งโรคอัลไซเมอร์ต่อเอนไซม์อะเซทิลโคลีนเอสเทอเรสของสารสกัด และองค์ประกอบทางเคมีจากรางแดง (*Ventilago denticulata*) เมื่อทำการแยกสารพบว่าสามารถแยกสารในกลุ่มควิโนนที่มีรายงานโครงสร้างแล้ว 7 สาร ประกอบด้วย ventiloquinone I (1) 2-hydroxyislandicin (2) chrysophanol (3) physcion (4) emodin (5) questin (6) และ ventilatone A (7) รวมกับการพบสารสีส้มชัน 7 สาร แต่ไม่พบสารประกอบกลุ่มควิโนนที่มีรายงานโครงสร้าง 7 สาร ประกอบด้วย ventiloquinone I (1) 2-hydroxyislandicin (2) emodin (5) questin (6) และ ventilatone A (7) รวมกับการพบสารสีส้มชัน 7 สาร แต่ไม่พบสารประกอบกลุ่มควิโนนที่มีรายงานโครงสร้าง.

การวิเคราะห์ข้อมูลทางสเปกโทรสโคปี โดยใช้เทคนิค NMR เป็นหลัก และโดยการเปรียบเทียบกับข้อมูลที่มีรายงานไว้แล้ว งานวิจัยนี้เป็นครั้งแรกที่สามารถแยกสารประกอบ 2 จาก V. denticulata และเป็นครั้งแรกที่รายงานการพบสาร 4 จากพืชในสกุล Ventilago จากการศึกษาฤทธิ์ยับยั้งโรคอัลไซเมอร์ของสารสกัดเปลือกเเทงแดง เพราะสารสกัดชันเอทิลออกซิตัล แสดงฤทธิ์ยับยั้งโรคอัลไซเมอร์ของสารสกัดเปลือกเเทงแดง (IC₅₀ 9.3±0.0001 ไมโครกรัมต่อมิลลิกรัม) ได้ ดีกว่าชันแมกนวล (IC₅₀ 36.3±0.0003 ไมโครกรัมต่อมิลลิกรัม) ประมาณ 4 เท่า ส่วนสารบริงาที่แยกได้ พบว่า เสาร์สาร 7 เท่านั้นที่มีฤทธิ์ยับยั้งต่อเอนไซม์อะเซทิลโคลีนเอสเทอเรส ที่ค่า IC₅₀ 21.99±0.14 ไมโครกรัม
ABSTRACT

The anti-alzheimer property against enzyme acetylcholinesterase of Thai medicinal plant *Ventilago denticulata* extracts and its chemical constituents was investigated. Isolation and purification of *V. denticulata* roots extracts afforded seven known quinone compounds: ventiloquinone I (1), 2–hydroxyislandicin (2), chrysophanol (3), physcion (4), emodin (5), questin (6) and ventilatone A (7) along with a large quantity of a mixture of common plant sterol, long chain fatty acids and tannin. The structures of quinones were assigned from combination of spectral data analysis especially 1D– and 2D NMR technique, and by comparison with the reported data. Compounds 2 and 4 were isolated for the first time from *V. denticulata* and from the genus *Ventilago*, respectively. The crude EtOAc extract (IC$_{50}$ 9.3±0.0001 µg/mL) revealed about 4 times more potent anti–AChE ability than that of the MeOH soluble fraction (IC$_{50}$ 36.3±0.0003 µg/mL), and only compound 7 exhibited effective inhibitory activity at IC$_{50}$ value of 21.99±0.14 µM.

INTRODUCTION

The genus *Ventilago* is a climbing shrubs or semi–scandent shrub that belonging to Rhamnaceae family with about 40 species worldwide (Flora of China, 2007) and only nine species have found in Thailand that including: *V. cristata* (Thao khan lek), *V. denticulata* [synonym *V. calyculata* (Raang–Daeng)], *V. harmandiana* (Khrua plok), *V. laotica*, *V. leiocarpa*, *V. maingayi*, *V. malaccensis* (Kong keep), *V. oblongifolia*, *V. ochrocarpa* (Khrua khao klaep) (Smitinand 2014). The plant *V. denticulata* or *V. calyculata* has been found in middle elevations; Bhutan, India, Nepal, Vietnam and Thailand (Flora of China, 2007). It has been commonly known in Thai as Raang–Daeng (central) or Plok glaep (Buriram), a climbing shrubs or semi–scandent shrub or lianas on the trees that distributed in Thailand (Smitinand 2014). It is also known as Phaya–Ngoo–khieo in Khon Kaen province where it was collected. *Ventilago* plants have been widely used in Asian folk medicine for the treatment of many kinds of diseases. The decoction of Indian *V. calyculata* roots (Balakrishnan and Singh, 2013) and of Thai *V. harmandiana* heart woods and stem barks (Panthong et al., 2004), has been used for the treatment of diabetes. Vines and leaves of *V. denticulata* were used as tea as a tonic for treatment of the pains of ligament and bone.
in Thai traditional medicine (Caichompoo et al., 2012).

Previous phytochemical studies revealed the isolation of anthraquinone (Rao et al., 1983), naphthalene, napthoquinone, benziso–chromanquinone (pyranapthoquinone), napthoquinone–lactone (Hanumaiah et al., 1985a; 1985b; 1985c), triterpenoid, sterol and glycoside (Hanumaiah et al., 1983). A number of anthraquinone has been reported for its biological activities such as anti–cancer (Asche, 2005) and anti–bacterial (Hatano et al., 1999) abilities. The insect antifeedant potency of the quinones isolated from V. madaraspatana was investigated, and ventiloquinone A exhibited antifeedant activity (Krishnakumari et al., 2001). Extracts of the heart woods, stem barks and stem woods of Thai V. harmandiana was shown as phosphodiesterase inhibitor (Temkitthawon et al., 2004). Ethanolic extract of Thai V. denticulata leaves exhibited anti–HSV–1 (Lipipun et al., 2003).

Alzheimer’s disease (AD) is the most common neurodegenerative disease in the elderly. It associated with loss of acetylcholine neurotransmitter, which was characterized by a neuro dysfunction resulting in loss of memory and cognitive disorder (Walsh and Selkoe, 2004). As part of the ongoing project to search for bioactive compound from Thai plants, the root extracts of V. denticulate were screened and exhibited in vitro anti–acetylcholinesterase (AChE) activity. Emodin and physcion isolated from Rheum emodi rhizomes were found to be inhibitors for treatment of AD by effect on phosphatidylinositol 3–kinase (Sun and Liu, 2015) and β–amyloid aggregation (Ho et al., 2015). The related anthraquinone, aloe emodin or 1,8–dihydroxy–3–(hydroxymethyl)–9,10–anthraquinone possessed 57.2% inhibition against acetylcholinesterase (AChE) (Orhan et al., 2008). However, no previous work has published on anti–AChE or anti–AD ability of crude extracts and chemical constituents from the genus Ventilago.

EXPERIMENT

Plant Material, The air–dried roots of V. denticulata was collected from Nam Phong District, Khon Kaen Province, Thailand in October, 2009. A voucher specimen (Jannarin Nonthakham 002) has been deposited at the Natural Product Research Unit, Department of Chemistry, Faculty of Science, Srinakharinwirot University, and was identified by comparison with the authentic V. denticulata voucher specimens of the Forest Herbarium, Royal Forest Department, Bangkok, Thailand.

Extraction and isolation

The roots of V. denticulata (3.5 kg) were extracted with EtOAc then MeOH (each 5 L x 3). Each extract was combined and concentrated under vacuum to yield EtOAc–
and MeOH extracts in 202 g (5.8%, based on dried plant material) and 144 g (4.1%), respectively. Anti–AChE property of EtOAc– and MeOH extracts was tested against *Electrophorus electricus* AChE resulting in IC$_{50}$ values of 9.3±0.0001 and 36.3±0.0003 µg/mL, respectively. Both crude extracts were further investigated for their bioactive compounds.

The EtOAc extract (80 g) was subjected to quick column chromatography (QCC) over silica gel eluting with gradient system of n–hexane, EtOAc and MeOH to give twelve main fractions (A–L). A mixture of β–sitosterol and stigmasterol was collected from fraction A as a colorless solid (343 mg, 0.01%) which gave a purple coloration with anisaldehyde–H$_2$SO$_4$ reagent on TLC. Fraction B (11 g) was chromatographed over silica gel column using gradient of EtOAc in n–hexane and fifteen fractions (B1–B15) were collected. Subfraction B10 was subjected to column chromatography (CC) over silica gel using gradient CH$_2$Cl$_2$ : n–hexane and compound 1 (252 mg, 0.007%) was then obtained from subfraction B10.6. Compound 2 (11 mg, 0.0003%) was obtained from the isolation of subfraction B11 with gradient system of CH$_2$Cl$_2$ : n–hexane. Fraction C (2.6 g) was separated by CC (silica gel) eluting with gradient of CH$_2$Cl$_2$ in n–hexane to afford five subfractions (C1–C5) and subfractions C–3 and C–4 were proved to be compound 3 (93 mg, 0.003%) and a mixture of fatty acids as pale orange viscous oil (1.5 g, 0.043%), respectively. Fraction I (877 mg) was further chromatographed with gradient eluting solvent of EtOAc : n–hexane to afford nine subfractions (I1–I9) and compound 4 was yielded from subfraction I5 (31 mg). By using the same manner reporting by Pegg et al. (Pegg et al., 2008), the most abundant of tannin was identified from fractions J (8.6 g, 0.25%) and K (1.8 g, 0.16%) by TLC screening technique (silica gel plate, a mixture of CHCl$_3$: methanol: water (65:35:10, v/v/v) as the mobile phase system, followed by an orange color development with a 0.5% (w/v) vanillin solution prepared in 4% (w/v) HCl). A portion of MeOH extract was fractionated by QCC using gradient system of n–hexane, CH$_2$Cl$_2$, EtOAc and MeOH to obtain five fractions (M–Q). Fraction P (3.3 g) was further subjected to silica gel CC twice (with a gradient of n–hexane, CH$_2$Cl$_2$, EtOAc and MeOH) and compounds 5 (121 mg, 0.0035%), 6 (51 mg, 0.0015%) and 7 (16 mg, 0.0005%) were collected from subfractions P6.2–P6.4. TLC chromatogram of the most polar fraction P8 (23.4 g, 0.67%) and P9 (28.0 g, 0.8%) indicated for the presence of tannin as a brownish solid and as major constituent of this plant species.

**Ventilquinone I** (1): yellow amorphous solid, 252 mg, mp 138–140 °C; R$_o$ 0.42 (50% EtOAc : CH$_2$Cl$_2$); UV (MeOH) $\lambda_{max}$ (log ε):
441 (4.14), 300 (4.39), 263 (4.58) and 222 (4.70) nm; IR (CHCl₃) \( \lambda_{\text{max}} \) cm\(^{-1}\): 3333, 2976, 1746, 1651, 1607, 1416, 1380, 1208, 1093, 868 and 756; \(^1\)H NMR (CDCl₃, 300 MHz): \( \delta \) 13.55 s (1-H, 1H), 7.79 br s (8–OH, 1H), 6.25 s (1H, H–5), 5.06 q \( (J = 6.4 \text{ Hz}, 1 \text{H, H–1}) \), 3.84 s (3H, 7–OCH₃), 3.61 ddq \( (J = 10.4, 6.1, 1.7 \text{ Hz}, 1 \text{H, H–3}) \), 2.93 dt \( (J = 17.1, 1.7, 1 \text{H, H–4a}) \), 2.46 ddd \( U = 17.1, 10.4, 2.1 \text{ Hz}, 1 \text{H, H–4b}) \), 1.64 d \( U = 6.4 \text{ Hz}, 3 \text{H, 1–CH₂} \) and 1.40 d \( U = 6.1 \text{ Hz}, 3 \text{H, 3–CH₃} \); \(^{13}\)C NMR (CDCl₃, 75 MHz): \( \delta \) C 191.1 (C–9), 179.3 (C–6), 157.1 (C–8), 156.4 (C–10), 152.9 (C–7), 141.0 (C–4a), 139.9 (C–10a), 111.3 (C–5a), 110.2 (C–9a), 108.9 (C–5), 70.9 (C–1), 68.9 (C–3), 61.0 (7–OCH₃), 31.5 (C–4), 21.4 (1–CH₃) and 20.8 (3–CH₃); ESIMS: –ve m/z [M–H]⁺ 303.5 (100).

2-Hydroxyislandicin (2) or 1,2,4,8-tetrahydroxy–3–methyl–9,10–anthraquinone: fluorescence red–pink fine needles, 11 mg, mp 208–210 °C; R \(_t\) 0.35 (20% EtOAc : n-hexane x 2); UV (MeOH) \( \lambda_{\text{max}} \) (log \( \epsilon \)) \( = \): 522 (3.64), 493 (3.64), 364 (3.11), 258 (3.90) and 219 (3.97) nm; IR (KBr) \( \lambda_{\text{max}} \) cm\(^{-1}\): 3446, 2923, 1618, 1594, 1458, 1295, 1201, 831 and 738; \(^1\)H NMR (CDCl₃ + CD₃OD, 300 MHz): \( \delta \) 13.93 s (1H, 1–OH), 12.72 s (1H, 8–OH), 12.21 s (1H, 4–OH), 7.85 br dd \( U = 7.7, 0.8 \text{ Hz}, 1 \text{H, H–5}) \), 7.69 t \( U = 7.7 \text{ Hz}, 1 \text{H, H–6}) \), 7.25 dd \( U = 7.7, 0.8 \text{ Hz}, 1 \text{H, H–7}) \) and 2.24 s (3H, 3–CH₃); \(^{13}\)C NMR (CDCl₃ + CD₃OD, 75 MHz): \( \delta \) C 187.3 (C–9), 183.9 (C–10), 161.9 (C–8), 160.7 (C–2), 160.5 (C–4), 152.5 (C–1), 136.7 (C–6), 131.6 (C–10a), 123.3 (C–7), 121.9 (C–3), 118.9 (C–5), 115.8 (C–8a), 105.0 (C–4a), 96.8 (C–9a) and 8.2 (3–CH₃); \(^1\)H NMR (DMSO–d₆, 300 MHz): \( \delta \) H 13.87 s (1H, 1–OH), 11.93 br s (1H, 8–OH), 7.73 t \( U = 7.8 \text{ Hz}, 1 \text{H, H–6}) \), 7.63 d \( U = 7.8 \text{ Hz}, 1 \text{H, H–7}) \), 7.25 d \( U = 7.8 \text{ Hz}, 1 \text{H, H–5}) \) and 2.03 s (3H, 3–CH₃); \(^{13}\)C NMR (DMSO–d₆, 75 MHz): \( \delta \) C 189.7 (C–9), 183.1(C–10), 161.5 (C–8), 159.9 (C–2), 154.0 (C–4), 147.9 (C–1), 137.3 (C–6), 133.7 (C–10a), 123.5 (C–7), 120.8 (C–3), 118.6 (C–5), 115.7 (C–8a), 109.9 (C–4a), 104.6 (C–9a) and 8.6 (3–CH₃); ESIMS: +ve m/z [M+H]⁺ 282.3 (100).

Chrysophanol (3) or 1,8-dihydroxy–3–methyl–9,10–anthraquinone: orange amorphous solid, 93 mg, mp 160–162 °C; R \(_t\) 0.69 (30% EtOAc : hexane); UV (MeOH) \( \lambda_{\text{max}} \) (log \( \epsilon \)) \( = \): 427 (3.75), 286 (3.74), 276 (3.73), 256 (4.03) and 224 (4.25) nm; IR (CHCl₃) \( \lambda_{\text{max}} \) cm\(^{-1}\): 3395, 3016, 2923, 1736, 1626, 1462, 1215 and 757; \(^1\)H NMR (CDCl₃, 300 MHz): \( \delta \) H 12.12 s (1H, 8–OH), 12.01 s (1H, 1–OH), 7.83 d \( U = 7.5 \text{ Hz}, 1 \text{H, H–5}) \), 7.67 dd \( U = 8.5, 7.5 \text{ Hz}, 1 \text{H, H–6}) \), 7.65 s \( U = 8.5 \text{ Hz}, 1 \text{H, H–7}) \), 7.10 s \( U = 8.5 \text{ Hz}, 1 \text{H, H–7}) \) and 2.46 s (3H, 3–CH₃); \(^{13}\)C NMR (CDCl₃, 75 MHz): \( \delta \) C 192.5 (C–9), 182.0 (C–10), 162.4 (C–8), 162.7 (C–1), 149.3 (C–3), 136.9 (C–6), 133.6 (C–4a), 133.3 (C–10a), 124.5 (C–7), 124.3 (C–2), 121.3 (C–4), 119.9 (C–5), 115.9 (C–8a), 113.7 (C–9a) and 22.2 (3–CH₃); ESIMS: –ve m/z [M–H]⁺ 253.9 (100).
Physcion (4) or 1,8-dihydroxy-3-methoxy-6-methyl-9,10-anthraquinone:
Yellow fine needles, 31 mg, mp 190–192 °C; R_v 0.66 (30% EtOAc : hexane); UV (MeOH) \( \lambda_{\text{max}} \) (log \( \epsilon \)): 433(4.11), 286 (4.3), 265 (4.28) and 222 (4.58) nm; IR (CHCl_3) \( \delta_{\text{max}} \) cm\(^{-1}\): 3401, 2923, 1632, 1613, 1443, 1390, 1261, 1220 and 760; \(^1\)H NMR (CDCl_3, 300 MHz): \( \delta \)H 12.30 s (1H, 1-OH), 12.10 s (1H, 8-OH), 7.61 d (J = 1.0 Hz, 1H, H-5), 7.36 d (J = 2.5 Hz, 1H, H-4), 7.07 d (J = 1.0 Hz, 1H, H-7), 6.68 d (J = 2.5 Hz, 1H, H-2), 3.93 s (3H, 3-OCH_3), 2.44 s (3H, 6-CH_3); \(^{13}\)C NMR (CDCl_3, 75 MHz): \( \delta \)C 190.7 (C-9), 182.0 (C-10), 166.5 (C-3), 165.1 (C-1), 162.5 (C-8), 148.4 (C-6), 135.2 (C-4a), 133.2 (C-10a), 124.4 (C-7), 121.2 (C-5), 113.6 (C-8a), 110.2 (C-9a), 108.2 (C-4), 106.7 (C-2), 56.0 (3-OCH_3), 22.1 (6-CH_3); ESIMS: \( +\)ve m/z [M+H]^+ 282.3 (100).

Emodin (5) or 1,3,8-trihydroxy-6-methyl-9,10-anthraquinone: 121 mg, orange fine needles, mp 254–256 °C; R_f 0.42 (30% EtOAc : n-hexane); UV (MeOH) \( \lambda_{\text{max}} \) (log \( \epsilon \)): 437 (4.24), 288 (4.47), 266 (4.41), 253 (4.39) and 221 (4.67) nm; IR (KBr) \( \delta_{\text{max}} \) cm\(^{-1}\): 3481, 3052, 2926, 1626, 1595, 1479, 1340, 1272, 1227, 1174, 858 and 768; 1H NMR (CDCl_3 + DMSO-d6, 300 MHz): \( \delta \)H 12.24 s (1H, 1-OH), 12.18 s (1H, 8-OH), 10.71 br s (1H, 3-OH), 7.57 s (1H, H-5), 7.29 d (J = 2.3 Hz, 1H, H-4), 7.05 s (1H, H-7), 6.64 d (J = 2.3 Hz, 1H, H-2), 2.46 s (3H, 6-CH_3); 13C NMR (CDCl_3 + DMSO-d6, 75 MHz): \( \delta \)C 190.0 (C-9), 181.7 (C-10), 161.9 (C-8), 165.4 (C-3), 164.9 (C-1), 147.6 (C-6), 132.8 (C-10a), 134.9 (C-4a), 123.8 (C-7), 120.5 (C-5), 109.4 (C-4), 113.3 (C-8a), 108.1 (C-2), 108.9 (C-9a), 21.7 (6-CH_3); ESIMS: –ve m/z [M-H]^- 269.9 (100).

Questin (6) or 3,8-dihydroxy-1-methoxy-6-methyl-9,10-anthraquinone: pale yellow fine needles, 93 mg, mp 246–248 °C; R_v 0.69 (50% EtOAc : n-hexane); UV (MeOH) \( \lambda_{\text{max}} \) (log \( \epsilon \)): 351 (3.74), 306 (4.01), 251 (4.19) and 234 (4.36) nm; IR (KBr) \( \delta_{\text{max}} \) cm\(^{-1}\): 3350, 2950, 1696, 1659, 1600, 1515, 1436, 1279, 1216, 859 and 775; \(^1\)H NMR (CDCl_3, 300 MHz): \( \delta \)H 12.28 s (1H, 8-OH), 6.79 d (J = 2.1 Hz, 1H, H-4), 6.73 d (J = 2.1 Hz, 1H, H-2), 6.59 s (1H, H-5), 6.48 s (1H, H-7), 3.89 s (3H, 1-OCH_3), 2.31 s (3H, 6-CH_3); \(^{13}\)C NMR (CDCl_3, 75 MHz): \( \delta \)C 179.5 (C-10), 169.4 (C-9), 161.1 (C-1), 163.5 (C-3), 157.8 (C-4a), 155.5 (C-10a), 148.0 (C-6), 134.7 (C-8), 111.1 (C-7), 112.7 (C-2), 106.9 (C-5), 109.9 (C-9a), 103.5 (C-4), 106.2 (C-8a), 52.7 (1-OCH_3), 22.2 (6-CH_3); ESIMS: –ve m/z [M-H]^- 282.6.

Ventilatone A (7): pale yellow fine needles, 16 mg, mp 256–258 °C (decomposed); R_v 0.45 (50% EtOAc : n-hexane); UV (MeOH) \( \lambda_{\text{max}} \) (log \( \epsilon \)): 278 (3.51), 305 (3.73), 268 (3.89) and 236 (414) nm; IR (CHCl_3) \( \delta_{\text{max}} \) cm\(^{-1}\): 2983, 1731, 1676, 1651, 1629, 1588, 1253, 1124, 875 and 749 nm; \(^1\)H NMR (CDCl_3, 300 MHz): \( \delta \)H 7.84 s (1H, H-5), 6.19 s (1H, H-8), 5.91 s (1H, H-13), 4.52 ddq (J = 10.5, 6.2, 3.1 Hz, H-3), 3.20 dd (J = 17.1, 3.1 Hz, H-4a), 3.03 dd (J =
Anti–AChE activity assays. The in vitro enzyme inhibitory activity of the isolated compounds was screened against *Electrophorus electricus* AChE by providing adopting the method of Ellman (Ellman et al., 1961) and galanthamine (IC_{50} 1.45 µM) was used as reference compound as our previously described report (Khammee et al., 2014).

RESULT AND DISCUSSION

The EtOAc extract obtained from of the roots of *V. denticulata* was fractionated by column chromatography to yield a mixture of β-sitosterol and stigmasterol (Pierre and Moses, 2015), a mixture of long chain fatty acids, tannin and four reported quinones 1–4 benzisochromanquinone (ventiloquinone I (1)), anthraquinoneones [2-hydroxylandicin (2), chrysophanol (3) and physcion (4)] whilst investigation of methanolic extract afforded two additional anthraquinones [emodin (5) and questin (6)] together with a naphthoquinone–lactone [ventilatone A (7)] and large amount of tannin. The structure of quinones were elucidated mainly on the basis of 1D– and 2D NMR data analysis and compared with those previously reported data. A mixture of β-sitosterol and stigmasterol, and fatty acids isolated were confirmed their occurring based on their ¹H NMR data analysis. The most abundant tannin obtained from the most polar fractions of both extracts which were examined by TLC screening technique (Pegg et al., 2008).

From 1D– and 2D NMR spectral data analysis, the major isolated quinone 1 was assigned to be a benzisochromanquinone. Its ¹H– and ¹³C NMR (CDCl₃) spectra showed signals of methylene (H–4a,b) protons at δ_{H} 3.61 ddq (J = 10.4, 6.1, 1.7 Hz, H–3), 2.93 dt (J = 17.1, 1.7, H–4a), 2.46 dddd (J = 17.1, 10.4, 2.1 Hz, H–4b). A chelated hydroxyl group on structure of 1 was found at δ_{H} 13.55 s (10–OH). ¹H NMR data was slightly different from those previous reported (CDCl₃, 220 MHz) at H–5 (δ_{H} 7.37 s) and 7–OCH₃ (δ_{H} 4.19) (Hanumaiah et al., 1985c). Its benzisochromanquinone (or pyranapthoquinone) backbone was confirmed by 2D NMR data analysis. Correlations of H–1 to 1–CH₃, of H–4 to H–3, and of H–3 and 3–CH₃ were observed on COSY spectrum. HMBC correlations of H–5 to carbonyl at C–6 (δ_{C} 179.3) and C–9 (δ_{C} 191), C–5a (δ_{C} 111.3) and C–9a (δ_{C} 110.2), and of 10–OH to C–9, as well as correlations of H–4a and H–4b to C–10a (δ_{C} 139.9) and C–4a (δ_{C} 141.0), suggested ring
connection. From those evidences compound 1 was figured out as ventiloquione I (Hanumaiah et al., 1985c).

$^1$H- and $^{13}$C NMR spectra of 2 (CDCl$_3$ + CD$_3$OD) showed three singlet chelated hydroxyl signals at $\delta_h$ 13.93 (1-OH), 12.72 (8-OH) and 12.21 (4-OH), and other signals at 7.85 br dd ($J = 7.7$, 0.8 Hz, H–5), 7.69 $d$ ($J = 7.7$ Hz, H–6), 7.25 dd ($J = 7.7$, 0.8 Hz, H–7) and a singlet methyl group at $\delta_h$ 2.24 (3–CH$_3$). The $^1$H NMR data recorded in DMSO–$d_6$, however, showed two chelated hydroxyls at $\delta_h$ 13.87 $s$ (1H, 1–OH) and 11.93 br $s$ (1H, 8–OH). It is noteworthy that the methyl group at C–3 was detected at the upfield chemical shift of $\delta_c$ 8.2 ppm compare to that of 3 ($\delta_c$ 22.2). The COSY correlations of H–6 to H–5 and H–7 and HMBC correlations of 3–CH$_3$ to C–1 ($\delta_c$ 152.5), C–2 ($\delta_c$ 160.7), C–3 ($\delta_c$ 121.9) and C–4 ($\delta_c$ 160.5), of H–7 to C–8a (115.8) and of H–5 to C–10 ($\delta_c$ 183.9) and C–10a ($\delta_c$ 131.6) confirmed the connections on structure of 2. Its spectral data corresponded to a reported quinone, 2-hydroxyislandicin which was first isolated from the root bark of V. maderaspatana (Rao et al., 1983) and it was then found here for the first time from V. denticulata.

From 1D- and 2D NMR (CDCl$_3$) data analysis, 3 showed seven proton signals at $\delta_h$ 12.12 $s$ (8–OH), 12.01 $s$ (1–OH), 7.83 $d$ ($J = 7.5$ Hz, H–5), 7.67 $dd$ ($J = 8.5$, 7.5 Hz, H–6), 7.65 $s$ (H–4), 7.29 $d$ ($J = 8.5$ Hz, H–7), 7.10 $s$ (H–2) and 2.46 $s$ (3–CH$_3$). Correlations of H–2 to H–4 and 3–CH$_3$, and H–6 to H–5 and H–7 on COSY spectrum as well as correlations of H–2 to carbonyl at C–9 ($\delta_c$ 192.5) and C–9a ($\delta_c$ 113.7), of H–4 to carbonyl carbon at C–10 ($\delta_c$ 182.0), C–4a ($\delta_c$ 133.6) and C–9a, of H–5 and H–7 to C–8a ($\delta_c$ 115.9) and of H–5 to C–10. The NMR data of 3 corresponded to those of chrysophanol (Li et al., 2000).

$^1$H NMR data of 4 were found for signals of two chelated hydroxyl groups [12.30 $s$ (1H, 1–OH), 12.10 $s$ (1H, 8–OH)], four aromatic protons [7.61 $d$ ($J = 1.0$ Hz, H–5), 7.36 $d$ ($J = 2.5$ Hz, H–4), 7.07 $d$ ($J = 1.0$ Hz, H–7), 6.68 $d$ ($J = 2.5$ Hz, H–2)] and a singlet methyl 6–CH$_3$ at $\delta_h$ 2.46. In comparison with $^1$H- and $^{13}$C NMR spectra of 5, an additional singlet methoxy group at C–3 displayed at $\delta_h$ 3.93 ($\delta_c$ 56.0) in 4. Its anthraquinone scaffold was confirmed by COSY correlations of H–2 to H–4 and 3–OCH$_3$, and of H–5 to H–7 and 6–OCH$_3$, as well as HMBC correlations of 1–OH and H–2 to C–9a ($\delta_c$ 110.2), of H–4 to C–4a ($\delta_c$ 135.2), carbonyl at C–10 ($\delta_c$ 182.0) and C–10a ($\delta_c$ 133.2), of H–7 to C–8a ($\delta_c$ 113.6) and carbonyl at C–9 ($\delta_c$ 190.7), and of H–4 to C–9a and C–10. NMR data of 4 was similar to those of phycnon (Chu et al., 2005).

$^1$H- and $^{13}$C NMR data of compound 5 (CDCl$_3$ + DMSO–$d_6$) displayed similar signals related to structure 4 (CDCl$_3$). Compound 5
has two chelated hydroxyl groups \([\delta_h 12.24 \, \text{s} (1–OH), 12.18 \, \text{s} (8–OH)]\), four aromatic protons \([\delta_h 7.57 \, \text{s} (H–5), 7.29 \, d \, (J = 2.3 \, \text{Hz}, \ H–4), 7.05 \, s \, (H–7), 6.64 \, d \, (J = 2.3 \, \text{Hz}, H–2)]\) and singlet methyl of 6–CH\(_3\) at \(\delta_h 2.46\). COSY and HMBC correlations of 5 were similar to those of 4, which also confirmed the ring connections. NMR data of 5 corresponded to those of emodin (Chu et al., 2005), a natural anthraquinone was commonly found from rhubarb \((\text{Rheum emodi})\) and it is also present in many herbs (Cohen and Towers, 1995).

\(^1\text{H}\) NMR spectrum of 6 showed one singlet chelated hydroxyl group at \(\delta_h 12.28\) (1–OH). Four aromatic protons \([\delta_h 6.79 \, d \, (J = 2.1 \, \text{Hz}, H–4), 6.73 \, d \, (J = 2.1 \, \text{Hz}, H–2), 6.48 \, s \, (H–7), 6.59 \, s \, (H–5)]\) and singlet methyl \([\delta_h 2.31 \, (6–CH\(_3\)])\) displayed in the similar regions for those of compounds 4–5. A singlet methyl at \(\delta_h 3.89 \, (1–OCH\(_3\))\) was confirmed by its HMBC correlation to carbonyl at C–9 \((\delta_c 169.4)\). Correlations of 8–OH to C–8a \((\delta_c 106.2)\) and of H–4 to C–4a \((\delta_c 157.8)\), C–9a \((\delta_c 109.9)\), C–10 \((\delta_c 179.5)\), as well as of H–5 to C–9a and C–10 confirmed the basic structure of anthraquinone. Compound 6 was assigned to be questitin (Fujimoto et al., 2004) which was found to be the first isolation from plant that belongs to the genus \textit{Ventilago}.

1D- and 2D NMR analysis of compound 7 confirmed its structure to be a naphthoquinone–lactone related to 1. \(^1\text{H}\)- and \(^{13}\text{C}\) NMR (CDCl\(_3\)) spectra of 7 showed different signals from those of 1 at methine (H–3) and each methylene (H–4a,b) protons at \(\delta_c 4.52 \, \text{ddq} \, (J = 10.5, 6.2, 3.1 \, \text{Hz}, \ H–3), 3.20 \, \text{dd} \, (J = 17.1, 3.1 \, \text{Hz}, \ H–4a), 3.03 \, \text{dd} \, (J = 17.1, 10.5 \, \text{Hz}, H–4b).\)

In comparison with 1, the absence of the chelated hydroxyl group in \(^1\text{H}\) NMR data of 7 and an additional carbonyl signal at \(\delta_c 161.3\) of ring D shown in \(^{13}\text{C}\) NMR spectrum. COSY correlations of H–4 to H–3 and H–5, and between H–3 and 3–CH\(_3\) were observed to confirm the connection between those protons. Correlations of H–4 to C–4a \(137.6)\) and C–5 \(120.3)\), of H–5 to carbonyl at C–6 \((\delta_c 179.5)\), C–9a \((\delta_c 116.8)\) and C–10a \((\delta_c 117.2)\) were observed on HMBC spectrum corresponding to benzisochromanquinone (or pyranaptoquinone) scaffold related to 1. HMBC correlations of H–11 to C–1 \((\delta_c 162.6)\) and carbonyl at C–12 \(161.3)\) were also observed the present of lactone at C–1 and C–10. Those results led to elucidate structure of 7 to be ventilatone A (Hanumaiah et al., 1985a).

The inhibitory activity of the crude extracts and all isolated compounds was investigated against AChE. Compounds 1–4 were inactive against AChE although they were isolated from EtOAc extract which possesses moderate anti-AChE property IC\(_{50}\) values of 9.3±0.0001 \(\mu\text{g/mL}\). The MeOH extracts
exhibited weak anti–AChE effect at IC₅₀ 36.3±0.0003 µg/mL. Amongst the quinones isolated, only compound 7 obtained from MeOH extract exhibit anti–AChE effect with IC₅₀ 21.99±0.14 µM while compounds 5–6 inactive in the same test. The presence of lactone group at ring D of quinone scaffold 7 might be important for the activity.

Figure Structures of compounds 1–7 isolated from the roots of V. denticulate

In all tested in this study, the EtOAc extract exhibited more potent anti–AChE activity than the MeOH soluble fraction and the pure compound 7, and other quinones were inactive. These results could be due to the synergism of a total set of phytochemical compounds within medicinal plant (Ncube et al., 2012). Plant fatty acid was shown to have anti-AChE activity (Farag et al., 2016). Stigmasterol from Rhazya stricta was reported to be an AChE inhibitor with IC₅₀ of 644.0±11.75 µM (Sultana and Khalid, 2010). In addition, tannin has been reported for anti–AChE activity (Pithayanukul et al., 2005). The presence of large amount fatty acids (0.04%), steroidal triterpenoid (0.01%) and the highest quantity of tannin (more than 1%), when compared with the minor quantity of quinones obtained, might play important role for synergistic anti–AChE action of plant extracts. Although compounds 1–6 displayed inactive effect against AChE, however, emodin and physcion were shown to be β–amyloid aggregation inhibitors (Sun and Liu, 2015; Ho et al., 2015).

CONCLUSION

The chemical investigation of Thai medicinal plant V. denticulata roots led to isolation of seven previously reported quinones. This is the first report on the isolation of 2–hydroxyislandicin (2) from this plant species and questin (4) from the genus Ventilago. Amongst isolates, only 7 exhibited AChE inhibitory activity. The anti–AChE activity of crude extracts demonstrates the potential phytotherapeutic value of this medicinal plant for its anti–AD ability.

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