ฤทธิ์ยับยั้งเอนไซม์อะเซทิลโคลีนเอสเทอเรสของสารสกัดจากรางแดงและองค์ประกอบทางเคมี

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

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บทคัดย่อ

ได้ทำการศึกษาฤทธิ์ยับยั้งโรคอัลไซเมอร์ ต่อเอนไซม์อะเซทิลโคลีนเอสเทอเรส ของสารสกัด และองค์ประกอบทางเคมีจากรางแดง (*Ventilago denticulata*) เมื่อทำการแยกสาร พบว่าสามารถแยกสารในกลุ่มควิโนนที่มีรายงานโครงสร้างแล้ว 7 สาร ประกอบด้วย ventiloquinone I (**1**) 2-hydroxyislandicin (**2**) chrysophanol (**3**) physcion (**4**) emodin (**5**) questin (**6**) และ ventilatone A (**7**) ร่วมกับการพบสารสเตียรอล กรดไขมัน ที่พบได้ทั่วไป และ แทนนินในปริมาณมาก การพิสูจน์โครงสร้างของสารควิโนนได้จากการวิเคราะห์ข้อมูลทางสเปกโทรสโกปี โดยใช้เทคนิค NMR เป็นหลัก และโดยการเปรียบเทียบกับข้อมูลที่มีรายงานไว้แล้ว งานวิจัยนี้เป็นครั้งแรกที่สามารถแยกสารประกอบ **2** จาก *V. denticulata* และเป็นครั้งแรกที่รายงานการพบสาร **4** จากพืชในสกุล *Ventilago* จากการศึกษาฤทธิ์ยับยั้งเอนไซม์อะเซทิลโคลีนเอสเทอเรส พบว่าสารสกัดชั้นเอทิลอะซิเตต แสดงฤทธิ์ยับยั้งเอนไซม์อะเซทิลโคลีนเอสเทอเรส (IC_{50} 9.3±0.0001 ไมโครกรัมต่อมิลลิกรัม) ได้ดีกว่าชั้นเมทานอล (IC_{50} 36.3±0.0003 ไมโครกรัมต่อมิลลิกรัม) ประมาณ 4 เท่า ส่วนสารบริสุทธิ์ที่แยกได้ พบว่าเฉพาะสาร **7** เท่านั้นมีฤทธิ์ยับยั้งต่อเอนไซม์อะเซทิลโคลีนเอสเทอเรส ที่ค่า IC_{50} 21.99±0.14 ไมโครโมลาร์

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.

คำสำคัญ: รางแดง ควิโนน สารยับยั้งเอนไซม์อะเซทิลโคลีนเอสเทอเรส โรคอัลไซเมอร์

Keywords: *Ventilago denticulata*, Quinone, Acetylcholinesterase inhibitor, Alzheimer's disease.

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* (khruea plok), *V. laotica*, *V. leiocarpa*, *V. maingayi*, *V. malaccensis* (Kong keep), *V. oblongifolia*, *V. ochrocarpa* (Khruea 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), naphthoquinone–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 phosphatidy-linositol 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–anthra quinone possessed 57.2% inhibi-tion 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_2SO_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_2Cl_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_2Cl_2 : *n*–hexane. Fraction C (2.6 g) was separated by CC (silica gel) eluting with gradient of CH_2Cl_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_2Cl_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_2Cl_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.

Ventiloquinone I (**1**): yellow amorphous solid, 252 mg, mp 138–140 °C; R_f 0.42 (50% EtOAc : CH_2Cl_2); UV (MeOH) λ_{max} (log ε):

441 (4.14), 300 (4.39), 263 (4.58) and 222 (4.70) nm; IR ($CHCl_3$) λ_{max} cm^{-1}: 3333, 2976, 1746, 1651, 1607, 1416, 1380, 1208, 1093, 868 and 756; ^1H NMR ($CDCl_3$, 300 MHz): δ_H 13.55 *s* (1–OH, 1H), 7.79 *br s* (8–OH, 1H), 6.25 *s* (1H, H–5), 5.06 *q* (*J* = 6.4 Hz, 1H, H–1), 3.84 *s* (3H, 7–OCH_3), 3.61 *ddq* (*J* = 10.4, 6.1, 1.7 Hz, 1H, H–3), 2.93 *dt* (*J* = 17.1, 1.7, 1H, H–4a), 2.46 *ddd* (*J* = 17.1, 10.4, 2.1 Hz, 1H, H–4b), 1.64 *d* (*J* = 6.4 Hz, 3H, 1–CH_3) and 1.40 *d* (*J* = 6.1 Hz, 3H, 3–CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_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–OCH_3), 31.5 (C–4), 21.4 (1–CH_3) and 20.8 (3–CH_3); 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_f 0.35 (20% EtOAc : *n*–hexane x 2); UV (MeOH) λ_{max} (log ε): 522 (3.64), 493 (3.64), 364 (3.11), 258 (3.90) and 219 (3.97) nm; IR (KBr) λ_{max} cm^{-1}: 3446, 2923, 1618, 1594, 1458, 1295, 1201, 831 and 738; ^1H NMR ($CDCl_3$ + CD_3OD, 300 MHz): δ_H 13.93 *s* (1H, 1–OH), 12.72 *s* (1H, 8–OH), 12.21 *s* (1H, 4–OH), 7.85 *br dd* (*J* = 7.7, 0.8 Hz, 1H, H–5), 7.69 *t* (*J* = 7.7 Hz, 1H, H–6), 7.25 *dd* (*J* = 7.7, 0.8 Hz, 1H, H–7) and 2.24 *s* (3H, 3–CH_3); ^{13}C NMR ($CDCl_3$ + CD_3OD, 75 MHz): δ_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–CH_3,); ^1H NMR (DMSO–d_6, 300 MHz): δ_H 13.87 *s* (1H, 1–OH), 11.93 *br s* (1H, 8–OH), 7.73 *t* (*J* = 7.8 Hz, 1H, H–6), 7.63 *d* (*J* = 7.8 Hz, 1H, H–7), 7.25 *d* (*J* = 7.8 Hz, 1H, H–5) and 2.03 *s* (3H, 3–CH3); ^{13}C NMR (DMSO–d_6, 75 MHz): δ_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_f 0.69 (30% EtOAc : hexane); UV (MeOH) λ_{max} (log ε): 427 (3.75), 286 (3.74), 276 (3.73), 256 (4.03) and 224 (4.25) nm; IR ($CHCl_3$) λ_{max} cm^{-1}: 3395, 3016, 2923, 1736, 1626, 1462, 1215 and 757; ^1H NMR ($CDCl_3$, 300 MHz): δ_H 12.12 *s* (1H, 8–OH), 12.01 *s* (1H, 1–OH), 7.83 *d* (*J* = 7.5 Hz, 1H, H–5), 7.67 *dd* (*J* = 8.5, 7.5 Hz, 1H, H–6), 7.65 *s* (1H, H–4), 7.29 *d* (*J* = 8.5 Hz, 1H, H–7), 7.10 *s* (1H, H–2) and 2.46 *s* (3H, 3–CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_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–CH_3); 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_f 0.66 (30% EtOAc : hexane); UV (MeOH) λ_{max} (log ε): 433(4.11), 286 (4.3), 265 (4.28) and 222 (4.58) nm; IR ($CHCl_3$) λ_{max} cm^{-1}: 3401, 2923, 1632, 1613, 1443, 1390, 1261, 1220 and 760; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_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): δ_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; Rf 0.42 (30% EtOAc : n–hexane); UV (MeOH) λ_{max} (log ε): 437 (4.24), 288 (4.47), 266 (4.41), 253 (4.39) and 221 (4.67) nm; IR (KBr) λ_{max} cm^{-1} : 3481, 3052, 2926, 1626, 1595, 1479, 1340, 1272, 1227, 1174, 858 and 768; 1H NMR (CDCl3 + DMSO–d6, 300 MHz): δ_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–CH3); 13C NMR (CDCl3 + DMSO–d6, 75 MHz): δC 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–CH3); 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_f 0.69 (50% EtOAc : *n*–hexane); UV (MeOH) λ_{max} (log ε): 351 (3.74), 306 (4.01), 251 (4.19) and 234 (4.36) nm; IR (KBr) λ_{max} cm^{-1} : 3350, 2950, 1696, 1659, 1600, 1515, 1436, 1279, 1216, 859 and 775; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_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): δ_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_f 0.45 (50% EtOAc : *n*–hexane); UV (MeOH) λ_{max} (log ε): 278 (3.51), 305 (3.73), 268 (3.89) and 236 (414) nm; IR ($CHCl_3$) λ_{max} cm^{-1} : 2983, 1731, 1676, 1651, 1629, 1588, 1253, 1124, 875 and 749 nm; ^{1}H NMR ($CDCl_3$, 300 MHz): δ_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*

= 17.1, 10.5 Hz, H–4b), 3.03 *dd* (*J* = 17.1, 10.5 Hz, 1H, H–4b) and 1.58 *d* (*J* = 6.2 Hz, 3H, 3–CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ_C 181.9 (C–9), 179.5 (C–6), 168.6 (C–7), 162.6 (C–1), 161.3 (C–12), 151.6 (C–10), 137.6 (C–4a), 133.7 (C–5a), 120.3 (C–5), 117.2 (C–10a), 116.8 (C–9a), 112.0 (C–8), 96.2 (C–11), 74.0 (C–3), 56.49 (7–OCH_3), 34.5 (C–4), 20.5 (3–CH_3); ESIMS: +ve *m/z* $[M+H]^+$ 453.9 (100).

Anti–AChE activity assays. The *in vitro* enzyme inhibitory activity of the isolated compounds was screened against *Electrophorus electricus* AChE by provided 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**)], anthraquinenones [2–hydroxyislandicin (**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 ^{1}H 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 ^{1}H– and ^{13}C NMR ($CDCl_3$) 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 *ddd* (*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). ^{1}H NMR data was slightly different from those previous reported ($CDCl_3$, 220 MHz) at H–5 (δ_H 7.37 s) and 7–OCH_3 (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–CH_3, of H–4 to H–3, and of H–3 and 3–CH_3 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 ventiloquinone I (Hanumaiah et al., 1985c).

^{1}H– and ^{13}C NMR spectra of **2** ($CDCl_3$ + CD_3OD) showed three singlet chelated hydroxyl signals at δ_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 *t* (*J* = 7.7 Hz, H–6), 7.25 *dd* (*J* = 7.7, 0.8 Hz, H–7) and a singlet methyl group at δ_H 2.24 (3–CH_3). The ^{1}H NMR data recorded in DMSO–d_6, however, showed two chelated hydroxyls at δ_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 δ_C 8.2 ppm compare to that of **3** (δ_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 (δ_C 152.5), C–2 (δ_C 160.7), C–3 (δ_C 121.9) and C–4 (δ_C 160.5), of H–7 to C–8a (115.8) and of H–5 to C–10 (δ_C 183.9) and C–10a (δ_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 δ_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 (δ_C 192.5) and C–9a (δ_C 113.7), of H–4 to carbonyl carbon at C–10 (δ_C 182.0), C–4a (δ_C 133.6) and C–9a, of H–5 and H–7 to C–8a (δ_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 [δ_H 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 δ_H 2.46. In comparion with ^{1}H– and ^{13}C NMR spectra of **5**, an additional singlet methoxy group at C–3 displayed at δ_H 3.93 (δ_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 (δ_C 110.2), of H–4 to C–4a (δ_C 135.2), carbonyl at C–10 (δ_C 182.0) and C–10a (δ_C 133.2), of H–7 to C–8a (δ_C 113.6) and carbonyl at C–9 (δ_C 190.7), and of H–4 to C–9a and C–10. NMR data of **4** was similar to those of physcion (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 [δ_H 12.24 *s* (1–OH), 12.18 *s* (8–OH)], four aromatic protons [δ_H 7.57 *s* (H–5), 7.29 *d* (*J* = 2.3 Hz, H–4), 7.05 *s* (H–7), 6.64 *d* (*J* = 2.3 Hz, H–2)] and singlet methyl of 6–CH_3 at δ_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 anthraquonone was commonly found from rhubarb (*Rheum emodi*) and it is also present in many herbs (Cohen and Towers, 1995).

^{1}H NMR spectrum of **6** showed one singlet chelated hydroxyl group at δ_H 12.28 (1–OH). Four aromatic protons [δ_H 6.79 *d* (*J* = 2.1 Hz, H–4), 6.73 *d* (*J* = 2.1 Hz, H–2), 6.48 *s* (H–7), 6.59 *s* (H–5)] and singlet methyl [δ_H 2.31 (6–CH_3)] displayed in the similar regions for those of compounds **4**–**5.** A singlet methyl at δ_H 3.89 (1–OCH_3) was confirmed by its HMBC correlation to carbonyl at C–9 (δ_C 169.4). Correlations of 8–OH to C–8a (δ_C 106.2) and of H–4 to C–4a (δ_C 157.8), C–9a (δ_C 109.9), C–10 (δ_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 questin (Fujimoto et al., 2004) which was found to be the first isolation from plant that belongs to the genus *Ventilago*.

1D– and 2D NMR analysis of compound **7** confirmed its structure to be a naphthoquinone–lactone related to **1**. ^{1}H– and ^{13}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 δ_H 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* = 17.1, 10.5 Hz, H–4b).

In comparison with **1**, the absence of the chelated hydroxyl group in ^{1}H NMR data of **7** and an additional carbonyl signal at δ_C 161.3 of ring D shown in ^{13}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 (δ_C 179.5), C–9a (δ_C 116.8) and C–10a (δ_C 117.2) were observed on HMBC spectrum corresponding to benzisochromanquinone (or pyranaptoquinone) scaffold related to **1**. HMBC corelations of H–11 to C–1 (δ_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 µg/mL. The MeOH extracts

exhibited weak anti–AChE effect at IC_{50} 36.3±0.0003 µg/mL. Amongst the quinones isolated, only compound **7** obtained from MeOH extract exhibit anti–AChE effect with IC_{50} 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.

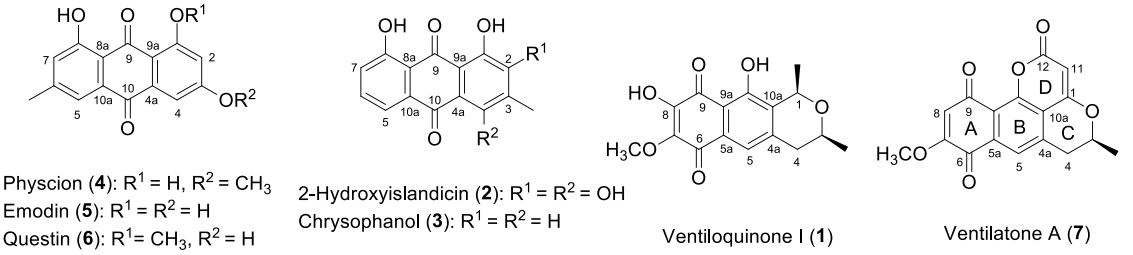

Figure Structures of compounds **1**–**7** isolated from the roots of *V. denticulate*

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 IC_{50} 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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REFERENCES

Asche, C. (2005). Antitumour quinones. Mini Rev Med Chem. 16(18): 449–467.

Balakrishnan, N. and Singh, B. (2013). Anti–diabetic activity of roots of *Ventilago calyculata* Tul. in alloxan induced diabetic rats. Asian J Chem. 25(5): 2438–2440.

Caichompoo, W., Zhang, Q.–Y., Thangthaisong, T. and Phadungkit, M. (2012). Pharmacognostic specification and phytochemical screening of a traditional formula used to improve bone Inter J Pharm Res. 4(1): 89–93.

Chu, X., Sun, A. and Liu, R. (2005). Preparative isolation and purification of five compounds from the Chinese medicinal herb *Polygonum cuspidatum* Sieb. et Zucc by high–speed counter–current chromatography. J Chromatogr A. 1097(1–2): 33–39.

Cohen, P.A. and Neil Towers, G.H. (1995). The anthraquinones of *Heterodermia obscurata*. Phytochemistry 40(3): 911–915.

Ellman, G.L., Courtney, K.D., Andres, V.Jr. and Feather–Stone, R.M. (1961). A new and rapid colorimetric determination of acetyl-cholinesterase activity. Biochem Pharmacol. 7(2): 88–95.

Farag, M.A., Ezzat, S.M., Salama, M.M. and Tadros, M.G. (2016). Anti–acetylcholinesterase potential and metabolome classification of 4 Ocimum species as determined via UPLC/qTOF/MS and chemometric tools. J Pharm Biomed Anal. 125(2016): 292–302.

Flora of China, 12: 164–166. (2007). *Ventilago* Gaertner, Fruct Sem Pl. 1(223): 1788.

Fujimoto, H., Nakamura, E., Okuyama, E. and Ishibashi, M. (2004). Six immunosuppressive features from an ascomycete, *Zopfiella longicaudata*, found in a screening study monitored by immunomodulatory activity. Chem Pharm Bull. 52(8): 1005–1008.

Hanumaiah, T., Marshall, D.S., Rao, B.K., Rao, J.U.M., Rao, K.V.J. and Thomson, R.H. (1985a). Naphthoquinone–lactones and extended quinones from *Ventilago calyculata*. Phytochemistry 24(11): 2669–2672.

Hanumaiah, T., Rao, B.K., Rao, C.P., Rao, G.S.R., Rao, J.U.M., Rao, K.V.J., Marshall, D.S. and Thomson, R.H. (1985b). Naphthalenes and naphthoquinones from *Ventilago* species. Phytochemistry 24(8): 1811–1815.

Hanumaiah, T., Rao, Kesava, B. and Rao, K.V.J. (1983). Chemical examination of *Ventilago calyculata*, Tulasne. Acta Ciencia Indica, Chemistry 9(1–4): 209–211.

Hanumaiah, T., Marshall, D.S., Rao, B.K., Rao, C.P., Rao, G.S.R., Rao, J.U.M.; Rao, K.V.J.H. and Thomson, R. (1985c). Benzisochro-manquinones in *Ventilago* species. Phytochemistry 24(10): 2373–2378.

Hatano, T., Uebayashi, H., Ito, H., Shiota, S., Tsuchiya, T. and Yoshida, T. (1999). Phenolic constituents of Cassia seeds and antibacterial effect of some naphthalenes and anthraquinones on methicillin–resistant *Staphylococcus aureus*. Chem Pharm Bull. 47(8): 1121–1127.

Ho, S.L., Poon, C.Y., Lin, C., Yan, T., Kwong, D.W., Yung, K.K., Wong, M.S., Bian, Z. and Li, H.W. (2015). Inhibition of β–amyloid aggregation by albiflorin, aloe–emodin and neohesperidin

and their neuroprotective effect on primary hippocampal cells against β–amyloid induced toxicity. Curr Alzheimer Res. 12(5): 424–433.

Khammee, T., Athipornchai, A., Upamaia, W., Jaisin, Y. and Suksamrarn, S. (2014). Synthesis of hydroxyxanthones and evaluations for their acetylcholinesterase inhibitory and neuro-toxicity activities. KKU Sci J. 42(2): 212–220.

Krishnakumari, G.N., Bhuvaneswari, B. and Raja Swapna, I., (2001). Antifeedant activity of quinones from *Ventilago madaraspatana*. Fitoterapia 72, 671–675.

Li, C., Shi, J.–G., Zhang, Y.–P. and Zhang, C.–Z. (2000). Constituents of *Eremurus chinensis*. J Nat Prod. 63(5) : 653–656.

Lipipun, V., Kurokawa, M., Suttisri, R., Taweechotipatr, P., Pramyothin, P., Hattori, M. and Shiraki, K. (2003). Efficacy of Thai medicinal plant extracts against herpes simplex virus type 1 infection in vitro and in vivo. Antiviral Res. 60(3): 175–180.

Ncube, B., Finnie, J.F. and Van Staden, J. (2012). In vitro antimicrobial synergism within plant extract combinations from three South African medicinal bulbs. J Ethnopharmacol. 139(1): 81–89.

Orhan, I., Tosun, F. and Sener, B. (2008). Coumarin, anthraquinone and stilbene derivatives with anticholinesterase activity. Z Naturforschung C. 63(5–6): 366–370.

Panthong, A., Kanjanapothi, D., Taesotikul, T., Phankummoon, A., Panthong, K. and Reutrakul, V. (2004). Anti–inflammatory activity of methanolic extracts from *Ventilago harmandiana* Pierre. J Ethnopharmacol. 91(23): 237–242.

Pegg, R.B., Rybarczyk, A., Amarowicz, R. (2008). Chromatographic separation of tannin fractions from a bearberry–leaf (*Arctostaphylos Uva–Ursi* L. Sprengel) extract by SE–HPLC – A short report. Pol. J. Food Nutr Sci. 58(4): 485–490.

Pierre, L.L. and Moses, M.N. (2015). Isolation and characterisation of stigmasterol and β–sitosterol from *Odontonema strictum* (Acanthaceae). JIPBS. 2(1): 88–95.

Pithayanukul, P., Ruenraroengsak, P., Bavovada, R., Pakmanee, N., Suttisri, R. and Saen–oon, S., (2005). Inhibition of Naja kaouthia venom activities by plant polyphenols. J Ethnopharmacol. 97(3): 527–533.

Rao, B.K.; Hanumaiah, T., Rao, C.P.; Rao, G.S.R.; Rao, K.V.J. and Thomson, R.H. (1983). Anthraquinones in *Ventilago species*. Phytochemistry 22(11): 2583–2585.

Smitinand, T. 2014. *In* Thai plant names (Revised edition 2014). Bangkok: The forest herbarium, Royal Forest Department. p. 578.

Sultana, N. and Khalid, A. (2010). Phytochemical and enzyme inhibitory studies on indigenous medicinal plant *Rhazya stricta*. Nat Prod Res. 24(4): 305–314.

Sun, Y.–P. and Liu, J.–P. (2015). Blockade of emodin on amyloid–β25–35–induced neurotoxicity in a βpp/PS1 mice and pc12 cells through activation of the class iii phospha-tidylinositol 3–kinase/beclin–1/b–cell lym-phoma 2 pathway. Planta Med. 81(2): 108–115.

Temkitthawon, P., Viyoch, J., Limpeanchob, N., Pongamornkul, W., Sirikul, C., Kumpila, A.., Suwanborirux, K and Ingkaninan, K., (2008). Screening for phosphodiesterase inhibitory activity of Thai medicinal plants. J. Ethnopharmacol. 119, 214–217.

Walsh, D.M. and Selkoe D.J. (2004). Deciphering the molecular basis of memory failure in Alzheimer's disease. Neuron 44(1): 181–193.

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