CHEMICAL CONSTITUENTS OF THE RHIZOMES OF *Zingiber collinsii* Mood &Theilade (ZINGIBERACEAE) GROWING IN VIETNAM

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ABSTRACT

A chemical investigation of the rhizomes of *Zingiber collinsii* Mood & Theilade (Zingiberaceae) resulted in the isolation and identification of seven compounds, including a sesquiterpenoid (zerumbone(**1**)), acoumarin (scopoletin (**2**)), two flavonoids (quercetin (**3**), rutin (**4)**),afurfural derivative (5-(hydroxymethyl)furfural (**5**)), and two curcuminoids (bisdemethoxycurcumin (**6**),demethoxycurcumin (**7**)). The chemical structures of the seven compounds were determined on the basis of NMR and MS analyses.

Keywords:Zingiber collinsii, zerumbone, furfural, scopoletin, flavonoid, curcuminoid.

1. INTRODUCTION

About 25 species of Zingiberaceae are used to cure multiple disorders in human and animals [1]. In addition to their medicinal activities, Zingiberaceae plants extracts may also serve as a natural larvicidal agent. Ginger extracts have anti-inflammatory, anti-oxidant and anti-cancer effects. Shogaol can significantly attenuate a variety of neuro inflammatory responses in cortical astrocytes [2,3]. Ginger supplementations significantly reduce severity of acute chemotherapy-induced nausea in adult cancer patients. The genus *Zingiber* Boehm has been represented by 13 species in Indo-China. Among them 10 species were reported from Vietnam. Hoincluded 11 species in his Illustrated Flora of Vietnam [4]. The major pungent compounds in ginger are active gingerols and their derivatives, viz. shogaols, zingerone, and parado [5-8]. The ginger volatile oil is consisted of monoterpenes camphene, cineole, linalool, limonene, citral, geraniol, citronellol and borneol and sesquiterpenes, α-zingiberene, ar-curcumene, β-bisabolene, β-sesquiphellandrene, zingiberol, and zingiberenol along with some aliphatic aldehydes and alcohols [9-13].Ginger is added to a wide range of food as an indispensable curry powder or

sauce. It is often used to flavour bread, tea, carbonated drinks, biscuits, pickles, and otherconfectionaries because of its aroma and flavour. During a field trip to Vietnam in 1980 Mark Collins collected a most remarkable new species of *Zingiber*. *Zingiber collinsii* Mood & Theilade (Zingiberaceae), a deciduous species, was recently collected in Vietnam and introduced by Mark Collins [14]. Literature information is scanty on its volatile and non-volatile constituents as well as the biological potential of this plant [15].

In this paper we reported on the isolation and identification of seven compounds from the plant rhizomes.

2. EXPERIMENTS

2.1. General

Melting points were determined using Yanagimoto MP-S3 apparatus. NMR spectra were obtained on the Bruker AV-500 NMR spectrometer, with tetramethylsilane (TMS) as the internal standard and chemical shifts were reported in δ values (ppm). The electrospray ionization mass spectra (ESI-MS) were determined using an Agilent 1200 LC-MSD Trap spectrometer. Column chromatography (CC) was perforssmed on silicagel (Kieselgel 60, 70 - 230 mesh and 230 - 400 mesh, E. Merck). Thin layer chromatography (TLC) was conducted on precoatedKieselgel 60 F_{254} plates (Merck) and the compounds were visualized by spraying with 10 % (v/v) H_2SO_4 followed by heating at 110 °C for 10 min.

2.2. Plant materials

The rhizomes of *Zingiber collinsii* Mood & Theilade (Zingiberaceae) were collected in Pu Mat, Nghe An in September 2013. They were identified by Assoc. Prof. Dr. Tran Huy Thai (Institute of Ecology and Biological Resources, Vietnam Academy of Science and Technology). Voucher specimens are kept at the Faculty of Biology, Vinh University.

2.3. Extraction and isolation

The dried powdered rhizomes of *Zingiber collinsii* (4.0 kg) were extracted three times with methanol at room temperature. The methanol extract was evaporated under vacuum to dryness as a dark brown mass (180 g). The methanol residue (180 g) was suspended in water and partitioned with ethylacetate and *n*-butanol to afford ethyl acetate (53 g) and *n*-butanol (30 g) extracts, respectively.

The ethyl acetate extract (53 g) was applied to silicagel column chromatography with a*n*-hexan: acetone step gradient system (100:0; 50:1; 39:1; 30:1; 20:1; 15:1; 9:1; 4:1; 2:1; 1:1) to afford minor fractions which were combine into ten fractions. Fraction 1 was subjected to silicagel column chromatography (200 g, 60 × 3 cm) eluting with *n*-hexane-acetone (15:1; 9:1) to afford ten subfractions. Subfraction 1.1 was purified with silicagel column chromatography (200 g, 60 × 5 cm) eluting with *n*-hexane:acetone (15:1)to yield compound **5** (55,2 mg). Subfraction 1.3 was purified with silicagel column chromatography (60 g, 60 × 2 cm) eluting with a *n*-hexane:acetone step gradient system(9:1; 4:1) to yield compounds **1** (35 mg) and **2** (29 mg). Subfraction 1.4, eluting with chloroform: methanol (20:1), was further fractionated and purified with silicagel column chromatography (200 g, 60 × 3 cm) to afford compound **3** (83 mg).

The *n*-butanol extract (30 g) was applied to silicagel column chromatography with a cloroform:metanol step gradient system (30:1, 20:1, 10:1, 5:1) to give ten fractions. Fraction 5, eluting with chloroform:methanol (10:1; 6:1), was purified by silicagel column chromatography (200 g, 60 × 3 cm) to give compound **4** (34 mg). Fraction 6, eluting with chloroform:methanol (7:1), was purified with silicagel column chromatography to give compounds **6** (112 mg) and **7** (92 mg).

Zerumbone (**1**): yellowish crystals, m.p. 66 – 67 °C; ESI-MS *m/z* 218$[M]^+$;^{1}H-NMR (CD_3OD, 500 MHz), δ (ppm): 6.12 (1H, *d*, *J* = 11.0 Hz, H-6), 5.99 (1H, *s*, H-10), 5.33 (1H, *dd*, *J* = 6.0, 10.0 Hz, H-9), 2.46-2.56 (1H, *m*, H-5), 2.38-2.44 (1H, *m*, H-4), 2.26-2.31 (1H, *m*, H-1), 1.93 (1H, *d*, *J* = 12.5 Hz, H-2), 1.79 (3H, *s*, 13-CH_3), 1.59 (3H, *s*, 12-CH_3), 1.26 (3H, *s*, 15-CH_3), 1.08 (3H, *s*, 14-CH_3); ^{13}C-NMR (CD_3OD, 125 MHz), δ (ppm): 206.8 (C-8), 163.2 (C-10), 151.2 (C-6), 138.9 (C-7), 137.6 (C-3), 128.1 (C-9), 126.1 (C-2), 43.3 (C-1), 40.4 (C-4), 38.8 (C-11), 29.8 (C-15), 25.4 (C-5), 24.5 (C-14), 15.3 (C-12), 11.8 (C-13).

Scopoletin (**2**): yellowish crystals, m.p. 203 – 204 °C; ESI-MS *m/z* 193 $[M+H]^+$; ^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 7.60 (1H, *d*, *J* = 9.5 Hz, H-4), 6.92 (1H, *s*, H-5), 6.85 (1H, *s*, H-8), 6.28 (1H, *d*, *J* = 9.5 Hz, H-3), 3.95 (3H, *s*, 6-OCH_3); ^{13}C-NMR ($CDCl_3$, 125 MHz), δ (ppm): 161.5 (C-2), 150.3 (C-7), 149.8 (C-9), 144.1 (C-6), 143.3 (C-4), 113.4 (C-5), 111.5 (C-10), 107.5 (C-3), 103.2 (C-8), 56.6 (6-OCH_3).

Quercetin (**3**): yellow needles, m.p. 313 – 314 °C; ESI-MS *m/z*302 $[M]^+$;^{1}H-NMR (DMSO-d_6, 500 MHz), δ (ppm): 7.67 (1H, *d*, *J* = 2.5 Hz, H-2'), 7.54 (1H, *dd*, *J* = 7.5, 2.5 Hz, H-6), 6.88 (1H, *d*, *J* = 8.5 Hz, H-5'), 6.40 (1H, *d*, *J* = 2.0 Hz, H-8), 6.18 (1H, *d*, *J* = 2.0 Hz, H-6); ^{13}C-NMR (DMSO-d_6, 125 MHz), δ (ppm): 175.9 (C-4), 163.9 (C-7), 160.8 (C-5), 156.2 (C-9), 147.7 (C-4'), 146.9 (C-2), 145.1 (C-3'), 135.8 (C-3), 122.0 (C-1'), 120.0 (C-6'), 115.7 (C-5'), 115.1 (C-2'), 103.1 (C-10), 98.2 (C-6), 93.4 (C-8).

Rutin (**4**): yellow crystals, m.p. 214 – 215 °C; ESI-MS *m/z*611 $[M]^+$;^{1}H-NMR (DMSO-d_6, 500 MHz), δ (ppm): 7.54 (2H, *dd*, *J* = 2.5, 9.0 Hz, H-2', 6'), 6.84 (1H, *d*, *J* = 8.0 Hz, H-5'), 6.38 (1H, *d*, *J* = 2.0 Hz, H-8), 6.19 (1H, *d*, *J* = 2.0 Hz, H-6), 5.34 (1H, *d*, *J* = 7.0 Hz, glc H-1"), 4.38 (1H, *brs*, rham H-1'"), 3.71-3.05 (*m*,12H of sugar moieties), 1.00 (3H, *d*, *J* = 6.0, rham-CH_3);^{13}C-NMR (125 MHz, DMSO-d_6), δ ppm: 177.5 (C-4),164.2 (C-7), 161.3 (C-5), 156.7 (C-2), 156.5 (C-9), 148.5 (C-4'), 144.8 (C-3'), 133.4 (C-3), 121.7 (C-6'), 121.3 (C-1'), 116.4 (C-5'), 115.3 (C-2'), 104.0 (C-10), 101.3 (C-1''), 100.8 (C-1'''), 98.8 (C-6), 93.7 (C-8), 76.5 (C-3''), 76.0 (C-5''), 74.2 (C-2''), 71.9 (C-4'''), 70.7 (C-3'''), 70.5 (C-2'''), 70.1 (C-4''), 68.3 (C-5'''), 67.1 (C-6''), 17.8 (C-6''').

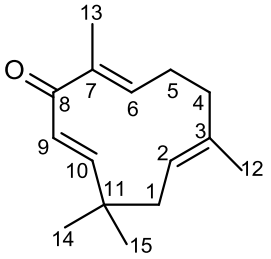
5-(Hydroxymethyl)furfural (**5**): colorless oil; ESI-MS *m/z* 127 $[M+H]^+$;^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 9.49 (1H, *s*, H-1), 7.24 (1H, *d*, *J* = 3.5 Hz, H-3), 6.51 (1H, *d*, *J* = 3.5 Hz, H-4), 4.66 (2H, *s*, H-6); ^{13}C-NMR ($CDCl_3$, 125 MHz), δ (ppm): 177.8 (C-1), 161.2 (C-5), 151.8 (C-2), 123.8 (C-3), 109.9 (C-4), 56.9 (C-6).

Bisdemethoxycurcumin (**6**): yellow powder, m.p. 220 – 22 °C; ESI-MS *m/z* 309 $[M+H]^+$;^{1}H-NMR ($CDCl_3$, 500 MHz), δ (ppm): 7.57 (2H, *d*, *J* = 16.0 Hz, H-4, 4'), 7.42 (4H, *d*, *J* = 9 Hz, H-6, 6', 10, 10'), 6.87 (4H, *d*, *J* = 9 Hz, H-7, 7', 9, 9'), 6.46 (2H, *d*, *J* = 16.0 Hz, H-3, 3'), 5.80(1H, *s*, H-1);^{13}C-NMR ($CDCl_3$, 125 MHz), δ (ppm): 183.1 (C-2, 2'), 159.4 (C-8, 8'), 140.2 (C-4, 4'), 129.6 (C-6, 6', 10, 10'), 126.1 (C-5, 5'), 120.6 (C-3, 3'), 115.9 (C-7, 7', 9, 9'), 100.8 (C-1).

Demethoxycurcumin (**7**): yellow powder,m.p. 160 – 161 °C; ESI-MS*m/z* 339 $[M+H]^+$;^{1}H-NMR ($CDCl_3$, 500 Hz), δ (ppm): 7.57 (2H, *dd*, *J* = 9.0, 16.0 Hz, H-4, 4'), 7.42 (2H, *d*, *J* = 8.5 Hz, H-6, 6'), 7.07 (2H, *dd*, *J* = 2.0, 4.0 Hz, H-10, 10'), 6.90 (1H, *d*, *J* = 8.5 Hz, H-7'), 6.87 (2H,

d, *J* = 8.5 Hz, H-9, 9'), 6.47 (2H, dd, J = 6.0, 15.5 Hz, H-3, 3'), 5.80 ppm (1H, *s*, H-1), 3.93 (3H, *s*, 7-OCH_3); ^{13}C-NMR ($CDCl_3$, 125 Hz), δ (ppm): 183.1 (C-2), 182.7 (C-2'), 159.4 (C-8), 148.5 (C-8'),147.4 (C-7), 140.3 (C-4), 140.2 (C-4'), 129.6 (C-6'), 126.8 (C-5), 126.0 (C-5'), 122.6 (C-10, 10'), 120.9 (C-3), 120.5 (C-3'), 115.9 (C-9), 115.2 (C-7', 9'), 109.9 (C-6), 100.8 (C-1), 55.6 (7-OCH_3).

3. RESULTS AND DISCUSSION



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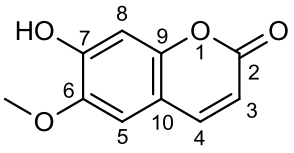

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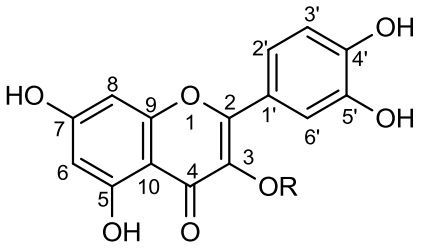
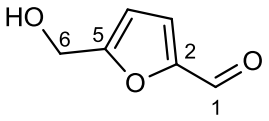


3 R = H
4 R = α-L-rhamnopyranosyl-(1➔6)-β-D-glucopyranose



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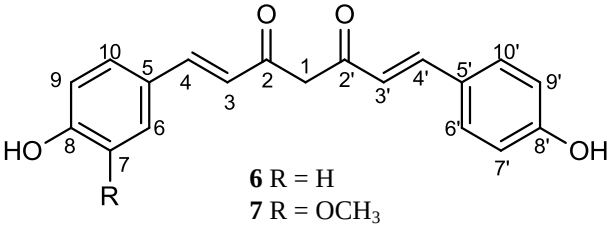

6 R = H
7 R = OCH_3

Compound **1** was isolated as yellowish crystals, m.p. 66 – 67 °C. The ESI-MS spectrum showed a pseudomolecular ion peak $[M]^+$ at *m/z* 218 ($C_{15}H_{22}O$). The ^{1}H-NMR spectrum showed four singlets of four methyl groups at δ_H 1.79 (13-CH_3), 1.59 (12-CH_3), 1.26 (15-CH_3) and 1.08 (14-CH_3), three methylene groups signals (H-1, H-4 and H-5) between δ 2.26-2.56 and a broad doublet at 6.12 (*d*, *J* = 11.0 Hz, H-6) of olefinic proton. The ^{13}C-NMR and DEPT spectrum exhibited 15 carbons, including 4 quarternary carbons, three methylene groups, four methine and four methyl groups. In the very low field of the ^{13}C-NMR spectrum, there appeared one signal at δ_C 206.8 (C-8) corresponding to a carbonylcarbon. The signals of four methyl groups appeared at δ_C 11.8 (C-13), 15.3 (C-12), 24.5 (C-14), and 29.8 (C-15). In addition, four downfield signals at δ_C 126.0, 151.2, 128.1 and 163.2 were assigned to methine carbons C-2, C-6, C-9 and C-10, respectively. This analysis suggested that the compound **1** is zerumbone. This is also confirmed by comparison of spectral data of **1** with literature [16].

Compound **2** was isolated as yellowish crystals, m.p. 203 – 204 °C. The ESI-MS spectrum showed a pseudomolecular ion peak $[M+H]^+$ at *m/z* 193 ($C_{10}H_8O_4$). The ^{1}H-NMR spectrum showed two specific doublets at δ_H 6.28 (*d*, *J* = 9.5 Hz, H-3) and 7.60 (*d*, *J* = 9.5 Hz, H-4) featuring two protons of pyrone ring of a coumarin. In low field, two aromatic protons present at 6.92 (*s*, H-5) and 6.85 (*s*, H-8) ppm. Besides, a signal of methoxy group appeared at δ_H 3.95 (*s*, 6-CH_3). The ^{13}C-NMR spectrum showed 10 carbons, including signals at δ_C 161.5 (C-2) and 150.3 (C-7) corresponding to carbonyl group and phenolic carbon. The methoxy group also appeared at δ_C 56.0 ppm. These data proved that **2** has coumarin skeleton. The comparison of spectra of **2** with literature data [17] determined **2** asscopoletin.

Compound **3** was isolated as yellow needles, m.p. 313 – 314 °C.The ESI-MS spectrum showed a pseudomolecular ion peak $[M]^+$ at *m/z* 302 ($C_{15}H_{10}O_7$). The ^{1}H-NMR spectrum showed two doublets of two aromatic protons at δ_H 6.18 (*d*, *J* = 2.0 Hz, H-6) and 6.40 ppm (*d*,*J* = 2.0 Hz, H-8). Also, three protons at 7.67 (*d*, *J* = 2.5 Hz, H-2'), 7.54 (*dd*, *J* = 7.5, 2.5 Hz, H-6') and 6.88 ppm (*d*, *J* = 8.5 Hz, H-5') showed ABX-type signals. The ^{13}C-NMR spectrum of compound **3** showed 15 signals between δ 93.4-175.9 ppm of the flavonoid skeleton. The comparison of spectral data of **3** with literaturedata [18] determined **3** as quercetin.

Compound **4** was isolated as yellow crystals, m.p. 214 – 215 °C. The ESI-MS spectrum showed a pseudomolecular ion peak $[M]^+$ at *m/z* 611 ($C_{27}H_{30}O_{16}$). The structure of compound **4** can be elucidated by the comparison of its spectra with the NMR spectra of **3**. The ^{1}H-NMR spectrum of **4** showed signals of one tri-substituted aromatic ring at 7.54 (*m*, H-2', 6'), and 6.84 (*d*, *J* = 8.0 Hz, H-5') and two anomeric protons at 5.34 (*d*, *J* = 7.5 Hz, glc H-1") and 4.38 (*d*,*J* = 1.0 Hz, rham H-1'").This is evinced that compound **4** has the same aglycone moiety as**3**. However, the ^{1}H-NMR spectrum of compound **4** showed the presence of proton signals of two glycosides at 3.71-3.05 (*m*, 12H of sugar moieties) and a methyl group at 0.99 (3H, *d*, *J* = 6.0, rham-CH_3). The ^{13}C-NMR and DEPT spectra of **4** showed signals of 27 carbons, including 15 carbons of flavonoid skeleton and 12 carbons of the two sugar moieties of rutin (β-D-glucose: δ_C 101.3, 74.2, 76.5, 70.1, 76.0, 67.1 and α-L-rhamnose: δ_C 104.0, 74.1, 71.9, 70.7, 70.5, 17.8). The comparison of spectral data of **4** with literature data [18] confirmed **4** to be rutin (quercetin-3-O-[α-L-rhamnopyranosyl-(1→6)-β-D-glucopyranoside).

Compound **5** was isolated as colorless oil. It had a pseudomolecular ion peak $[M+H]^+$ at *m/z* 127 in ESI-MS spectrum. The ^{1}H-NMR spectrum showed a signal of an aldehyde proton at δ 9.49 ppm, two olefinic protons at δ_H 7.24 (*d*, *J* = 3.5 Hz) and 6.51 (*d*, *J* = 3.5 Hz). The ^{13}C-NMR spectrum showed 6 carbon signals, including an aldehyde at 177.8, 4 olefinic carbons at 161.2 (C-5), 151.8 (C-2), 123.8 (C-3), and 109.9 (C-4), and an aliphatic oxygen-bearing carbon at 56.9 (C-6). Based on these data and the comparison with literature data [19], compound **5** is suggested to be 5-(hydroxymethyl)furfural.

Compounds **6** and **7** were isolated as yellow powders. They were identified as curcuminoids. The ^{1}H-NMR spectrum of compound **6** indicated the presence of a methine proton in the enolic form at 5.80 ppm (1H, *s*, H-1). Two doublets with a large coupling constant at δ 7.57 (2H, *d*, *J* = 16.0 Hz, H-4, 4') and 6.46 (2H, *d*, *J* = 16.0 Hz, H-3, 3') showed a transolefinic system. At downfield, it showed a presence of AB-type signals at δ 7.42 (4H, *d*, *J* = 9 Hz, H-6, 6', 10, 10') and 6.87 (4H, *d*, *J* = 9 Hz, H-7, 7', 9, 9'). The ^{13}C-NMR spectrum of **6**showed 8 signals of 19 carbons. At very low field, one signal of two carbonyl groups presented at δ 183.1 (C-2, 2') and one signal at δ 159.4 (C-8, 8') corresponding to two phenolic carbons. The ^{13}C-NMR spectrum also exhibited signals of aromatic carbons at δ 129.6 (C-6, 6', 10, 10'), 126.1 (C-5, 5'), 115.9 (C-7,7', 9, 9') and olefinic carbons at δ 140.2 (C-4, 4'), 120.6 (C-3, 3'). The spectral data of compound **7** are similar with **6**, but with difference in emergence of a methoxy group at δ 3.93 (3H, *s*) in ^{1}H-NMR spectrum and 55.6 in ^{13}C-NMR spectrum. These spectral data proved that **6** and **7** were bisdemethoxycurcumin and demethoxycurcumin, respectively. The structures of these compounds were reaffirmed based on the comparison with literature data [20].

4. CONCLUSIONS

In the present study, thechemical constituentsof the rhizomes of *Zingiber collinsii* Mood & Theilade (Zingiberaceae) collected in Pumat, Nghe An province was investigated, resulting in the isolation and identification of seven compounds such aszerumbone, scopoletin, quercetin,

rutin, (5-(hydroxymethyl)furfural), bisdemethoxycurcumin, and demethoxycurcumin. Their structures were determined based on NMR and MS analyses.

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TÓM TẮT

THÀNH PHẦN HÓA HỌC CỦA RỄ LOÀI GỪNG COLLINS
(*Zingiber collinsii* Mood & Theilade (ZINGIBERACEAE)) Ở VIỆT NAM

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Nghiên cứu thành phần hóa học của rễ loài gừng Collins (*Zingiber collinsii* Mood & Theilade (Zingiberaceae)) bằng các phương pháp sắc kí đã phân lập được 7 hợp chất bao gồm sesquiterpenoid (zerumbone (**1**)), cumarin (scopoletin (**2**)), flavonoid (quercetin (**3**), rutin (**4**)), dẫnxuất furfural (5-(hydroxymethyl) furfural (**5**)) và curcuminoid (bisdemethoxycurcumin (**6**), demethoxycurcumin (**7**))Các hợp chất này được xác định cấu trúc bằng các phương pháp phổ khối lượng (MS) và phổ cộng hưởng từ hạt nhân (NMR).

Từ khóa:Zingiber collinsii, zerumbone, furfural, coumarin, flavonoid, curcuminoid.