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FURTHER STUDY ON CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *ALPINIA CONCHIGERA* GRIFF. (ZINGIBERACEAE)

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SUMMARY

*β-Sitosterol, stigmasterol, cardamomin (**1**), chalconaringenin 2′-O-methyl ether (**2**), alpinetin (**3**), and naringenin 5-O-methyl ether (**4**) were isolated from the MeOH extract of Alpinia conchigera Griff. (Zingiberaceae). Their structures were determined on the basis of spectroscopic analyses. The antimicrobial and free radical scavenging activities of the isolates **1-4** were evaluated in correlation with those of the soluble fractions.*

Key words: *Alpinia conchigera*; Zingiberaceae; chalcone; flavanone; antimicrobial activity; free radical scavenging activity

I - INTRODUCTION

The medicinal plant *Alpinia conchigera* Griff. of family Zingiberaceae (Vietnamese name: Rieng rung) is a very attractive target for the chemical and biological studies. Flavones, flavanones, chalcones, diarylheptanoids, phenylpropanoids, and carbohydrates were isolated from the rhizomes and fruits of the species [1-3]. Our previous investigation revealed the isolation of the chalcone cardamomin (**1**) as the main constituent of the EtOH extract of the rhizomes of *A. conchigera* growing in Vietnam [1]. The finding will be of high interest since the blockade of nuclear factor-kappa B signaling pathway and anti-inflammatory activity of cardamomin were clearly demonstrated in our study [4]. In a continuation of the study we report in this paper the isolation of *β*-sitosterol, stigmasterol, cardamomin (**1**), chalconaringenin 2′-*O*-methyl ether (**2**), alpinetin (**3**), and naringenin 5-*O*-methyl ether (**4**) from the MeOH extract of *A. conchigera* and the antimicrobial and free radical scavenging activities of the isolates **1-4** in correlation with those of the soluble fractions of the MeOH extract.

II - EXPERIMENTAL

1. General Procedure

^{1}H-NMR (500 MHz) and ^{13}C-NMR (125 MHz) spectra were obtained on a Bruker Avance 500 spectrometer with tetramethyl silane (TMS) as reference. EI-MS (70 eV) spectra were measured on a Hewlett-Packard 5989B mass spectrometer. Silica gel (63 - 100 μm, Merck) was used for open column (CC) and flash column (FC) chromatography. TLC was performed on precoated DC Alufolien 60 F_{254} plates (Merck) and detected by UV light (254 nm) and/or by spraying with 1% vanillin in conc. H_2SO_4 and 5% $FeCl_3$ in EtOH.

2. Plant Material

The rhizomes of *A. conchigera* Griff. (Zingiberaceae) were collected and identified by Dr Nguyen Hoanh Coi, a botanist of the Military Institute of Pharmaceutical Control and Research, in October 2003 in province Thai Nguyen, Vietnam.

3. Extraction and Isolation

The fresh rhizomes of *A. conchigera* (20 kg) were sliced, air-dried and then oven-dried at 50°C to give a dry material (1.5 kg). The material was powdered and then extracted with MeOH by percolation at room temperature. The combined MeOH extracts were concentrated under reduced pressure and the residue was suspended in H_2O and extracted with *n*-hexane, EtOAc, and *n*-BuOH, successively. On removing the organic solvents the corresponding *n*-hexane- (**CH**) (24.5 g, 1.63% yield on the basis of the dry weight of the rhizomes), EtOAc-(**CE**) (33.2 g, 2.21% yield), and *n*-BuOH-soluble (**CB**) fractions (11.3 g, 0.75% yield) were obtained. Part of the *n*-hexane-soluble fraction (10 g) was repeatedly chromatographed on silica gel CC and FC, using *n*-hexane-EtOAc gradients, to give β-sitosterol and stigmasterol as a mixture and cardamomin (**1**) (2.1g). Part of the EtOAc-soluble fraction (10 g) was repeatedly chromatographed on silica gel CC and FC, using $CHCl_3$-$(CH_3)_2CO$ gradients to give cardamomin (**1**) (1.4 g), chalconaringenin 2′-*O*-methyl ether (**2**) (5 mg), alpinetin (**3**) (5 mg), and naringenin 5-*O*-methyl ether (**4**) (5 mg).

Cardamomin (1): Yellow needles. mp. 203-205°C (Lit. [4]: 205-208°C). R_f=0.72 (*n*-hexane-EtOAc, 5:2). UV, IR, EI-MS, ^{1}H-, and ^{13}C-NMR spectroscopic data are comparable with the literature values [5].

Chalconaringenin 2′-O-methyl ether (2): Orange cylindrical crystals. mp. 158 - 160°C. R_f=0.52 [$CHCl_3$-$(CH_3)_2CO$, 5:1]. UV (EtOH) λ_{max}: 200, 367. UV (EtOH+$AlCl_3$) λ_{max}: 207, 368. IR ν_{max} (film) cm^{-1}: 3307, 1602, 1551, 1509, 1443, 1347, 1214, 1170, 1113, 1037, 978, 825. ^{1}H-NMR (CD_3OD): δ 3.93 (3H, s, 2'-OCH_3), 5.94 (1H, d, *J* = 2 Hz, H-3'), 6.01 (1H, d, *J* = 2 Hz, H-5'), 6.84 (2H, d, *J* = 8.5 Hz, H-3, H-5), 7.51 (2H, d, *J* = 8.5 Hz, H-2, H-6), 7.68 (1H, d, *J* = 15.5 Hz, H-α) 7.78 (1H, d, *J* = 15.5 Hz, H-β). ^{13}C-NMR (CD_3OD): δ 56.3 (q, 2′-OCH_3), 92.5 (d, C-5′), 97.1 (d, C-3′), 106.6 (s, C-1′), 116.9 (d, C-3, C-5), 125.6 (d, C-α), 128.4 (s, C-1), 131.3 (d, C-2, C-6), 143.7 (d, C-β), 161.1 (s, C-4), 164.7 (s, C-2'), 166.5 (s, C-4'), 168.6 (s, C-6′), 194.0 (s, CO). EI-MS (70 eV): *m/z* (%) 286 ($C_{16}H_{14}O_5$, $[M]^{+\cdot}$) (44.4), 193 (45.2), 166 (100), 138 (63.6), 123 (32.1), 103 (31.9), 95 (26.6), 77 (39.0).

Alpinetin (4): Colorless needles. mp. 231 - 232°C (Lit. [4]: 223 - 227°C). R_f = 0.78 [$CHCl_3$-$(CH_3)_2CO$, 5:1]. UV, IR, EI-MS, ^{1}H-, and ^{13}C-NMR spectroscopic data are comparable with the literature values [5].

Naringenin 5-O-methyl ether (5): Colorless needles. mp. > 260°C. R_f = 0.66 [$CHCl_3$-$(CH_3)_2CO$, 5:1]. UV, IR, EI-MS, ^{1}H, and ^{13}C-NMR spectroscopic data are comparable with the literature values [6].

Evaluation of antimicrobial activity and determination of minimum inhibitory concentration (MIC)

The broth microdilution method was used for preliminary evaluation and MIC determination [7]. The strains of bacteria, fungi and yeasts were listed in the table 1.

Evaluation of free radical scavenging activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method [8] was employed in the evaluation of the free radical scavenging activity of the soluble fractions **CH**, **CE**, and **CB**, and the pure compounds **1** - **4**.

III-RESULTS AND DISCUSSION

1. Extraction, Isolation, and Structure determination of compounds 1-4

The dried rhizomes of *A. conchigera* were extracted with MeOH, and the resultant MeOH extract was fractionated into *n*-hexane-(**CH**), EtOAc-(**CE**), and *n*-BuOH-soluble (**CB**) fractions. β-Sitosterol, stigmasterol, and

cardamomin (**1**) were isolated from **CH**, and cardamomin (**1**), chalconaringenin 2′-*O*-methyl ether (**2**), alpinetin (**3**), and naringenin 5-*O*-methyl ether (**4**) were isolated from **CE** by repeated CC and FC.

Compound **2** was isolated as orange cylindrical crystals, mp. 158 - 160°C. Its molecular formula was determined as $C_{16}H_{14}O_5$ from EI-MS spectrum (*m/z* 286, $[M]^{+\cdot}$). The IR spectrum of **2** showed the presence of hydroxyl groups (ν_{max} 3307 cm^{-1}) and aromatic rings (ν_{max} 1602, 1509, and 1443). The ^{1}H- and ^{13}C-NMR spectroscopic data (see Experimetal) showed two benzene moieties, one of which was a tetrasubstituted benzene nucleus [δ_H 5.94 (d, J = 2 Hz), 6.01 (d, J = 2 Hz)] and the other was a 1,4-disubstituted benzene nucleus [δ_H 6.84 (2H, d, J = 8.5 Hz), 7.51 (2H, d, J = 8.5 Hz)], a *trans*-α,β-unsaturated carbonyl group [δ_H 7.68 (1H, d, J = 15.5 Hz, H-α), 7.78 (1H, d, J = 15.5 Hz, H-β); δ_C 125.6 (d, C-α), 143.7 (d, C-β), and 194.0 (s, CO)] and a methoxyl group [δ_H 3.93 (s)]. Comparison of the ^{13}C chemical shifts of **2** and cardamomin (**1**) revealed the structure of **2** as 4,4′,6′-trihydroxy-2′-methoxychalcone. Since the addition of $AlCl_3$ resulted in no shifts in UV spectrum the location of the methoxyl group was confirmed at C-2′.

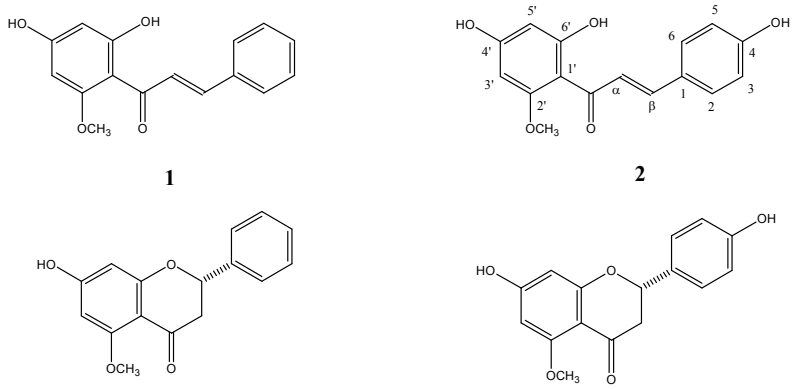

Figure 1: Chemical structures of compounds **1** - **4**

Therefore the structure of **2** was determined as chalconaringenin 2′-*O*-methyl ether, which was further confirmed by the analysis of ^{1}H-^{1}H COSY, HMQC, and HMBC spectra (Fig. 2).

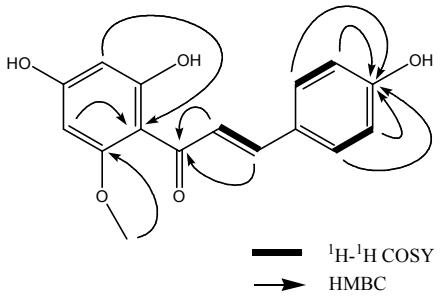

Figure 2: Selected ^{1}H-^{1}H COSY and HMBC correlations of **2**

Compound **1**, **3**, and **4** were determined by comparing their spectroscopic data (UV, IR, EI-MS, ^{1}H-, and ^{13}C-NMR spectra) with those reported in the literature [4, 5]. Their isolation from the EtOH extract of the rhizomes of *A. conchigera* was reported elsewhere [1].

Evaluation of the in-vitro antimicrobial activity

As shown in table 1 compounds **1** - **4** were found to be active against *Escherichia coli* and *Staphylococcus aureus* in a good correlation with the activity demonstrated by **CH** and **CE**. Compound **1** was shown to be more active (MIC 50 µg/ml) than **CH** (MIC 100 µg/ml) against *Bacillus subtilis*. Compounds **2** and **3** inhibited the growth of *B. subtilis* but they were isolated from the non-active **CE**. Compounds **1**-**4** were not active against the fungi and the yeasts

tested. However **CE** and **CB** can be used as plant-derived preparations against *Fusarium oxysporum*, *Candida albicans*, and *Saccharomyces cerevisiae*.

Table 1: Antimicrobial activity of the soluble fractions CH, CE, and CB and pure isolates **1**-**4** from *A. conchigera*

No.	Sample	Minimum Inhibitory Concentration (MIC, µg/ml)							
		Gram (-) bacterium		Gram (+) bacterium		Fungus		Yeast	
		Escherichia coli	*Pseudomonas aeruginosa*	*Bacillus subtillis*	*Staphylococcus aureus*	*Aspergillus niger*	*Fusarium oxysporum*	*Candida albicans*	*Saccharomyces cerevisiae*
1	**CH**	200	100	100	n.a[a)]	n.a[a)]	n.a[a)]	n.a[a)]	n.a[a)]
2	**CE**	100	n.a[a)]	n.a[a)]	50	n.a[a)]	n.a[a)]	50	100
3	**CB**	n.a[a)]	n.a[a)]	n.a[a)]	100	n.a[a)]	100	50	100
4	**1**	25	n.a[a)]	50	25	n.a[a)]	n.a[a)]	n.a[a)]	n.a[a)]
5	**2**	50	n.a[a)]	25	25	n.a[a)]	n.a[a)]	n.a[a)]	n.a[a)]
6	**3**	50	n.a[a)]	50	50	n.a[a)]	n.a[a)]	n.a[a)]	n.a[a)]
7	**4**	50	n.a[a)]	n.a[a)]	50	n.a[a)]	n.a[a)]	n.a[a)]	n.a[a)]

[a)] n. a.: the sample did not show activity in the preliminary test.

2. Evaluation of the free radical scavenging activity

The antioxidant potentials of **CH**, **CE**, **CB**, and **1**-**4** were evaluated using a chemical reaction with the stable radical species 2,2-diphenyl-1-picrylhydrazyl (the DPPH method). Table 2 showed the free radical scavenging activity of **CE** (10.78 µg/ml) and **CB** (2.45 µg/ml). However, **CH** and the pure isolates **1** - **4** were not active as DPPH free radical scavengers.

Table 2: Free radical scavenging activity of the soluble fractions **CH**, **CE**, and **CB** and pure isolates **1** - **5** from *A. conchigera*

No.	Sample	Scavenging activity, %	Scavenging activity, µg/ml
1	Positive reference	87.04±0.0	25.31
2	Negative reference	0.00±0.0	n.a[a)]
3	**CH**	46.18±0.3	n.a[a)]
4	**CE**	67.14±0.6	10.78
5	**CB**	68.50±0.6	2.45
6	**1**	3.28±0.9	n.a[a)]
7	**2**	15.41±0.4	n.a[a)]
8	**3**	5.48±1.2	n.a[a)]
9	**4**	4.30±1.0	n.a[a)]

[a)]n.a.: the sample did not show free radical scavenging activity in the DPPH method.

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