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STUDY ON CHEMICAL CONSTITUENTS AND BIOLOGICAL ACTIVITIES OF *ALPINIA KWANGSIENSIS* COLLECTED IN VIETNAM

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ABSTRACT

Three compounds named methyl-*trans*-*p*-coumarate (**1**), scopoletin (**2**), and (+)-gallocatechin (**3**) have been isolated from the ethyl acetate fraction of methanol extract of *Alpinia kwangsiensis* roots collected in Thai Nguyen province. Their structures were elucidated on the basis of spectroscopic data and by comparison with their spectral data reported in literature. This is the first isolation of three compounds from this species. The ethyl acetate fraction was found to be active against bacteria as *Staphylococcus aureus* and *Bacillus subtilis* with the IC_{50} values of 74.65 μg/ml and 80.54 μg/ml, respectively. This fraction also showed antioxidant activity through DPPH test with the EC_{50} value of 87.98 μg/ml.

Keywords: Alpinia kwangsiensis, coumaric, flavanol, antioxidant, DPPH, antibacterial.

1. INTRODUCTION

Alpinia kwangsiensis T. L. Wu and S. J. Chen (*A. kwangsiensis*) belongs to the family Zingiberaceae and is a perennial herb that grows widely in valley forests in Guangdong, Guangxi, Guizhou, Yunnan provinces of China and the northern mountainous areas of Vietnam. Its roots can cure abdominal, stomach, vomiting, etc. [1, 2, 3]. The chemical constituents of the essential oil from *A.kwangsiensis* rhizomes collected in China were identified by GC-MS method [4, 5]. The main compounds in the essential oil (camphor and eucalyptol) exhibited significant insecticidal activity on *Lasioderma serricorne* [5].

In Vietnam, this species is used only for food and medical treatment according to folklore [1, 6] and has never been reported on the phytochemicals and biological activities. This paper deals with the isolation and structure elucidation of methyl-*trans*-*p*-coumarate (**1**), scopoletin (**2**) and *(+)*-gallocatechin (**3**) from the ethyl acetate fraction of methanol extract of *A. kwangsiensis* roots as well as evaluation of antioxidant and antibacterial activities of this fraction.

2. MATERIALS AND METHODS

2.1. General procedure

Melting points (m.p.) were determined on a Botius melting point apparatus (Germany). ESI-MS spectra were recorded on ESI-LC/MS/MS-Xevo TQMS. The NMR experiments were carried out on a Bruker Avance 500 MHz spectrometer with tetramethylsilane (TMS) as a zero internal standard. Infrared spectra were recorded on a Nicolet Impact 410 FT-IR spectrophotometer. Optical rotation values were measured on a JASCO P-2000 digital polarimeter. For column chromatography (CC), silica gel (0.040-0.063mm, Merck, Germany) and Sephadex LH-20 (25-100μm, Sigma-Aldrich) were used. The TLC was performed on Merck pre-coated TLC DC-Alufolien silica gel $60F_{254}$ (Merck).

2.2. Plant material

The roots of *Alpinia kwangsiensis* T. L. Wu and S. J. Chen (Zingiberaceae) were collected in the Thai Nguyen province, Viet Nam, in April 2015. The species was identified by Dr. Nguyen Thi Phuong Anh, Vietnam National Museum of Nature, Vietnam Academy of Science and Technology (VAST). A voucher specimen (No. BK-05) has been deposited at the Hanoi University of Science and Technology, Vietnam. The roots were air-dried and powdered after collection.

2.3. Extraction and isolation

The dried powdered roots (2.8 kg) were extracted with 95% aqueous MeOH (5 L x 3 times) at room temperature for 24 h. The combined extracts were concentrated under vacuum to obtain a crude MeOH residue (163.0 g). The crude residue was then suspended in hot water (1L) and successively partitioned with *n*-hexane and ethyl acetate (EtOAc) to give 14.8 g of *n*-hexane and 42.1 g of EtOAc residues, respectively.

The EtOAc residue of the methanol extract (35 g) was subjected to silica gel CC with solvents of increasing polarity ranging from (0–100 % MeOH in CH_2Cl_2) to give 18 fractions (E1–E18). Fraction E3 (1.96 g) was subjected to silica gel CC with a mixture of CH_2Cl_2/acetone (50:1 to 7:1; v/v) to afford two subfractions (E3.1 and E3.2). The subfraction E3.1 was further purified by crystallization in *n*-hexane/acetone (5:1, v/v) to give compound **1** (43 mg). The subfraction E3.2 was subjected to silica gel CC with a mixture of *n*-hexane/acetone (10:1, v/v) and followed by crystallization in *n*-hexane/acetone (5:1, v/v) to yield compound **2** (15 mg). Fraction E5 (0.85g) was subjected to silica gel CC with a mixture of CH_2Cl_2/MeOH (15:1, v/v) to afford two subfractions (E5.1 and E5.2). The subfraction E5.2 was chromatographed on a Sephadex LH-20 column eluted with MeOH and followed by crystallization in *n*-hexane/acetone (4:1, v/v) to give compound **3** (29 mg).

Methyl-*trans*-*p*-coumarate (1): white crystals; m.p. 136-137 ^oC; R_f 0.21 on silica gel (*n*-hexane/acetone, 5:1, v/v); IR (KBr) ν_{max} (cm^{-1}): 3484, 1634, 2911, 2894, 1564; ^1H NMR (500 MHz, $CDCl_3$) δ_H (ppm): 7.63 (1H, d, J = 16.0 Hz, H-7), 7.42 (2H, d, J = 8.5 Hz, H-2, H-6), 6.86 (2H, d, J = 8.5 Hz, H-3, H-5), 6.30 (1H, d, J = 16.0 Hz, H-8), 6.03 (1H, s, 4-O<u>H</u>), 3.80 (3H, *s*, -OCH_3); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C (ppm): 168.1 (C-9), 158.0 (C-4), 144.8 (C-7), 130.0 (C-2, C-6), 127.0 (C-1), 115.9 (C-3, C-5), 115.1 (C-8), 51.7 (9-OCH_3); ESI-MS (negative): *m/z* 177 $[M–H]^-$ for formula $C_{10}H_{10}O_3$.

Scopoletin (**2**): light yellow crystals, m.p. 203-205 ^{o}C; R_f 0.33 on silica gel (*n*-hexane/acetone, 4:1, v/v); IR (KBr) ν_{max} (cm^{-1}): 3427, 1637, 1560, 1432, 1115, 965; ^{1}H NMR (500MHz, CD_3OD) δ_H (ppm): 6.22 (1H, d, *J* = 9.5Hz, H-3), 7.85 (1H, d, *J* = 9.5Hz, H-4), 7.10 (1H, s, H-5), 6.77 (1H, s, H-8), 3.92 (3H, s, 6-OCH_3), 4.85 (1H, br s, 7-OH); ^{13}C-NMR (125MHz, CD_3OD) δ_C (ppm): 56.8 (7-OCH_3), 103.9 (C-8), 109.9 (C-5), 112.5 (C-10), 112.6 (C-3), 146.1 (C-4), 147.1 (C-6), 151.4 (C-7), 152.9 (C-9), 164.0 (C-2); ESI-MS (positive): *m/z* 193 $[M + H]^+$ for formula $C_{10}H_8O_4$.

(+)-Gallocatechin (**3**): white powder; m.p. 189-190 ^{o}C; $[\alpha]_D^{25}$ = +17.5 (MeOH, *c* 0.5); R_f 0.57 on silica gel (CH_2Cl_2/MeOH, 4:1, v/v); ^{1}H NMR (500MHz, CD_3OD) δ_H (ppm): 4.56 (1H, d, *J* = 7.5 Hz, H-2), 3.95 (1H, ddd, *J* = 10.0, 7.5, 5.5 Hz, H-3), 2.84 (1H, dd, *J* =16.0, 5.5 Hz, H-4α), 2.53 (1H, dd, *J* = 16.0, 7.5 Hz, H-4β), 5.95 (1H, d, *J* = 2.0 Hz, H-6), 5.89 (1H, d, *J* = 2.0 Hz, H-8), 6.43 (1H, s, H-2', H-6'); ^{13}C NMR (125MHz, CD_3OD) δ_C (ppm): 82.8 (C-2), 68.8 (C-3), 28.1 (C-4), 157.6 (C-5), 96.3 (C-6), 157.8 (C-7), 95.5 (C-8), 156.8 (C-9), 100.8 (C-10), 131.6 (C-1'), 107.2 (C-2'), 146.8 (C-3'), 134.0 (C-4'), 146.8 (C-5'), 107.2 (C-6'); ESI-MS (negative): *m/z* 305 $[M–H]^-$ for formula $C_{15}H_{14}O_7$.

2.4. Assay for antioxidant and antibacterial activities

2.4.1. Antibacterial screening

Antimicrobial assay of the EtOAc fraction was carried out by multi-microdilution method [7]. Microbial strains were used to evaluate the antimicrobial properties as *Staphylococcus aureus* (ATCC 13709), *Bacillus subtilis* (ATCC 6633), *Lactobasillus fermentum* (N4), *Enterococus faecium* (B650), *Pseudomonas aeruginosa* (ATCC 15442) and *Escherichia coli* (ATCC 25922). Antimicrobial activity was tested with the standard procedure at Institute of Chemistry, VAST.

2.4.2. DPPH radical scavenging activity

The antioxidant activity of the EtOAc fraction was evaluated by its scavenging capacity of DPPH (1,1-diphenyl-2-picrylhydrazyl) radical. Briefly, tested sample at various concentrations (128, 32, 8, 2, 0.5 µg/ml) was mixed with 1 mM DPPH. After 30 min, absorbance was measured at 517 nm and compared with the standard. (+)-Catechin was used as positive control. Scavenging activity was expressed as the percentage inhibition [8].

3. RESULTS AND DISCUSSION

3.1. Structure determinations

The methanol extract from the roots of *A. kwangsiensis* was partitioned into *n*-hexane and ethyl acetate-soluble fractions. Chromatography and crystallization methods of the ethyl acetate-soluble fraction led to the isolation of three compounds (**1**–**3**) (Figure 1).

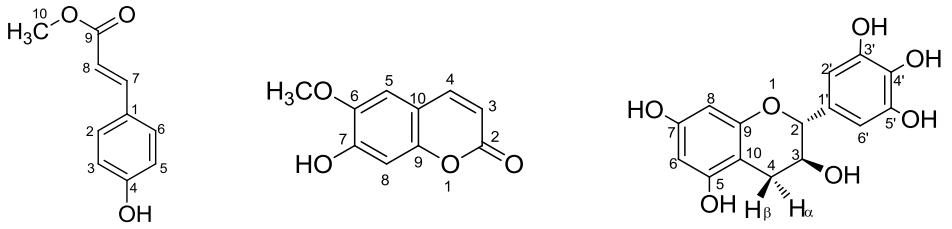
Compound **1** was obtained as white crystals. The molecular formula of **1** was clarified as $C_{10}H_{10}O_3$ based on the ion at *m/z* = 177 $[M–H]^-$ in negative ESI-MS. The infrared (IR) spectra showed absorption bands at 3484 and 1634 cm^{-1}, attributable to hydroxyl and carbonyl groups, respectively. The ^{1}H NMR spectrum indicated the presence of an ester methyl-*p*-coumarate. It showed prominent signals for *trans*-*p*-coumarate moiety at δ_H 7.42 (2H, *d*, *J* = 8.5 Hz, H-2, 6),

δ_H 6.86 (2H, d, *J* = 8.5 Hz, H-3, 5), suggested a *para* substituted benzene ring. Together with two olefinic proton signals at δ_H 7.63 (1H, d, *J* = 16.0 Hz, H-7) and δ_H 6.30 (1H, d, *J* = 16.0 Hz, H-8). The coupling constant of 16.0 Hz between H-7 and H-8 confirmed the *trans* geometry. In addition, the peak at δ_H 3.80 (3H, s, OCH_3) for methyl was adjacent to oxycarbonyl function. ^{1}H NMR spectrum of **1** was almost identical with that isolated previously [9], having a methyl-*p*-courmarate, thus confirming **1** as methyl-*trans*-*p*-coumarate. The ^{13}C NMR spectrum of **1** was in close agreement with the given structure. It showed signals for methyl-*trans*-*p*-coumarate. Methine carbon peaks observed at δ_C 130.0 and δ_C 115.9 corresponding to two pairs of unsubstituted aromatic carbons (C-2, 6 and C-3, 5, respectively), two peaks at δ_C 158.0 and δ_C 127.0 for quaternary aromatic carbons (C-1 and C-4. respectively), two olefinic carbon peaks at δ_C 144.8 (C-7) and δ_C 115.1 (C-8) and a peak for an ester carbonyl at δ_C 168.1 (C-9). Compound **1** also showed a signal for a methyl attached to oxycarbonyl function at δ_C 51.7ppm. In view of the data, compound **1** was identified as ester methyl-*trans*-*p*-coumarate. This ester was also isolated from the genus *Isatis tinctoria* and it shows insecticidal and antifeedant activities against termites and fungicidal activity against the brown-rot fungus [9]. This compound was isolated from the roots of *A. kwangsiensis* for the first time.

Compound **2** was isolated as light yellow crystals and gave a molecular formula of $C_{10}H_8O_4$ according to its ESI MS and NMR spectra. IR absorptions at 3427 cm^{-1} and 1637 cm^{-1} and a ^{13}C NMR signal at δ_C 164.0 suggested that hydroxyl and carbonyl lactone groups could be present in **2**. The ^{1}H NMR spectrum of compound **2** displayed signal characteristic of a 6,7-dioxygenated coumarin. The spectrum revealed two doublets at δ_H 6.22 (1H, d, *J* = 9.5 Hz) and δ_H 7.85 (1H, d, *J* = 9.5Hz) characteristic of H-3 and H-4 protons respectively of the pyrone ring of a coumarin. The presence of two aromatic proton singlets at δ_H 7.10 and δ_H 6.77 were attributable to H-5 and H-8 respectively. A three proton singlet at δ_H 3.92 was assigned for protons of methoxy group at C-6 and a broad singlet of one proton at δ_H 4.85 was assigned for proton of hydroxyl group at C-7. The ^{13}C NMR and DEPT spectra showed signals for one methoxyl group at δ_C 56.8, one carbonyl lactone group at δ_C 164.0, four methine carbons at δ_C 103.9 (C-8), 109.9 (C-5), 112.6 (C-3), 146.1 (C-4) and four quaternary carbon atoms at δ_C 112.5 (C-10), 147.1 (C-6), 151.4 (C-7), 152.9 (C-9). Therefore, based on the above evidence and comparision with those in the literature [10], compound **2** was elucidated as scopoletin. Scopoletin was isolated from some plants and has been reported to have antioxidant activity [11]. This compound was isolated from the roots of *A. kwangsiensis* for the first time.

Compound **3** was obtained as an amorphous solid. The ESI-MS spectrum of **3** indicated a pseudomolecular ion peak at *m/z* = 305$[M-H]^-$, corresponding to $C_{15}H_{14}O_7$ based on MS and NMR data. The ^{13}C NMR spectrum showed signals of 15 carbon atoms with the characteristics of a 3-flavanol skeleton including one methylene δ_C 28.1 (C-4); two hydroxymethines δ_C 82.8 (C-2) and δ_C 68.8 (C-3); four aromatic methines (δ_C 95.5 to 107.2 ppm); and eight aromatic quaternary carbon atoms (δ_C 100.8 to δ_C 157.8). The ^{1}H NMR spectrum of compound **3** displayed a doublet appearing at δ_H 4.56 (1H, d, *J* = 7.5Hz, H-2) and a signal at 3.95 (1H, ddd, *J* = 10.0, 7.5, 5.5 Hz, H-3) with the coupling constant of 7.5Hz between H-2 and H-3 confirmed the *trans* geometry, two double doublets at δ_H 2.84 (1H, dd, *J* = 16.0, 5.5 Hz, H-4α) and δ_H 2.53(1H, dd, *J* = 16.0, 7.5 Hz, H-4β) which are characteristic signals of ring A and B from catechin nucleus. Two doublets at δ_H 5.95(1H, d) and δ_H 5.89 (1H, d) with J_{metha} = 2.0 Hz are assigned to H-6 and H-8 protons, respectively. The appearance of a singlet signal at δ_H 6.43(1H, *s*, H-2', 6') suggested the presence of C-3', C-4' and C-5' trihydroxy group substitutions in ring C. Comparison of ^{1}H and ^{13}C NMR data of **3** with those in the literature [12] allowed to propose the

structure of compound **3** as *(+)*-Gallocatechin. This compound was isolated from the roots of *A. kwangsiensis* for the first time. Gallocatechin was found in green tea, bananas, persimmons, pomegranates, etc. It is one of the antioxidant chemicals found in food. Gallocatechin was also reported to have antimicrobial activities against bacteria (both Gram positive and Gram negative) and fungi [13].



1 **2** **3**

Figure 1. Structure of compounds **1**-**3**.

3.2. Antimicrobial and antioxidant activities

The ethyl acetate fraction of methanol extract of *A.kwangsiensis* roots was found to be active against two Gram-positive bacteria (*Staphylococcus aureus and Bacillus subtilis)* with the IC_{50} values of 74.65 μg/ml and 80.54 μg/ml, respectively. This fraction also showed significant DPPH scavenging activity with the EC_{50} value of 87.98 μg/ml.

4. CONCLUSIONS

The roots of *A.kwangsiensis* from Vietnam were collected to investigate. Three compounds including methyl-*trans*-*p*-coumarate (**1**), scopoletin (**2**) and (+)-gallocatechin (**3**) have been isolated from the ethyl acetate fraction of methanol extract of *Alpinia kwangsiensis* roots. These compounds (**1**–**3**) were isolated from the roots of *A. kwangsiensis* for the first time. This fraction were tested against two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*) and showed significant DPPH scavenging activity.

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