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Phenolics from the Rhizomes of *Kaempferia galanga* L. and Their Antioxidant Activity

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Authors' contributions

This work was carried out in collaboration between all authors. Authors XH, YW, FY and XZ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors FY and XZ managed the analyses of the study and managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: This research aimed at exploring phenolics from the *Kaempferia galanga* L. and evaluating their antioxidant effect.
Research Design: Phenolics were isolated and identified from the rhizomes of *Kaempferia galanga* L. Their radical scavenging ability was assayed by using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay method.
Place and Duration of Study: Research was conducted at the School of Pharmacy, Guangdong Pharmaceutical University, between at October 2016 and December 2017.
Methodology: Phenolics were isolated and purified by silica gel, ODS middle pressure liquid chromatography (MPLC) and high-performance liquid chromatography (HPLC), identified utilizing 1D, 2D nuclear magnetic resonance (NMR) and mass spectrum (MS). The radical scavenging ability of the isolated compounds was investigated via DPPH method.
Results: Ten phenolics were isolated and identified from the rhizomes of *Kaempferia galanga* L. (**1-10**). All compounds exhibited different levels of antioxidant effects in radical scavenging. Among them, compound **2** exhibited pronounced inhibitory effects in DPPH radical scavenging assay.

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Conclusion: In this study, ten phenolics were isolated from the rhizomes of sand ginger (*Kaempferia galanga* L.). DPPH radical scavenging was carried out to assess their antioxidant activity. The results indicated that some phenolics showed moderate antioxidant activity compared to those of ascorbic acid in DPPH. And these also demonstrated that phenolics may be partially responsible for the rhizomes of sand ginger's antioxidant activity. The discovery of phenolics and their antioxidant bioactivities is beneficial for understanding the scientific basis of the effects of *Kaempferia galanga* L.

Keywords: Kaempferia galanga L.; phenolics; antioxidant; DPPH.

ABBREVIATIONS

MPLC: Middle pressure liquid chromatography; HPLC: High performance liquid chromatography; NMR: Nuclear magnetic resonance; MS: Mass spectrum; EI: Electron ionization; DPPH: 2,2-diphenyl-1-picrylhydrazyl; CC: Column chromatography; TLC: Thin layer chromatography.

1. INTRODUCTION

Kaempferia galanga L., also known as Sand Ginger, belongs to the family Zingiberaceae. It is an aromatic herb and a popular spice used as a condiment in Asian cuisine. In China, the rhizomes of the sand ginger are sold as food flavouring agent, and are used to plain chicken and other braised food. The aromatic rhizomes of *K. galanga*, is used as expectorants and carminatives, aromatic stomachic and incense [1-3]. As a folk medicine, this plant has also been used for treating hypertension, pectoral and abdominal pains, headache, toothache, rheumatism, dyspepsia, coughs, inflammations, and tumors. A literature research revealed that the main chemical composition of *K. galanga* rhizomes were essential oils, which include ethyl cinnamate, *trans*-ethyl *p*-methoxycinnamate, *cis*-ethyl *p*-methoxycinnamate, *p*-methoxycinnamic acid, and a monoterpene ketone compound, 3-carene-5-one. However, there is scanty information about the polar components of this plant. In view of the widespread use of *K. galanga* rhizomes in traditional medicine, perfumery, and food flavoring, a systematic phytochemical investigation has been carried out in this study [4-7].

In this study, we reported the separation, structural elucidation and antioxidant activities of known 10 phenolics (**1-10**) from *K. galanga* rhizomes.

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Silica gel (200–300 mesh, Anhui Liangchen Silicon Material Co. Ltd. Anhui, China), ODS (40–60 *μ*m, Merck KGaA, Darastadt, Germany) and Sephadex LH-20 (Pharmacia, Pittsburgh, PA, USA) were used for column chromatography. HPLC-grade methanol was provided by Oceanpak Chemical Co. (Gothenburg, Sweden).

2.2 Instrumentation

IR spectra were obtained on a Perkin Elmer 100 spectrometer (Perkin Elmer Inc., Waltham, MA, USA) with KBr pellets. UV spectra were acquired on a Shimadzu UV-2550 spectrophotometer (Shimadzu Inc., Kyoto, Japan). 1D, 2D NMR were recorded on Bruker Avance III-400 NMR spectrometer (Bruker Inc., Falanden, Switzerland). Semi-preparative HPLC was applied with a Shimadzu HPLC system, including a pump model LC-20AR and an UV detector model SPD-20A (Shimadzu Inc., Kyoto, Japan). Two Cosmosil HPLC columns (5C18-MS-II, 10ID × 250 mm; 5C18-AR-II, 10ID × 250 mm; Nacalai Tesque, Kyoto, Japan) were applied in the purification. Microplate reader (ST-360) was the product of Kehua Technologies, Inc. (Shanghai, China).

2.3 Plant Material

The rhizomes of sand ginger were purchased from Qingping herbal medicine market, Guangzhou, China and identified by Prof. X.J. He of Guangdong Pharmaceutical University. A voucher specimen was deposited in the School of Pharmacy, Guangdong Pharmaceutical University, Guangzhou, China.

2.4 Extraction, Isolation and Purification

20 kg of dried rhizomes of sand ginger was chopped into chips and extracted four times with 75% ethanol (3 × 45 L) for 2 h at reflux.

Evaporation of ethanol under lowered pressure gave an ethanol-free extract (15 L). The extract was successively partitioned with the same volume of cyclohexane, $CHCl_3$, EtOAc and *n*-BuOH for three times, respectively. Compound **1** (12.9 g) was recrystallized in the $CHCl_3$-soluble fraction (172.5 g), and the rest of $CHCl_3$ extract was chromatographed by a silica gel column chromatography (CC) using $CHCl_3$−MeOH as eluent (100:1 to 0:1, v/v) to obtain 17 fractions (Frs. 1−17). Fraction 8 (2.5 g) was further isolated by silica gel, Sephadex LH-20 CC, and finally purified by RP-HPLC (C18 column, 10 × 250 mm) to get compounds **10** (9.4 mg) and **2** (12.9 mg).

The EtOAc extract (44.6 g) was subjected by a silica gel CC using $CHCl_3$−MeOH as eluent (100:1 to 0:1, v/v) to yield 11 fractions (Frs. 1−11). Compound **7** (40.0 mg) was also recrystallized from the fraction 5 (1.03 g). The rest of the fraction 5 was further purified by RP-HPLC to afford compound **3** (8.5 mg). Fraction 4 (4.8 g) was separated by the ODS CC, Sephadex LH-20 CC, preparative thin layer chromatography (TLC) and finally purified by RP-HPLC (C18 column, 10 × 250 mm) to obtain compounds **5** (23.6 mg), **6** (10.7 mg), **4** (27.1 mg), **9** (45.4 mg), **8** (5.2 mg), respectively.

2.5 DPPH Radical Scavenging Activity

The free radical scavenging capacity was determined using the DPPH radical discoloration method [8-10]. DPPH was dissolved in methanol at the concentration of 0.2 mmol/L, and 100 μL of this solution was mixed to 100 μL of the compound (dissolved in methanol) at the concentration of 2.5−400 μM. These two solutions were mixed and kept in dark at room temperature for 30 min. Then the absorbance was measured at 510 nm against a blank sample lacking scavenger.

2.6 Statistical Analysis

Experimental values were presented as mean ± SD.

3. RESULTS AND DISCUSSION

3.1 Isolation of Compounds 1-10 from Sand Ginger

The dried rhizome of sand ginger was extracted with 75% EtOH-H_2O, and the EtOH was removed to yield a concentrated solution. The solution was successively separated by silica gel CC, ODS CC, Sephadex LH-20 CC and finally purified using semi-preparative HPLC to yield compounds **1-10**. Compounds **1**, **2** and **10** were isolated from the $CHCl_3$-soluble fraction. Compounds **3-9** were isolated from the EtOAc-soluble fraction. Their chemical structures were shown in Fig. 1.

3.2 Structural Elucidation

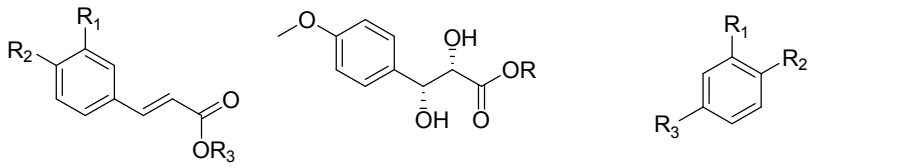
Compound **1** was obtained as white columnar crystals, IRν_{max} (KBr) cm^{-1}: 1710 (C=O), 1605, 1513, 829 (Ar-H), UV ($CHCl_3$) λ_{max} 280 nm. Electron ionization (EI)-MS molecular ion peak at *m/z* 206. In the lower field of ^{1}H NMR spectrum, a typical AA'BB' spin system of aromatic protons was observed at δ_H 7.46 (2H, d, *J* = 8.7 Hz), 6.88 (2H, d, *J* = 8.7 Hz). Two signals at δ_H 7.63 (1H, d, *J* = 16.0 Hz) and 6.29 (1H, d, *J* = 16.0 Hz) indicated the existence of a pair of olefinic protons in *trans* configuration. Moreover, one quartet and one triplet of ethyoxyl at δ_H 4.24 (2H, q, *J* = 7.1 Hz), 1.32 (3H, t, *J* = 7.1 Hz) were observed in the ^{1}H NMR spectrum, respectively. And there was one singlet of methoxyl at δ_H 3.82 (3H, s) were appeared in the higher field of ^{1}H NMR spectrum. There were 12 carbons in the ^{13}C NMR spectrum. In the lower field, δ_C 167.4 proved the existence of a carbonyl group. Signals at δ_C 161.5, 129.8, 129.8, 127.3, 111.4 and 111.4 were six signals of a phenyl unit, and the signals at δ_C 161.5 was one oxyphenyl of the phenyl unit. A pair of olefinic carbons was observed at δ_C 144.3, 115.9 in the ^{13}C NMR spectrum. Combining with its ^{1}H NMR, one methoxyl was confirmed at δ_C 55.4. Meanwhile, two signals at δ_C 60.4 and 14.5 were contributed to one ethyoxyl. Above ^{1}H and ^{13}C NMR data was consistent with the literature [11], thus compound **1** was established to be *trans*-4-Methoxy ethyl cinnamate.

The other nine phenolic compounds, ferulic acid (**2**) [12], *trans*-*p*-hydroxy-cinnamic acid (**3**) [13], *trans*-4-methoxycinnamic acid (**4**) [14], methyl (2R,3S)-2,3-dihydroxy-3-(4-methoxyphenyl) propanoate (**5**) [15], ethyl (2R,3S)-2,3-dihydroxy-3-(4-methoxyphenyl) propanoate (**6**) [15], *p*-hydroxybenzoic acid (**7**) [16], *p*-methoxybenzoic acid (**8**) [17], vanillic acid (**9**) [18], and methyl 3,4-dihydroxybenzoate (**10**) [19] were identified by spectroscopic analyses and comparison of their reported spectroscopic data.

3.3 DPPH Radical Scavenging Activity

In this study, the antioxidant activities of compounds **1-10** were evaluated using the

DPPH radical scavenging assay with ascorbic acid as the positive control. When DPPH radical scavenging rate was beyond 50%, the compounds were investigated to determine the corresponding IC_{50} values. The result indicated that most compounds showed weak antioxidant activities in radical scavenging (Fig. 2). Only compound **2** exhibited pronounced inhibitory effects in DPPH radical scavenging assay and the calculated IC_{50} values was 111.99 μM. Structure-DPPH radical scavenging activity relationship of phenolic showed various principles. It could be inferred that compounds with more phenolic hydroxyl group were beneficial for DPPH radical scavenging activity.



1 R_1=H, R_2=OCH_3, R_3=CH_2CH_3
2 R_1=OCH_3, R_2=OH, R_3=H
3 R_1=H, R_2=OH, R_3=H
4 R_1=H, R_2=OCH_3, R_3=H

5 R=CH_3
6 R=CH_2CH_3

7 R_1=H, R_2=OH, R_3=COOH
8 R_1=H, R_2=OCH_3, R_3=COOH
9 R_1=OCH_3, R_2=OH, R_3=COOH
10 R_1=OH, R_2=OH, R_3=$COOCH_3$

Fig. 1. Chemical structures of phenolics 1–10 isolated from *K. galanga* rhizomes

Table 1. ^{13}C NMR (101 MHz) data of compounds 1-10

No	δ_C (ppm)									
	1[a]	**2**[b]	**3**[c]	**4**[c]	**5**[a]	**6**[a]	**7**[c]	**8**[c]	**9**[c]	**10**[b]
1	127.3	127.5	125.3	126.9	130.8	130.8	121.8	121.9	121.7	122.8
2	129.8	111.4	130.1	130.0	127.7	127.8	131.9	132.5	115.1	113.7
3	111.4	148.7	115.8	114.4	113.8	113.8	115.5	113.9	147.3	152.1
4	161.5	149.9	159.6	161.0	159.6	159.6	161.9	164.1	151.1	148.2
5	111.4	116.0	115.8	114.4	113.8	74.7	115.5	113.9	112.8	124.6
6	129.8	123.8	130.1	130.0	127.7	74.9	131.9	132.5	123.6	115.0
7	144.3	145.9	144.0	143.8	74.7	172.4				167.7
8	115.9	116.0	115.5	116.6	75.0					
9	167.4	168.5	168.1	167.9	172.7					
$OCH_2\underline{C}H_3$	14.5					14.1				
$O\underline{C}H_2CH_3$	60.4					62.0				
OCH_3	55.4	56.3		55.3	52.5, 55.3	55.4		55.6	55.6	56.1
COOH							167.6	171.6	167.3	

a: measured in $CDCl_3$; b: measured in Acetone-d_6; c: measured in DMSO-d_6

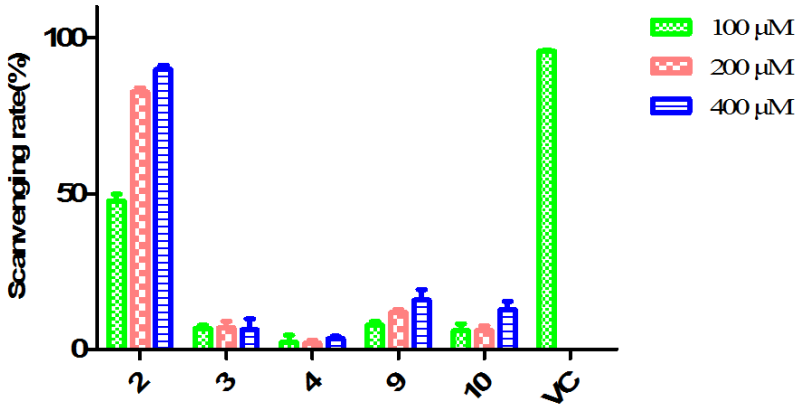


Fig. 2. DPPH radical scavenging effects of the phenolics 1-10

Table 2. Inhibitory effects of compound 2 from *K. galanga* rhizomes on DPPH radical scavenging

Compound	2	Ascorbic acid (VC) [b]
IC_{50} [a] (μM)	111.99 ± 2.82	24.02 ± 0.51

a Values are presented as means ± SD (n = 3).
b Positive control

4. CONCLUSION

In this study, ten phenolics were isolated from the rhizomes of *Kaempferia galanga* L. (**1-10**). Their structures were determined on the basis of MS and NMR data. Compound **2** exhibited pronounced inhibitory effects in DPPH radical scavenging assay. The discovery of phenolics and their antioxidant bioactivities is beneficial for understanding the scientific basis of the effects of *K. galanga*. These results demonstrated the potential utilisation of the aromatic rhizomes of *K. galanga* in functional foods and cosmetics.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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