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白皮锦鸡儿酚类化合物及其生物活性

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摘　要:从豆科植物白皮锦鸡儿(*Caragana leucophloea* Pojark.)地上部分分离到 3 个酚类化合物,经理化方法和波谱分析鉴定为鹅掌楸苷(**1**)、香草酸(**2**)和绿原酸(**3**)。化合物 **1** 和 **2** 表现出较好的抗细菌活性,半抑制浓度(IC_{50})为 8.11 ~22.88 μg/mL。**2** 和 **3** 则表现出一定的抗真菌活性,对稻瘟菌孢子萌发的 IC_{50} 值分别为 105.04 μg/mL 和 32.26 μg/mL,对西瓜枯萎病菌生长的 IC_{50} 值为 108.45 μg/mL 和 45.26 μg/mL。**2** 和 **3** 对秀丽隐杆线虫也有一定的抑制活性,当处理线虫 48 h 时,IC_{50} 值分别为 46.57 μg/mL 和 55.17 μg/mL。此外,**2** 具有一定的抗氧化活性,对羟基自由基清除的 IC_{50} 值为 67.96 μg/mL;对 Fe^{2+} 表现出一定的螯合能力,IC_{50} 值为 93.59 μg/mL。上述酚类化合物均为首次从白皮锦鸡儿中分离得到。

关键词:豆科;白皮锦鸡儿;鹅掌楸苷;香草酸;绿原酸;生物活性

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Phenolic Compounds from *Caragana leucophloea* and Their Biological Activities

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Abstract: Three phenolic compounds were isolated from the aerial parts of *Caragana leucophloea* Pojark. (Leguminosae). They were identified as liriodendrin (**1**), vanillic acid (**2**) and chlorogenic acid (**3**) by physicochemical properties and spectrometric methods. Compounds **1** and **2** exhibited strong antibacterial activity with the median inhibitory concentration (IC_{50}) values from 8.11 to 22.88 μg/mL. Both **2** and **3** demonstrated inhibitory activity on the spore germination of *Magnaporthe oryzae* with IC_{50} values of 105.04 and 32.26 μg/mL and on the mycelial growth of *Fusarium oxysporum* f. sp. *niveum* with IC_{50} values of 108.45 and 45.26 μg/mL, respectively. **2** and **3** also exhibited antinematodal activity on *Caenorhabditis elegans*. When the incubation time was 48 h, the IC_{50} values were 46.57 and 55.17 μg/mL, respectively. Additionally, **2** showed hydroxyl radical scavenging activity with IC_{50} of 67.96 μg/mL and had inhibitory activity on ferrozine-Fe^{2+} complex formation with IC_{50} value of 93.59 μg/mL. These phenolic compounds were isolated from *C. leucophloea* for the first time.

Key words: Leguminosae; *Caragana leucophloea*; liriodendrin; vanillic acid; chlorogenic acid; biological activities

Introduction

Caragana leucophloea Pojark. belongs to Leguminosae, and is mainly distributed in the Provinces of Xinjiang, Gansu and Inner Mongolia of Northwest China. It is also distributed in the surrounding countries such as Tajikistan, Kyrgyzstan, Kazakhstan, and Mongolia. *C. leucophloea* has been cultivated mainly for livestock forage and dune-fixation [1]. Its roots have been used to cure irregular menstruation, leucorrhea, numbness and pain caused by arthritis, edema due to the deficiency of spleen, lactation insufficiency and traumatic injury as the traditional Chinese medicine [2], and its aerial parts were mainly used as forage [1]. In our previous studies, the crude ethanol extract of *C. leucophloea* exhibited antioxidant and antifungal activities [3,4]. Three phenol-

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ic compounds namely 3-*O*-methylkaempferol, 3-*O*-methylquercetin, and quercetin have been obtained from this plant, and showed antioxidant and antimicrobial activities [5,6]. In this study, another three phenolic compounds were isolated and identified from the *n*-butanol fraction of the crude ethanol extract of *C. leucophloea*. The bioactivities of these 3 phenolic compounds including antibacterial, antifungal, antinematodal and antioxidant activities were evaluated to provide data supporting the development and utilization of *C. leucophloea*.

Materials and Methods

General

Silica gel (60-80 and 200-300 mesh, Qingdao Marine Chemical Company, China), Sephadex LH-20 (Pharmacia), and C_{18} reversed-phase silica gel (YMC) were used for column chromatography (CC). Thin layer chromatography (TLC) plates (Qingdao Marine Chemical Company, China) were coated with 0.5 mm layer of silica gel (GF_{254}, 300-400 mesh). Melting points were determined on an XT4-100B microscopic melting-point apparatus (Tianjin Tianguang Optical Instruments Company, China) and were uncorrected. NMR spectra were recorded on a Bruker ARX-400 (^1H at 400 MHz and ^{13}C at 100 MHz). HR-ESI-MS spectra were recorded on a Bruker Apex IV FTMS mass spectrometer. A microplate spectrophotometer (PowerWave HT, BioTek Instruments, USA) was employed to measure the light absorption value. Carbendazim and streptomycin sulfate were purchased from Sigma-Aldrich (USA). 3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) was purchased from Amresco (USA). Salicylic acid (SA), ascorbic acid (AA) were from Beijing Chemical Company. All other chemicals and reagents were of analytical grade.

Plant material

The aerial parts of *C. leucophloea* Pojark. were collected in June 2008 at Kelamayi of Xinjiang Province of China, and was authenticated by Professor Pin Yan of Shihezi University of Xinjiang, where the voucher specimen of this plant was deposited. The plant materials were left to dry in the shade at room temperature to a constant weight.

Isolation and purification

The dry aerial parts of *C. leucophloea* were ground into powder, and 5 kg of the powder was soaked in 95% ethanol (EtOH) at room temperature for three times at an interval of 7 days (3 × 20 L). After filtration, the filtrate was concentrated under vacuum at 50 ℃, the brown residue (477.5 g, yield 9.55%, w/w) was suspended in water and extracted with equivoluminal petroleum ether, ethyl acetate (EtOAc) and *n*-butanol, successively. They were concentrated to yield petroleum ether fraction (160.0 g, yield 3.20%, w/w), ethyl acetate fraction (65.5 g, yield 1.31%, w/w), *n*-butanol fraction (210.0 g, yield 2.72%, w/w) and aqueous fraction (102.5 g, yield 2.05, w/w), respectively. The *n*-butanol fraction, was subjected to macroporous resin AB-8 column chromatography (CC), and eluted with H_2O, 30% and 90% EtOH, successively. The 30% EtOH eluted fraction (145.0 g) was separated by silica gel CC (EtOAc: MeOH = 4: 1, v/v) to obtain 6 fractions (FA, FB, FC, FD, FE and FF) by monitored with TLC. Fraction FD was further separated on a silica gel column eluted with EtOAc-MeOH (from 1: 0 to 0: 1, v/v; about 5-fold of the column volume for each eluent). Subfraction FD-68 was further recrystalization and then purified with semi-preparative HPLC (MeOH-H_2O = 40: 60, v/v; flow rate = 3.0 mL/min; detection wavelength = 266 nm; injection volume = 2.0 mL) to afford compound **1** (6.7 mg). Similarly, compound **3** (11 mg) was obtained from subfraction FD-11-15. Fraction FE was further separated on a silica gel CC eluted with EtOAc-MeOH (from 1: 0 to 0: 1, v/v; about 5-fold of the column volume for each eluent). Subfraction FE-50-62 was further purified with Sephadex LH-20 (MeOH-H_2O = 70: 30, v/v) and recrystalization to afford compound **2** (15 mg).

Structural identification

Liriodendrin (**1**) was obtained as white amorphous powder; mp. 259-261 ℃; $C_{34}H_{46}O_{18}$, HR-ESI-MS (*m/z* 743.27545 $[M+H]^+$, calcd. 743.27569); ^1H NMR (pyridine-d_5, 400 MHz) δ (ppm), 4.74 (2H, d, J = 3.7 Hz, H-2,6), 4.12 (2H, m, H-4,8), 6.70 (4H, s, H-2′, 6′, 2″, 6″), 5.44 (2H, brs), 3.59 (12H, s,

OCH_3-3′,5′,3″,5″); ^{13}C NMR (pyridine-d_5, 100 MHz) δ (ppm), 54.5 (C-1,5), 85.9 (C-2,6), 72.0 (C-4,8), 135.0 (C-1′,1″), 104.7 (C-2′,6′,2″,6″), 153.7 (C-3′,5′,3″,5″), 138.0 (C-4′,4″), 56.4 (OCH_3-3′,5′,3″,5″), 104.6 (glc-1,1′), 75.8 (glc-2,2′), 78.1 (glc-3,3′), 71.3 (glc-4,4′), 78.4 (glc-5,5′), 62.3 (glc-6,6′). The structure was confirmed by comparison with literature data[7].

Vanillic acid (**2**) was obtained as white amorphous powder; m. p. 263-268 ℃; $C_8H_8O_4$, HR-ESI-MS (*m/z* 167.03462 $[M-H]^-$, calcd. 167.03389); ^1H NMR (DMSO-d_6, 400 MHz) δ (ppm), 12.65 (1H, s, OH-5), 6.20 (1H, d, J = 2.0 Hz, H-6), 10.86 (1H, s, OH-7), 6.37 (1H, d, J = 1.9 Hz, H-8); ^{13}C NMR (DMSO-d_6, 100 MHz) δ (ppm), 121.6 (C-1), 115.0 (C-2), 147.2 (C-3), 55.5 (OCH_3-3), 151.1 (C-4), 112.7 (C-5), 123.5 (C-6), 167.2 (C-7). The structure was confirmed by comparison with literature data [8].

Chlorogenic acid (**3**) was obtained as white amorphous powder; m. p. 207-209 ℃; $C_{16}H_{18}O_9$, HR-ESI-MS (*m/z* 353.08653 $[M-H]^-$, calcd. 353.08671); ^1H NMR (CD_3COCD_3, 400 MHz) δ (ppm), 1.97-2.27 (1H, m, H-2,6), 5.37 (1H, m, H-3), 4.21 (1H, m, H-4), 3.74 (1H, m, H-5), 7.16 (1H, d, J = 2.0 Hz, H-2′), 6.86 (1H, d, J = 8.2 Hz, H-5′), 7.01 (1H, dd, J = 2.0, 8.2 Hz, H-6′), 7.55 (1H, d, J = 16.0 Hz, H-7′), 6.27 (1H, d, J = 15.9 Hz, H-8′); ^{13}C NMR (CD_3COCD_3, 100 MHz) δ (ppm), 74.8 (C-1) 36.6 (C-2), 72.2 (C-3), 70.2 (C-4), 70.1 (C-5), 37.8 (C-6), 174.2 (C-7), 126.1 (C-1′), 115.0 (C-2′), 145.1 (C-3′), 147.7 (C-4′), 114.3 (C-5′), 121.3 (C-6′), 144.7 (C-7′), 113.6 (C-8′), 166.2 (C-9′). The structure was confirmed by comparison with literature data [9].

Antibacterial activity assay

Three Gram-positive (*Staphylococcus haemolyticus* ATCC 29970, *Bacillus subtilis* ATCC 11562 and *Ralstonia solanacearum* ATCC 11696), and three Gram-negative (*Agrobacterium tumefaciens* ATCC 11158, *Pseudomonas lachrymans* ATCC 11921 and *Xanthomonas vesicatoria* ATCC 11633) bacteria were selected for antibacterial activity assay. They were grown in liquid LB medium (yeast extract 5 g/L, peptone 10 g/L, NaCl 5 g/L, pH 7.0) overnight at 28 ℃, and the diluted bacterial suspension (10^6 cfu/mL) was ready for detection. A modified broth dilution-colorimetric assay using the chromogenic reagent MTT was used to detect the antibacterial activity of the compounds [10]. Briefly, each compound was dissolved in 30% dimethyl sulfoxide (DMSO), and then was diluted with 30% DMSO to obtain concentrations ranging from 0.03125 mg/mL to 2.0 mg/mL. The test sample solutions (10 μL) and prepared bacterial suspensions (90 μL) containing 1×10^6 cfu/mL were added into each well of the 96-well microplate. The negative control well contained 90 μL of the inoculum (1×10^6 cfu/mL) and 10 μL of 30% DMSO. Streptomycin sulfate was used as the positive control. After the plates were agitated to mix the contents of the wells using a plate shaker and incubated in the dark for 24 h at 28 ℃, 10 μL of MTT (5 mg/mL in 0.2 mol/L, pH 7.2 phosphate-buffered saline) was added into each well, and the plates were incubated for another 4 h. The minimum inhibitory concentration (MIC) value was determined as reported previously[11]. For further determination of the IC_{50} values for antibacterial activity of the compounds, the microplates incubated with MTT were centrifuged at 1,500 × g for another 20 min. Then the supernatant was aspirated, 200 μL of DMSO was added into each well, and the colored formazan products were extracted for 30 min using a plate shaker. After complete extraction, the plate was centrifuged at 1,500 × g for another 20 min, and then 100 μL of the supernatant (DMSO solution) in each well was transferred to a corresponding well of another 96-well microplate to measure their light absorption values at 510 nm using a microplate spectrophotometer (PowerWave HT, BioTek Instruments, USA). The percentage (%) of bacterial growth inhibition was determined as $[(A_c - A_t)/A_c)] \times 100$, where A_c was an average of 6 replicates of light absorption at 510 nm of the negative controls, and A_t was an average of 6 replicates of light absorption at 510 nm of the samples. The median inhibitory concentration (IC_{50}) was calculated using the linear relation between the inhibitory probability and concentration logarithm[12].

Antifungal activity assay

Rice blast fungus (*Magnaporthe oryzae* P131) was maintained on oatmeal-tomato agar (oatmeal 30 g/L, tomato juice 150 mL/L, and agar 20 g/L) at 25 ℃. A spore germination assay was employed to detect the antifungal activity of the compounds [13]. Briefly, the spores were prepared from 7-day-old cultures of *M. oryzae*, according to our previous report [14]. The test compound-acetone solution (25 μL) was mixed with an equivalent volume of fungal spore suspension containing 2×10^6 spores/mL. The mixture was then placed on the separate concave glass slides. The final compound concentrations ranged from 3.125 μg/mL to 200 μg/mL in 5% (v/v) acetone. The negative control was 5% acetone, and the positive control was carbendazim with concentrations ranging from 0.78 μg/mL to 50 μg/mL. Three replicates were used for each treatment. Slides containing the spores were incubated in a moist chamber at 25oC for 7 h. Each slide was then observed under the microscope for observing spore germination status. About 100 spores per replicate were observed to detect spore germination according to the method by Fiori *et al*. [15]. The percentage of spore germination inhibition was determined as: Spore germination inhibition = $[(G_c - G_t)/G_c] \times 100\%$, where G_c is an average of three replicates of germinated spore number in the negative control, and G_t is an average of three replicates of germinated numbers in the treated sets. The IC_{50} value calculation for the spore germination inhibition was the same as that for antibacterial activity assay.

Fusarium oxysporum f. sp. *niveum* was selected for mycelia growth inhibition assay. The dilution-colorimetric assay was employed to evaluate antifungal activity [16]. The fungus was cultured in liquid potato dextrose (PD) medium overnight at 28 ℃, and the fungal spore suspension was diluted to 1×10^6 cfu/mL. The compound solution was prepared the same as in the above antibacterial activity assay to the final concentrations ranging from 0.05 to 0.30 mg/mL containing 3% ethanol. Carbendazim was used as the positive control and all other procedures were the same as for the antibacterial activity assay.

Antinematodal activity assay

Caenorhabditis elegans, which was kindly supplied by Dr. Yuanmei Zuo at the College of Resources and Environmental Sciences, China Agricultural University, was inoculated on the nematode growth medium (NGM) that was cultured previously with *Escherichia coli* OP_{50} according to our previous report [17]. The NGM plate was full of the worms after 4 to 5 days at 16 ℃ in darkness. In order to determine the IC_{50} values of the compounds, 5% acetone water solutions of each sample at 1, 5, 10, 25, 50, 100, and 200 μg/mL were assayed for antinematodal activity. The test nematode dilution (90 μL containing 40 to 50 nematodes) was added into each well of the sterile 96-well microplate and then, 10 μL of sample stock solution was added into each well and mixed thoroughly. 5% acetone-water solution was used as the negative control. Avemectin B1, which was kindly provided by Dr. Shankui Yuan at the Institute for the Control of Agrochemicals, Chinese Ministry of Agriculture, was used as the positive control with the purity of 97.2%. It was a mixture of avermectins B1a and B1b in the ratio of 9.5 to 0.5 (w/w). Five replicates were carried out for each treatment, and the experiments were repeated twice. Dead and active nematodes were counted after 12 h, 24 h and 48 h. The nematodes were considered to be dead when they did not move by treating with a fine needle as the physical stimuli. The mean percentage of mortality was then calculated. The net percentage of mortality was about 3% by using 5% acetone-water solution as the negative control after 12 h, 24 h and 48 h. Nematode recovery was not observed to the dead nematodes. The IC_{50} value calculation for the antinematodal activity was the same as that for antibacterial activity assay.

Antioxidant activity assay

The activity of the compounds to scavenge hydroxyl radical was determined according to the method of Thirunavukkarasu *et al* [18]. with a slight modification. Briefly, 25 μL of ferrous sulfate solution (2 mg/mL) was mixed with 50 μL of hydrogen peroxide solution (1%) to generate hydroxyl radical in the well of microplate. Then 50 μL of salicylic acid (SA) solution (1.5 mg/mL) and 50 μL of sample solution were add-

ed to each well of microplate. The reaction mixtures were shaken and incubated at 37 ℃ for 1 h. The absorbance at 526 nm was recorded. Ascorbic acid (AA) was used as the positive control. Lower absorbance of the reaction mixture indicates higher hydroxyl radical scavenging activity. All the tests were performed in triplicate. The hydroxyl radical scavenging activity was calculated by the following equation: Scavenging activity (%) = $[D_0-(D_1-D_2)] \times 100/D_0$, where D_0 is the absorbance of reaction solution without tested sample, D_1 is the absorbance of the sample, and D_2 is the absorbance of the sample under identical conditions as D_1 with water instead of SA solution.

Results and Discussion

Elucidation of the phenolic compounds

Three compounds (1-3) were isolated from the *n*-butanol fraction of the crude ethanol extract of the aerial parts of *C. leucophloea*. After comparing their physicochemical and spectrometric data with those reported in literatures [7-9], they were known phenolic compounds and confirmed as liriodendrin (**1**), vanillic acid (**2**), and chlorogenic acid (**3**), respectively.

Liriodendrin (**1**), a lignan, was also named as acanthoside D or syringaresinol-4′,4′′-di-*O*-*β*-D-glucopyranoside. It has been isolated from many plant species such as *Acanthopanax senticosus* (Araliaceae) [19], *Broussonetia papyrifera* (Moraceae) [20], *Ilex pubescens* (Aquifoliaceae) [21], *Jasminum lanceolarium* (Oleaceae) [22], *Kalopanax pictus* (Araliaceae) [23], *Luehea candicans* (Tiliaceae) [24], *Opuntia dillenii* (Cactaceae) [25], *Pittosporum brevicalyx* (Pittosporaceae) [26], *Stelleropsis antoninae* (Thymelaeaceae) [27]. Both vanillic acid (2) and chlorogenic acid (3) are widely distributed in plants [28,29]. They were thought as the precursors of polyphenolic acids (tannins) [30]. Chlorogenic acid (3) was also named as 3-*O*-caffeoylquinic acid that is one of the most abundant polyphenols in the human diet with coffee, fruits and vegetables. It is very rich in the flowers and buds of *Lonicera japonica* Thunb (Caprifoliaceae), and the leaves of *Eucommia ulmoides* (Eucommiaceae) [31].

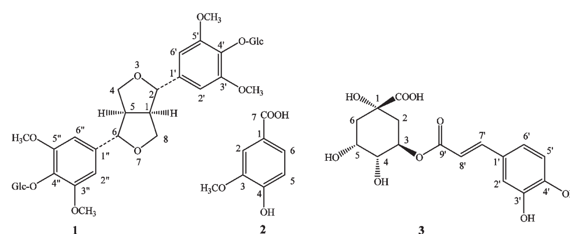

Fig. 1 Chemical structures of the phenolic compounds (1-3)

Antimicrobial activity

The isolated phenolic compounds (**1-3**) were tested for the antibacterial activities, and the corresponding minimum inhibitory concentration (MIC) and median inhibitory concentration (IC_{50}) values are summarized in Table 1. Among them, compound **1** was the most active compound with IC_{50} values from 8.11 to 15.43 μg/mL on the test bacteria. Compound **2** showed the moderate antibacterial activity with IC_{50} values from 10.90 to 22.88 μg/mL.

Table 1 Inhibitory activity of the compounds on bacteria

Tested bacterium	1		2		3		CK^+	
	MIC (μg/mL)	IC_{50} (μg/mL)	MIC (μg/mL)	IC_{50} (μg/mL)	MIC (μg/mL)	IC_{50} (μg/mL)	MIC (μg/mL)	IC_{50} (μg/mL)
B. subtilis	50	15.43 ± 0.02	75	19.62 ± 0.35	200	85.65 ± 1.51	50	15.73 ± 0.15
A. tumefaciens	50	8.11 ± 0.21	75	13.54 ± 0.20	200	88.06 ± 1.40	50	12.37 ± 0.21
S. haemolyticus	50	10.25 ± 0.02	75	18.44 ± 0.36	200	88.49 ± 1.89	50	8.09 ± 0.03
R. solanacearum	50	9.08 ± 0.02	75	10.97 ± 0.06	200	84.95 ± 0.44	50	13.73 ± 0.15
P. lachrymans	50	9.73 ± 0.06	75	17.82 ± 0.16	150	59.10 ± 0.07	75	12.97 ± 0.09
X. vesicatoria	50	10.63 ± 0.10	75	22.88 ± 0.33	150	112.46 ± 1.19	100	13.22 ± 0.22

Note: The positive control (CK^+) on bacteria was streptomycin sulfate.

Both vanillic acid (**2**), and chlorogenic acid (**3**) were screened to have antifungal activity on the spore germination of *M. oryzae* and mycelial growth of *F. oxysprorum* f. sp. *niverum* with their MIC and IC_{50} values shown in Table 2. For the two compounds, chlorogenic acid (**3**) exhibited stronger antifungal activity with the IC_{50} values as 32.26 μg/mL on *M. oryzae* and 45.26 μg/mL on *F. oxysprorum* f. sp. *niveum*.

Table 2 Inhibitory activity of the compounds on fungi

Test fungus	2		3		CK^+	
	MIC (μg/mL)	IC_{50} (μg/mL)	MIC (μg/mL)	IC_{50} (μg/mL)	MIC (μg/mL)	IC_{50} (μg/mL)
M. oryzae	200	105.04 ± 1.24	100	32.26 ± 2.79	50	6.25 ± 0.19
F. oxysporum f. sp. *niveum*	200	108.45 ± 1.43	100	45.26 ± 2.32	50	10.25 ± 0.29

Note: The positive control (CK^+) on fungi was carbendazim.

Antinematodal activity

Both compounds **2** and **3** exhibited antinematodal activity with their IC_{50} values shown in Table 3. Compound **2** showed slightly stronger antinematodal activity than **3**. When the incubation time was 48 h, the IC_{50} values of compounds **2** and **3** on *C. elegans* were 46.57 μg/mL and 55.17 μg/mL, respectively.

Table 3 Antinematodal activity of compounds 2 and 3 on *Caenorhadits elegans*

Compound	Incubation time (h)	IC_{50} (μg/mL)
2	12	54.89 ± 3.64
	24	50.64 ± 3.35
	48	46.57 ± 3.45
3	12	68.05 ± 3.56
	24	60.05 ± 2.63
	48	55.17 ± 2.54
CK^+	12	3.88 ± 1.54
	24	3.35 ± 1.39
	48	1.53 ± 1.27

Note: The positive control (CK^+) on *C. elegans* was avermectin B1.

Antioxidant activity

Among three compounds, only vanillic acid (**2**) was screened to have inhibitory activity on ferrozine-Fe^{2+} complex formation with IC_{50} value as 93.59 ± 4.64 μg/mL (IC_{50} value of the positive control EDTA as 11.88 ± 2.69 μg/mL). Vanillic acid (**2**) also showed hydroxyl radical scavenging activity with IC_{50} value as 67.96 ± 0.007 μg/mL (IC_{50} value of the positive control ascorbic acid as 15.15 ± 0.12 μg/mL).

Liriodendrin (**1**) possessed a variety of biological activities such as cytotoxic activity on HepG-2 [20], anti-arrhythmic activity on $CaCl_2$-induced arrhythmias in anesthetized rats [26], anti-inflammation and antinociception activities [32], and dopamine-induced cytotoxicity protective effect [33]. Both vanillic acid (**2**) and chlorogenic acid (**3**) also exhibited a variety of biological activities [34]. The antimicrobial and antioxidant activities of vanillic acid (**2**) and chlorogenic acid (**3**) in the present study were in accord with the previous reports [35,36].

Conclusion

Three phenolic compounds (**1-3**) were firstly isolated from the aerial parts of *C. leucophloea*, and were identified as liriodendrin (**1**), vanillic acid (**2**), and chlorogenic acid (**3**). Their biological activities were evaluated. Both liriodendrin (**1**) and vanillic acid (**2**) exhibited stronger antibacterial activity than chlorogenic acid (**3**). Furthermore, vanillic acid (**2**) and chlorogenic acid (**3**) were screened to have antifungal and antinematodal activity. Only vanillic acid (**2**) was screened to have antioxidant activity. They could be used as the potential antimicrobials, antinematodal or antioxidants agents. This is the first time to report the antimicrobial activity of liriodendrin (**1**), as well as the antinematodal activity of vanillic acid (**2**) and chlorogenic acid (**3**). The results could provide additional data for future development and utilization of *C. leucophloea*.

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