

Anti-inflammatory Compounds from the Heartwood of *Dalbergia melanoxylon*

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Abstract: A new chalcone compound, methyl 5-cinnamoyl-2-hydroxy-4-methoxybenzoate (**1**), and a new cinnamylphenol compound, methyl 3-cinnamyl-5-hydroxy-4-methoxybenzoate (**2**) together with four known compounds (**3-6**) were isolated from the CH₂Cl₂ fraction of *Dalbergia melanoxylon*. Their structures were elucidated by comprehensive spectral measurements including NMR techniques, mass spectrometry, single crystal X-ray diffraction and, together with comparison of the literature data. According to the determination results of compound **1** and **2** on RAW 264.7 cell viability, the IC₅₀ values of compounds were found to be 3.2 and 383.8 μM, respectively. Compared with LPS model group, compounds **1** and **2** were found to be significantly reduce the release of NO in the concentration ranges of 1.2~9.6 and 33.7 μM (*P*<0.01), and significantly inhibit the secretion of LDH in the range of 4.8~9.6 and 16.8~33.7 μM.

Keywords: *Dalbergia melanoxylon*; chalcone; cinnamylphenol; anti-inflammatory activity. © 2022 ACG Publications. All rights reserved.

1. Introduction

Dalbergia melanoxylon (Leguminosae), commonly known as African blackwood, or Mpingo, has great economic and medicinal value in Africa [1-2]. According to the literature, its roots, stems, leaves and bark can be used to treat abdominal pain, hernia, gonorrhoea, joint pain, wound cleaning and other [3-4]. Isolation of neoflavones, benzofurans, phenanthrenediones, N-cinnamoyl, quinoid derivatives and flavanones have been reported from *D. melanoxylon* [5-12]. In this study, a new chalcone compound (**1**), a new cinnamylphenol compound (**2**) together with four known compounds **3-6** were reported (Figure 1) by further phytochemical evaluation of the CH₂Cl₂ fraction of the *D. melanoxylon*. Furthermore, in vitro anti-inflammatory activities of compounds **1** and **2** on LPS-induced RAW264.7 cells inflammatory model was studied.

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2. Materials and Methods

2.1. General Experimental Procedures

Molecular weights of compounds were determined on a Thermo LTQ Orbitrap mass spectrometer. IR spectra were recorded on a Perkin Elmer FT-IR spectrometer. UV spectra were measured in MeOH, on a Waters e2695 series chromatograph. NMR spectra were recorded on a Bruker AVANCE 600 MHz spectrometer, and chemical shifts are expressed in ppm (δ) with TMS (tetramethylsilane) as an internal reference. X-ray diffraction analysis was performed on a Bruker Shelxtl, Dual, Eos diffractometer with the Cu K α radiation.

2.2. Plant Materials

The heartwood of *D. melanoxylon* was identified by Prof. Feng Xu, the Product Quality Inspection Center of Guangxi University. In July 2014, it was collected in Fang Cheng Gang, Guangxi of China, and voucher specimen (No. Liu-20140713) was deposited at School of Pharmacy, Jiangxi University of Chinese Medicine.

2.3. Extraction and Isolation

Extraction of dried heartwood of *D. melanoxylon* (50.0 kg) with 70% ethanol (solid-liquid ratio: 1:10, 3 times, 2 h) to give a crude extract (13.9 kg). This crude extract was dissolved in water and extracted with CH₂Cl₂, EtOAc and *n*-BuOH to obtain four fractions.

The CH₂Cl₂ soluble fraction (8.5 kg) was separated into 22 fractions (Fr.1-Fr.22) by silica gel CC (petroleum ether-EtOAc 50:1 to 1:5, v/v). Fr.1 (2.2 g) was chromatographed by silica gel CC (column chromatography) using petroleum ether-EtOAc solvent system 50:1-2:1 (v/v), and 3 fractions (Fr.1.A-Fr.1.C) were obtained. Fr.1.C (114.1 mg) was eluted with CH₂Cl₂-MeOH (1:1, v/v) to obtain **2** (10.1 mg) and **3** (6.3 mg) by Sephadex LH-20 CC. Fr.8 (147.4 g) was separated by silica gel CC with CH₂Cl₂-MeOH (100:1-10:1, v/v) as eluent to yield Fr.8.A-Fr.8.F. Frs.8.D (13.1 g) was purified on a silica gel CC eluted with a solvent system of petroleum ether-acetone (50:1, v/v) to afford **4** (277.9 mg). Fr.8.E (1.1 g) was eluted with CH₂Cl₂-MeOH (1:1, v/v) to yield **1** (4.1 mg) and **5** (3.4 mg) by Sephadex LH-20 CC. Frs.15 (83.2 g) was eluted with MeOH-H₂O (30:70-50:50, v/v) as eluent by ODS to obtain Frs.15.A-Frs.15.E. Frs.15.B (213.2 mg) was eluted with CH₂Cl₂-MeOH (1:1, v/v) to afford **6** (8.4 mg) by Sephadex LH-20 CC.

Methyl 5-cinnamoyl-2-hydroxy-4-methoxybenzoate (1): yellow crystals; NMR (600 MHz, Acetone-d₆) and ¹³C NMR (151 MHz, Acetone-d₆): Table 1; HR-ESI-MS: *m/z* 311.0927 [M-H]⁻ (calcd. 311.0925). Crystal data for **1**: C₁₈H₁₆O₅, M = 312.31, a = 15.5793(8) Å, b = 15.1761(9) Å, c = 6.6902(4) Å, α = 90°, β = 90°, γ = 90°, V = 1581.78(16) Å³, T = 100. (2) K, space group Pca21, Z = 4, μ (Cu K α) = 0.796 mm⁻¹, 10418 measured reflections 2448 independent reflections (R_{int} = 0.0599). The final R1 values were determined as 0.0346 (I > 2 σ (I)). The final wR(F2) values were 0.0793 (I > 2 σ (I)). The final R1 values were 0.0415 (all data). The final wR (F2) values were 0.0825 (all data). The goodness of fit on F2 was 1.073. Flack parameter = 0.23(14).

Methyl 3-cinnamyl-5-hydroxy-4-methoxybenzoate (2): yellow crystals; IR (KBr) ν_{\max} : 3514, 1654, 1608, 1530, 1510, 1259, 856, cm⁻¹; UV (MeOH) λ_{\max} : 210, 302.5 nm; ¹H NMR (600 MHz, CDCl₃) and ¹³C NMR (151 MHz, CDCl₃): Table 1; HR-ESI-MS: *m/z* 297.1135 [M-H]⁻ (calcd. 297.1132).

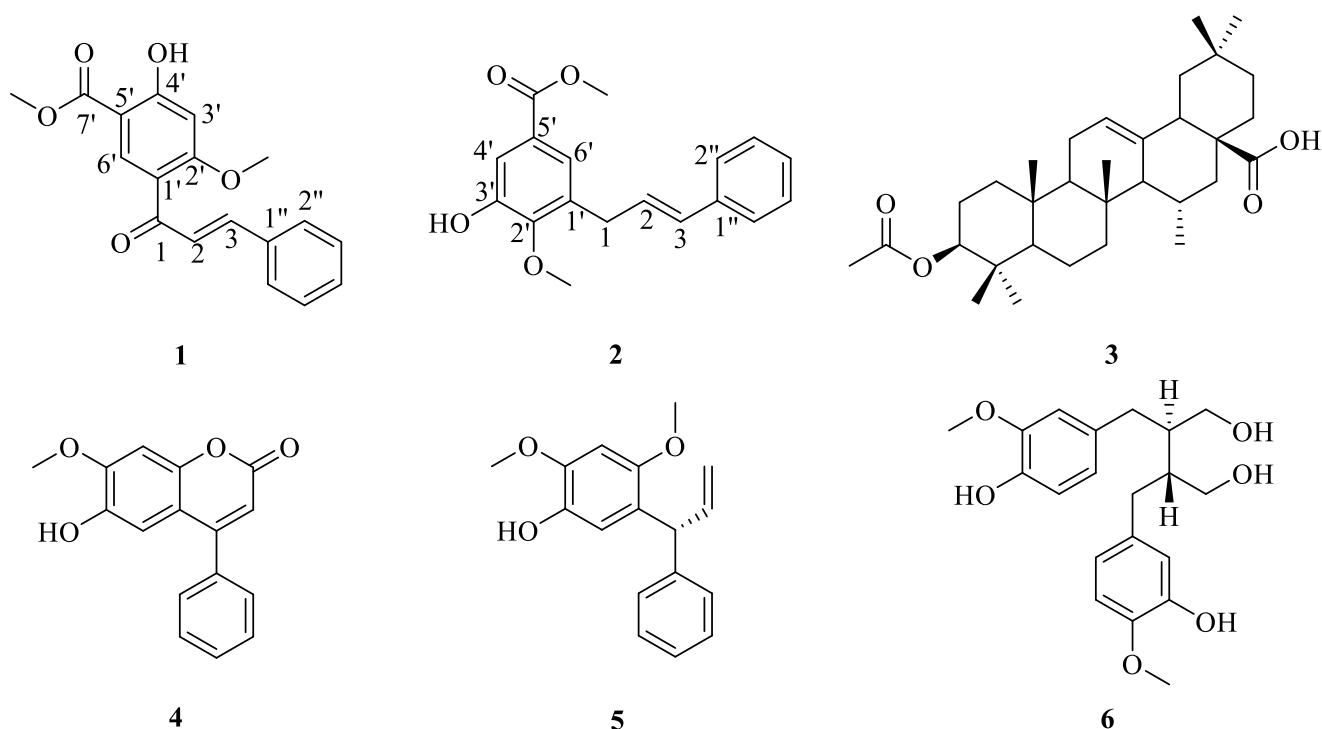


Figure 1. Structures of compounds **1-6**

2.4. Cell Viability Assay

RAW264.7 cells were cultured to the logarithmic growth phase, and the cell concentration was adjusted to 1×10^5 mL/cell. 96-well plates were used to culture cells, each well containing 100 μ L different concentrations of compounds **1** and **2**, the control group was added to the same volume without drugs containing 10% FBS. After 24 h culture, 100 μ L 10 % CCK-8 solution was added to each well, and then cultured for 1 hour. The absorbance of each well was measured at 450 nm [13-15].

2.5. Determination of NO and LDH

Cells were divided into blank group, LPS inflammatory model group and administration group. The complete medium was added to the blank group, 500 ng/mL LPS was added to the LPS group, and the following concentrations 1.2, 2.4, 4.8, 9.6 μ M for compound **1**, 4.2, 8.4, 16.8, 33.7 μ M for compound **2** together with 500 ng/mL LPS were added to drug administration groups. After 24 h of culture, the cell supernatant was collected and measurements performed by in strict accordance with the instructions of Nitric Oxide Assay kit and Lactate dehydrogenase assay kit [14-15].

3. Results and Discussion

3.1. Structure Elucidation

Compound **1** is a white crystal and its molecular formula is $C_{18}H_{16}O_5$. The unsaturation of the compound is 11 degrees, which is calculated by HR-ESI-MS m/z 311.0925 $[M-H]^-$ (calcd. 311.0925). The 1H NMR spectrum (Table 1) show that compound **1** has a hydroxyl group [δ_H 11.27 (1H, s, 4'-OH)] and two methoxy groups [δ_H 4.05 (3H, s, 2'-OMe) and 3.98 (3H, s, 7'-OMe)]. The ^{13}C NMR (Table 1) of **1** showed that it contains 14 aromatic carbon atoms, 2 methoxy carbons and 2 carbonyl carbons. These spectroscopic data were similar to 2'-methoxy-4'-hydroxychalcone [16]. The HMBC

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spectrum (Figure 2) shows that the CH₃OCO group was attached at C-5', which could be verified by the correlation between the methoxy group signal at δ_{H} 3.98 and the carbon signal at δ_{C} 170.0, while the correlation between the hydroxyl group signal at H-6' (δ_{H} 8.23), C-7' (δ_{C} 170.0), and H-3' (δ_{H} 6.69) to C-7' (δ_{C} 170.0) was unobvious. Moreover, the X-ray structure (Figure 3) also showed the hydroxyl group (δ_{H} 11.27) and the other methoxy group (δ_{H} 4.05) were substituted in C-4' and C-2', respectively. Based on this evidence, we identified **1** as methyl 5-cinnamoyl-2-hydroxy-4-methoxybenzoate.

Compound **2** is a white crystal. According to the HR-ESI-MS ion peak at m/z 297.1135 [M-H]⁻ (calcd. 297.1132), the molecular formula is C₁₈H₁₈O₄, indicating 10 degrees of unsaturation. The ¹H and ¹³C NMR spectra of compound **2** (Table 1) are similar to candenatenin H [17], except for a hydroxyl group of C-4'. Moreover, there was an extra CO group attached to C-5' in **2**. The strong correlations from two methoxyl groups at δ_{H} 3.89 and 3.92 to δ_{C} 163.4 and δ_{C} 170.5 further confirmed this point by HMBC (Figure 2). Furthermore, the correlations from the hydrogen signal at H-1 (δ_{H} 3.46) to C-2' (δ_{C} 163.4). So, the methoxy group (δ_{H} 3.89) should be placed at C-2'. Moreover, 3'-OH (δ_{H} 10.95) to C-2' (δ_{C} 163.4) implied that the hydroxy group was attached at C-3'. Further, there were two signals H-4' (δ_{H} 6.48) and H-6' (δ_{H} 7.62). The correlations from the hydrogen group signal at H-4' (δ_{H} 6.48) and H-6' (δ_{H} 7.62) to the carbon group signal (δ_{C} 170.5) implied that the CH₃OCO group was attached at C-5'. Thus, we identified the compound **1** as methyl 3-cinnamyl-5-hydroxy-4-methoxybenzoate.

Table 1. ¹H and ¹³C NMR data of Compound **1-2** (δ in ppm, J in Hz)

Position	1^a		2^b	
	δ_{H}	δ_{C}	δ_{H}	δ_{C}
1	-	188.5	3.46 (2H, d, J = 6.5 Hz)	30.7
2	7.59 (1H, d, J = 15.8 Hz)	141.8	7.22 (1H, t, J = 7.3 Hz)	128.5
3	7.66 (1H, d, J = 15.8 Hz)	126.7	6.35 (1H, m)	137.6
1'	-	130.2	-	130.8
2'	-	164.5	-	163.4
3'	6.69 (1H, s)	99.8	-	162.7
4'	-	166.1	6.48 (1H, s)	127.0
5'	-	105.5	-	120.5
6'	8.23 (1H, s)	133.5	7.62 (1H, s)	98.1
7'	-	170.0	-	170.5
1''	-	135.3	-	130.4
2'', 6''	7.75 (2H, dd, J = 7.2, 1.8 Hz)	128.4		128.4
3'', 5''		128.9	7.33 (5H, m)	126.1
4''	7.46 (3H, d, J = 5.9 Hz)	126.8		127.0
2'-OCH ₃	4.05 (3H, s)	56.0	3.89 (3H, s)	51.9
7'-OCH ₃	3.98 (3H, s)	52.0	3.92 (3H, s)	55.7
3'-OH	-	-	10.95 (1H, s)	-
4'-OH	11.27 (1H, s)	-	-	-

^a Measured in Acetone-*d*₆.

^b Measured in CDCl₃.

Additionally, four known compounds were identified as 3-*O*-acetyloleanolic acid (**3**)[18], dalbergin (**4**)[19], dalbergiphenol (**5**)[20] and (-)-secoisolaricresinol (**6**)[21] by comparing their NMR data with those reported in the literature, respectively (Figure 1).

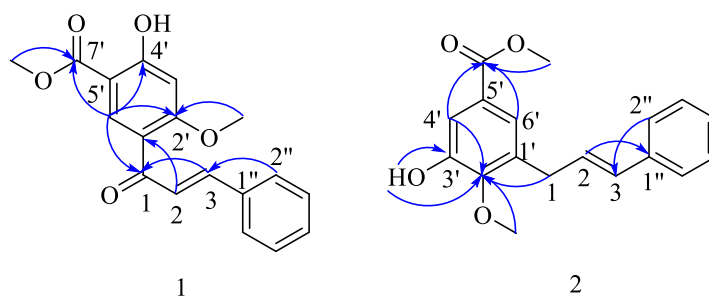


Figure 2. Selected HMBC correlations of compound **1**

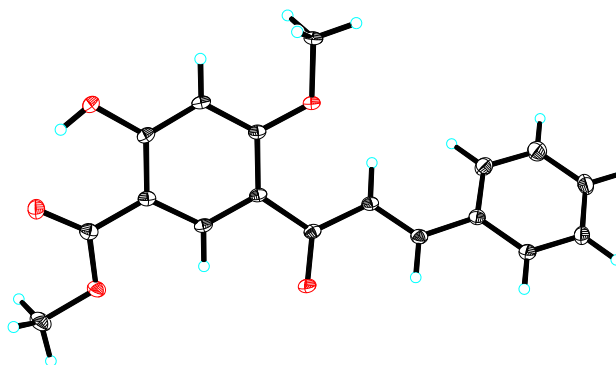


Figure 3. Single-crystal X-ray structure of compound **1**

3.2. Anti-inflammatory Activity

RAW264.7 cells were treated with different concentrations of compounds **1** and **2** *in vitro*, and the optimal concentration range without obvious effect on cell activity was explored with CCK-8 reagent. The cell viability test results showed that the IC_{50} values of compounds **1** and **2** were 13.2 and 383.8 μM , respectively (Table 2). And the effects of compounds **1** and **2** on LPS-induced secretion of NO and LDH in RAW264.7 cells were detected. Compared with the LPS model group, compound **1** and **2** could significantly reduce the release of NO in the concentration ranges of 1.2~9.6 and 33.7 μM ($P < 0.01$). Compounds **1** could significantly decrease LDH activity in 9.6 μM ($P < 0.01$) and 4.8 μM ($P < 0.05$), but 2.4 and 1.2 μM administration group significantly increased LDH activity. Compound **2** significantly reduced LDH activity in 33.7, 16.8 μM groups ($P < 0.01$) (Figure 4).

Table 2. Effect of compound **1** - **2** on RAW264.7 cell viability (n = 6)

Groups	Dose (μM)	Survival (%)	IC_{50} (μM)	Groups	Dose (μM)	Survival (%)	IC_{50} (μM)
Control	-	100 \pm 1.76		Control	-	100 \pm 2.8	
	10.0	102.64 \pm 2.17			42.1	100.47 \pm 3.14	
	20.1	30.09 \pm 2.52			84.2	91.84 \pm 1.18	
1	40.2	6.09 \pm 0.38	13.2	2	168.4	90.24 \pm 3.30	383.8
	80.4	3.23 \pm 0.68			336.7	49.13 \pm 2.84	
	160.8	0.47 \pm 0.50			673.4	27.83 \pm 1.37	

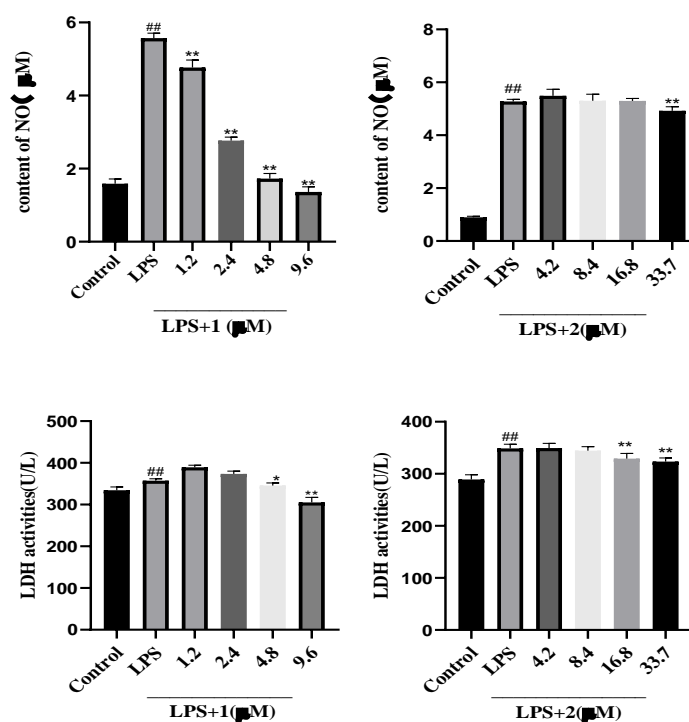
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Figure 4. Effect of compounds 1-2 on NO and LDH secretion by LPS-induced RAW264.7 cells ($\bar{x} \pm s$, n = 6)

Note: Compared with the blank group, ## $P < 0.01$, compared with the model group, ** $P < 0.01$, * $P < 0.05$.

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Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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References

- [1] T. M. Najeeb, T. O. Issa, Y. S. Mohamed, R. H. Ahmed, and T. O. Khider (2018). Phytochemical screening, antioxidant and antimicrobial activities of *Dalbergia melanoxylon* tree, *World Appl. Sci. J.* **36**, 826-833.

- [2] K. Nakai, and T. Yoshimura (2020). African blackwood (*Dalbergia melanoxylon*) and other local tanzanian tree species' biological performance against subterranean termites and wood decay fungi, *BioResources*. **15**, 2994-3005.
- [3] M. Gundidza, and N. Gaza (1993). Antimicrobial activity of *Dalbergia melanoxylon* extracts, *J. Ethnopharmacol*, **40**, 127.
- [4] E. Amri, and S. Juma (2016). Evaluation of antimicrobial activity and qualitative phytochemical screening of solvent extracts of *Dalbergia melanoxylon* (guill. & perr.), *Int. J. Curr. Microbiol. Appl. Sci*, **5**, 412-423.
- [5] D. M. X. Donnelly, J. O'Reilly, and W. B. Whalley (1975). Neoflavanoids of *Dalbergia melanoxylon*, *Phytochemistry* **14**, 2287-2290.
- [6] F.R.V. Heerden, E.V. Brandt, and D.G. Roux (1980). Isolation and synthesis of trans- and cis- (-)-clovamides and their deoxy analogues from the bark of *Dalbergia melanoxylon*, *Phytochemistry* **19**, 2125-2129.
- [7] S. Lin, R.H. Liu, G.Q. Ma, D.Y. Mei, F. Shao, and L.Y. Chen (2019). Two new compounds from the heartwood of *Dalbergia melanoxylon*, *Nat. Prod. Res.* **34**, 1-8.
- [8] Y. Liu, N. Zhang, J. W. He, L. Y. Chen, and R. H. Liu (2021). Two new compounds from the heartwood of *Dalbergia melanoxylon* and their protective effect on hypoxia/reoxygenation injury in H₉C₂, *Nat. Prod. Commun.* **16**, 1-7.
- [9] Y. Liu, J. Cheng, Shu, M. F. Wang, Z. J. Xu, L. Yang, X. W. Meng, W. B. Duan, N. Zhang, F. Shao, R. H. Liu, and L. Y. Chen (2021). Melanoxylonin A-G, neoflavanoids from the heartwood of *Dalbergia melanoxylon* and their cardioprotective effects, *Phytochemistry* **189**, 112845.
- [10] P. Mutai, M. Heydenreich, G. Thoithi, G. Mugumbate, K. Chibale, and A. Yenesew (2013). 3-hydroxyisoflavanones from the stem bark of *Dalbergia melanoxylon*: isolation, antimycobacterial evaluation and molecular docking studies, *Phytochem. Lett.* **6**, 671-675.
- [11] M. F. Wang, G. Q. Ma, F. Shao, R. H. Liu, L. Y. Chen, Y. Liu, Li. Yang, and X. W. Meng (2022). Neoflavanoids from the heartwood of *Dalbergia melanoxylon*, *Nat Prod Res.* **36**, 735-741.
- [12] Z. J. Xu, Y. Liu, X. W. Meng, F. Shao, R. H. Liu, L. Yang and L. Y. Chen (2021). Neoflavanoids from the heartwood of *Dalbergia melanoxylon*, *Rec Nat Prod.* **16**, 1-6.
- [13] Y. Lu and Y.F. Chen (2021). Eudesmane sesquiterpenoids from *Salvia plebeia*, *Rec. Nat. Prod.* **15**, 613-616.
- [14] Y.D. Wang, C. Wang, Y.H. Wang, Q. Hu and F.H. Wang (2022). A new isoflavan glucoside from the roots of *Astragalus membranaceus* var. *Mongholicus*, *Rec. Nat. Prod.* **16**, 404-408.
- [15] C.X. Shen, C.Y. Ouyang, Y.Zhang, Q. Zhu, R.H. Liu and L.Y.Chen (2022). Sesquiterpenoids from the Leaves of *Dalbergia odorifera*, *Rec. Nat. Prod.* doi /10.25135/rnp.347.2205.2466
- [16] H. Yang, D. Wang, L. Tong, and B. C. Cai (2009). Flavonoid aglycones of *Oxytropis falcata*, *Chem. Nat. Compd.* **45**, 239-241.
- [17] S. Cheenpracha, T. Ritthiwigrom, C. Karalai, and S. Laphookhieo (2012). Candenatenins G-K, phenolic compounds from *Dalbergia candenatensis* heartwood, *Phytochem. Lett.* **5**, 708-712.
- [18] U. Kolak, G. Topcu, A. Ulubelen, S. Birteksoz, and G. Otuk (2005). Terpenoids and steroids from the roots of *Salvia blepharochlaena*, *Turk J Chem.* **29**, 177-186.
- [19] G. C. Kite, P. Green, N. C. Veitch, M. C. Groves, P. E. Gasson, and M. Simmonds, (2010). Dalnigrin, a neoflavanoid marker for the identification of brazilian rosewood (*Dalbergia nigra*) in cites enforcement, *Phytochemistry*, **71**, 1122-1131.
- [20] N. Muangnoicharoen, and A. W. Frahm, (1982). Neoflavanoids of *Dalbergia parviflora*. *Phytochemistry*, **21**, 767-772.
- [21] S. S. Hong, X. H. Han, J. S. Hwang, K. S. Lee, and B. Y. Hwang, (2006). Lignans from the stem barks of *Kalopanax septemlobus*, *Nat. Prod. Sci.* **12**, 201-204.

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