SUPPLEMENTARY MATERIAL

Two new simple iridoids from the ant-plant *Myrmecodia tuberosa* and their antimicrobial effects

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Six iridoid derivatives (**1–6**), including two new compounds myrmecodoides A and B (**1** and **2**), were isolated from the ant-plant *Myrmecodia tuberosa*. Their structures were determined on the basis of spectroscopic data (¹H and ¹³C NMR, HSQC, HMBC, ¹H-¹H COSY, NOESY, and HR-ESI-MS) and by comparison with the literature values. Among isolates, **3** and **4** exhibit weak antibacterial effect against *Staphylococcus aureus* subsp. *aureus* with MIC value of 100.0 µg/mL.

Keywords: *Myrmecodia tuberosa*; Rubiaceae; ant-plant; iridoid; antimicrobial activity

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Antibacterial assay

The antibacterial activity of the pure compounds was tested using the micro-dilution method on 96-well microtiter plate according to the method of Vanden Berghe and Vlietinck (1991) and McKane and Kandel (1996).

Briefly:

Test microorganism strains:

- + Gram-(–) bacteria: *Escherichia coli* (ATCC 25922)
Pseudomonas aeruginosa (ATCC 25923)
- + Gram-(+) bacteria: *Bacillus subtilis* (ATCC 11774)
Staphylococcus aureus subsp. *aureus* (ATCC 11632)
- + Fungi: *Aspergillus niger* (439)
Fusarium oxysporum (M42)
- + Yeast: *Candida albicans* (ATCC 7754)
Saccharomyces cerevisiae (SH 20)

Positive controls:

- + Streptomycin for Gr(+) bacteria
- + Tetracycline for Gr(–) bacteria
- + Nystatin or Amphotericin B for fungi and yeast.

Antibiotic agents was dissolved in DMSO 100% with suitable concentrations.

Negative control:

Microorganisms without mixture of antibiotic agents and tested compounds.

Cultured media:

- + Media for maintain of strains: Sabouraud Dextrose Broth (SDB-Sigma) for fungi and yeast. Trypticase Soy Broth (TSB-Sigma) for bacteria.
- + Tested media: Eugon Broth (Difco, USA) for bacteria, Mycophil (Difco, USA) for fungi and yeast.

Experiment:

- + The strains were activated and diluted according to McFarland 0.5 standard for experiment.
- + The tested plates were incubated at 37°C for 24hrs with bacteria and at 30°C for 48hrs with fungi and yeast.

Calculation of the results:

Minimum inhibitory concentration (MIC) of tested samples: The samples were diluted with decrease of concentrations to reach minimum inhibitory concentration (MIC), the lowest concentration of each sample showing no growth.

The crude samples with $\text{MIC} \leq 200 \text{ } \mu\text{g/ml}$ and pure compounds with $\text{MIC} \leq 100 \text{ } \mu\text{g/ml}$ were considered to be active.

Figure S1. ¹H NMR spectrum (CD₃OD, 500 MHz) of myrmecodoide A (1)

Figure S2. ¹³C NMR spectrum (CD₃OD, 125 MHz) of myrmecodoide A (1)

Figure S3. HSQC spectrum (CD₃OD, 500 MHz) of myrmecodoide A (**1**)

Figure S4. COSY spectrum (CD₃OD, 500 MHz) of myrmecodoide A (**1**)

Figure S5. HMBC spectrum (CD_3OD , 500 MHz) of myrmecodoide A (**1**)

Figure S6. NOESY spectrum (CD_3OD , 500 MHz) of myrmecodoide A (**1**)

Figure S7. ¹H NMR spectrum (CD₃OD, 500 MHz) of myrmecodoide B (2)

Figure S8. ¹³C NMR spectrum (CD₃OD, 125 MHz) of myrmecodoide B (2)

Figure S9. HSQC spectrum (CD_3OD , 500 MHz) of myrmecodoide B (**2**)

Figure S10. COSY spectrum (CD_3OD , 500 MHz) of myrmecodoide B (**2**)

Figure S11. HMBC spectrum (CD_3OD , 500 MHz) of myrmecodoide B (**2**)

Figure S12. NOESY spectrum (CD_3OD , 500 MHz) of myrmecodoide B (**2**)

Figure S13. Key NOESY correlations of compounds **1** and **2**

Table S1. ^1H (CD_3OD , 500 MHz) and ^{13}C NMR (CD_3OD , 125 MHz) spectroscopic data of compounds **1** and **2**.

Pos.	1				2			
	$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$ mult. (J in Hz)	^1H - ^1H COSY	HMBC (H $\rightarrow$ C)	$\delta_{\text{C}}^{\text{a,b}}$	$\delta_{\text{H}}^{\text{a,c}}$ mult. (J in Hz)	^1H - ^1H COSY	HMBC (H $\rightarrow$ C)
1	70.3	4.07 dd (11.0, 11.5) 4.34 dd (6.5, 11.5)	9	3, 5, 8	176.5	-		
3	179.1	-			72.1	4.71 dd (1.0, 12.0) 4.87 br d (12.0)		1, 4, 5, 11
4	39.0	2.68 dq (11.0, 7.0)	5, 11	3, 5, 6, 11	143.5	-		
5	51.0	1.84 ddd (7.0, 10.5, 11.0)	4, 6, 9	1, 4, 11, 6, 9	39.0	3.21 m	6, 9	1, 4
6	80.0	3.99 ddd (6.0, 10.5, 11.0)	5, 7	4	37.7	1.62 m/2.51 m	5, 7	4, 5, 7, 8, 9
7	45.4	1.42 ddd (9.5, 11.0, 12.0) 2.14 dd (6.0, 12.0)	6, 8	6, 8, 10	84.9	4.00 ddd (6.0, 8.0, 10.0)	6, 8	10, 1'
8	34.7	1.73 m	7, 9, 10	1, 10	45.0	2.43 ddq (8.0, 10.5, 7.0)	7, 9, 10	1, 7, 9, 10
9	45.6	2.21 m	1, 5, 8	1, 10	50.0	2.74 dd (8.0, 10.5)	5, 8	1, 5, 8, 10
10	19.5	1.09 d (7.0)	8	7, 8, 9	17.9	1.21 d (7.0)	8	7, 8, 9
11	14.2	1.28 d (7.0)	4	3, 4, 5	114.1	5.12 d (1.5) 5.20 br s		3, 4, 5
1'					102.9	4.34 d (8.0)	2'	7
2'					75.1	3.19 dd (8.0, 9.0)	1', 3'	
3'					78.1	3.67 t (9.0)	2', 4'	
4'					71.7	3.30 t (9.0)	3', 5'	
5'					78.0	3.28 m	3', 6'	
6'					62.8	3.70 dd (5.0, 12.0) 3.88 dd (2.0, 12.0)	5'	

Note: All assignments were done by HSQC, COSY, HMBC, and NOESY experiments

References

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