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 myrmecoides 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.

*Culture 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 MIC ≤ 200 µg/ml and pure compounds with MIC ≤ 100 µg/ml were considered to be active.
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| Pos. | $\delta^c_{\text{a,b}}$ | $\delta^a_{\text{H,c}}$ | mult. ($J$ in Hz) | $^1$H-$^1$H COSY | HMBC (H $\rightarrow$ C) | $\delta^c_{\text{a,b}}$ | $\delta^a_{\text{H,c}}$ | mult. ($J$ in Hz) | $^1$H-$^1$H 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           | -               |                | 1               | 4, 5, 11         |
| 3    | 179.1           | -               | 72.1            | 4.71 dd (1.0, 12.0) | 4.87 br d (12.0) | 143.5           | -               |                | 1               | 4, 5, 11         |
| 4    | 39.0            | 2.68 dq (11.0, 7.0) | 5.7             | 3, 5, 6, 11      | 143.5           | -               | 37.7            | 1.62 m/2.51 m   | 5               | 4, 5, 7, 8, 9    |
| 5    | 51.0            | 1.84 ddd (7.0, 10.5, 11.0) | 1, 4, 11, 6, 9 | 39.0            | 3.21 m          | 1               | 4               | 1.4             | 1               | 4, 5, 7, 8, 9    |
| 6    | 80.0            | 3.99 ddd (6.0, 10.5, 11.0) | 5               | 4               | 1               | 4               | 1.4             | 1               | 4, 5, 7, 8, 9    |
| 7    | 45.4            | 1.42 ddd (9.5, 11.0, 12.0) | 6, 8            | 6, 8, 10        | 1               | 4               | 1.4             | 1               | 4, 5, 7, 8, 9    |
| 8    | 34.7            | 1.73 m           | 7, 9, 10        | 1               | 10             | 1               | 4               | 1.4             | 1               | 4, 5, 7, 8, 9    |
| 9    | 45.6            | 2.21 m           | 1, 5, 8         | 1               | 10             | 1               | 4               | 1.4             | 1               | 4, 5, 7, 8, 9    |
| 10   | 19.5            | 1.09 d (7.0)     | 8               | 7, 8, 9         | 1               | 4               | 1.4             | 1               | 4, 5, 7, 8, 9    |
| 11   | 14.2            | 1.28 d (7.0)     | 4               | 3, 4, 5         | 114.1           | 1               | 4               | 1.4             | 1               | 4, 5, 7, 8, 9    |
| 1'   | 102.9           | 4.34 d (8.0)     | 2               | 102.9           | 4.34 d (8.0)     | 2               | 10              | 1               | 4, 5, 7, 8, 9    |
| 2'   | 75.1            | 3.19 dd (8.0, 9.0) | 1               | 3               | 3               | 3               | 1               | 3'              | 1', 3'           |
| 3'   | 81.3            | 3.67 t (9.0)     | 2               | 4               | 3               | 3               | 1               | 3'              | 1', 3'           |
| 4'   | 71.7            | 3.30 t (9.0)     | 3               | 4               | 3               | 3               | 1               | 3'              | 1', 3'           |
| 5'   | 78.5            | 3.28 m           | 3               | 5               | 3               | 3               | 1               | 3'              | 1', 3'           |
| 6'   | 62.8            | 3.70 dd (5.0, 12.0) | 4               | 5               | 3               | 3               | 1               | 3'              | 1', 3'           |

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

References

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