Triterpenoid saponins from *Lysimachia candida* Lindl.

Xin Xia, Xiaoyi Wei, Lidong Lin

**Abstract**

Background: Identification of chemical constituents is very important for ensuring the function of traditional herbal medicine. The objectives of this study were to determine the activity chemical constituents of *Lysimachia candida* Lindl.

Methods: Solvent fractionation and chromatographic separation techniques were used to isolate the chemical constituents of *Lysimachia candida* Lindl.

Results: Three triterpenoid saponins were isolated from the antifungal activity fraction of *Lysimachia candida* Lindl. Their structures were elucidated on the basis of spectroscopic data. They were established as a new compound 3-0-[β-D-Xylopyranosyl-(1→2)]-β-D-Glucopyranosyl-[1→4]-[β-D-Glucopyranosyl-(1→2)]-α-L-arabinopyranoside-13,28-Epoxy-3,16,22-oleananetriol, named Lysimanoside (1), along with two known compounds Lysikokianoside I (2) and Anagallisin C (3).

Conclusion: Anagallisin C showed to be the main antifungal compound against *Aspergillus flavus* in *Lysimachia candida* Lindl.

1. Introduction

*Lysimachia candida* Lindl. is a folklore medicinal plant that grows in South China. The whole plant is used for treating fever, swelling, dermatitis and bone fracture. In the course of our investigation on antifungal natural products from plants, we found the crude extract of *L. candida* Lindl. showed activity against *Aspergillus flavus* with a dosage of about 500 μg on filter diffusion assay. The crude extract was suspended in H2O, and partitioned with CHCl3, EtOAc and n-BuOH successively. The n-BuOH extract showed activity against *A. flavus* with a dosage of about 200 μg on filter diffusion assay. In further investigation on the antifungal constituents of this plant, a new compound, 3-0-[β-D-Xylopyranosyl-(1→2)]-β-D-Glucopyranosyl-[1→4]-[β-D-Glucopyranosyl-(1→2)]-α-L-arabinopyranoside-13,28-Epoxy-3,16,22-oleananetriol, named Lysimanoside (1), along with two known compounds Lysikokianoside I (2) and Anagallisin C (3) were isolated from the stems of *L. candida* Lindl. Anagallisin C showed to be the main antifungal compound against *A. flavus* in *L. candida* Lindl.

2. Results and discussion

The MeOH extract of the stems of *L. candida* Lindl. was suspended in H2O, and partitioned with CHCl3, EtOAc and n-BuOH successively. Separation of n-BuOH extract by a combination of silica gel and ODS column chromatography and preparative HPLC afforded Lysimanoside (1), Lysikokianoside I (2) and Anagallisin C (3) (Fig. 1). Their chemical structures were determined on the basis of spectroscopic analysis and comparison to the reported values in literature.

Compound 1 was obtained as an amorphous white powder, 
\[\delta^1H = 13.9° (c = 1.0, MeOH).\] The molecular formula was determined as C52H86O22 from ESIMS ions at m/z 1085 [M + Na]+, 1101 [M + K]+ in positive and 1061 [M – H]–, 1097.5 [M + Cl]– in negative as well as an HRESIMS ion at m/z 1063.56616 [M + H]+ (Calcd for C52H86O22, 1063.56890). The negative-ion ESIMS showed fragment ion peaks at m/z 929 [M–C6H10O5–H]– due to the loss of a pentose unit, m/z 767 [M–C5H10O2–C6H10O5–H]– due to the loss of a pentose unit and a hexose unit and m/z 605 [M–C5H10O2–C6H10O5–C6H10O5–H]– due to the loss of a pentose unit and two hexose units. Compound 1 displayed 52 carbon signals in 13C NMR spectrum (Table 1), of which 30 carbon signals could be assigned to the signals of the aglycon. 13C NMR spectrum exhibited seven sp3 carbons at δ 16.69, 16.69, 18.79, 20.11, 26.02, 28.34 and 33.86 and signals for an oxygenated methylene protons at δ 3.37 (d, J = 8.0 Hz) and 3.66 (d, J = 8.0 Hz) indicated that the aglycon of 1 is based on a 13,28-epoxylleanane skeleton.

The 1H NMR and 13C NMR spectrum indicated the presence of one...
The 13C NMR spectrum exhibited seven sp3 carbons at δ 104.32, 105.59, and one β-dxylopyranosyl unit. The above analysis revealed that compound 1 was a triterpenoid saponin with four monosaccharide units. Compound 1 was identified as anagallisin C. Thus, compound 2 was identified as lysikokianoside I.

Compound 3 was obtained as an amorphous white powder. The molecular formula was determined as C37H68O22 from ESIMS ions at m/z 1085 [M + Na]+, 1063 [M + H]+ in positive and 1061 [M – H]–, 1097 [M + Cl]– in negative. The negative-ion ESIMS showed fragment ion peaks at m/z 929 [M–C4H9O2–H]– due to the loss of a pentose unit and m/z 767 [M–C6H12O4–C4H9O5–H]– due to the loss of a pentose unit and a hexose unit and m/z 605 [M–C6H12O4–C6H10O5–C6H10O5–H]– due to the loss of a pentose unit and two hexose units. Compound 3 displayed 52 carbon signals in 13C NMR spectrum (Table 1), of which 30 carbon signals could be assigned to the signals of the aglycon. 13C NMR spectrum exhibited six sp3 carbons at δ 17.19, 18.83, 19.81, 24.94 and 33.90 and signals for an oxygenated methylene at δ 0.70 (H-24) and δ 0.82 (H-26). These data coupled with information from the 1H NMR spectrum of six methyl singlets at δ 1.24, 1.14, 0.94, 0.92, 0.90 and 0.70 and a pair of oxygenated methylene protons at δ 3.34 (d, J = 8.0 Hz) and 3.70 (d, J = 8.0 Hz) indicated that the aglycon of 3 is based on a 13,28-epoxyoleanane skeleton. The 1H NMR data of the aglycon were similar to that of compound 2, but the methyl group signal of C-23 was replaced by a hydroxymethyl at δ 64.62. This assignment was confirmed by the HMBC correlation between δH 0.70 (H-24) and δC 64.62. 1H NMR spectrum showed four anomic proton signals at δ 4.99 (d, J = 8.0 Hz), 4.90 (d, J = 8.0 Hz), 4.68 (d, J = 4.0 Hz) and 4.51 (d, J = 4.0 Hz). The above analysis revealed that compound 2 was a triterpenoid saponin with four monosaccharide units. The sequence of the sugar chain was assigned with the same structure as that of compound 1 by comparing their 1H NMR and 13C NMR data. The 1H NMR and 13C NMR data agreed with the known compound lysikokianoside I. Thus, compound 2 was identified as lysikokianoside I.
uncorrected. Optical rotations were obtained on a Perkin–Elmer 341 polarimeter (Perkin–Elmer, Inc., Waltham, MA). HRESIMS data were obtained on a Bruker BioTOF IIIQ mass spectrometer (Bruker Biospin Gmbh, Rheistetten, Germany). ESIMS data were collected on an MDS SCIEX API 2000 LC/MS/MS instrument (Applied Biosystems, Inc., Forster, CA). The 1H (400 MHz), 13C (100 MHz), and 2D NMR spectra were recorded in CD3OD on a Bruker DRX-400 instrument using TMS as an internal reference (Bruker Biospin Gmbh, Rheistetten, Germany). Preparative HPLC was conducted with a Shimazu LC-6A pump and a Shimazu RID-10A refractive index detector (Shimazu Corp., Japan) using an XTerra prep MS C-18 column (10 μm, 300 × 19 mm, Waters Corp., Milford, MA). For column chromatography, silica gel 60 (100 – 200 mesh, Qingdao Marine Chemical Ltd., Qingdao, China), Developsil ODS (75 μm, Nomura Chemical Co. Ltd., Japan) and Sephadex LH-20 (GE healthcare, Upssala, Sweden) were used. TLC was performed on precoated plates (Kieselgel 60GF254, Merck, Darmstadt, Germany).

3.2. Plant material

The plant of L. candida Lindl. was collected from Dinghu mountain conservation and identified by Professor Huagu Ye, South

### Table 1

<table>
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<tr>
<th>Carbon no.</th>
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3.3. Extraction and isolation

The crude extraction (84 g) was suspended in H₂O and sequentially extracted with CHCl₃, EtOAc and n-BuOH. The extracts were evaporated under vacuum to yield a CHCl₃ fraction (0.9 g), an EtOAc fraction (0.35 g), an n-BuOH fraction (9.3 g), and a H₂O fraction. Each fraction was tested in vitro for antifungal activity against A. flavus. The n-BuOH fraction, exhibited antifungal activity against A. flavus, was chromatographed over silica gel column, eluted with CHCl₃:MeOH (7:3, 6:4, 5:5, and 4:6, each 2000 ml) to give six fractions (Fr.1: 0.69 g, Fr.2: 0.28 g, Fr.3: 0.26 g, Fr.4: 1.66 g, Fr.5: 0.36 g, and Fr.6: 0.74 g). The six fractions were tested in vitro for antifungal activity against A. flavus. Fr.5 (90 mg) that exhibited antifungal activity against A. flavus was separated on an ODS C-18 column, eluted with 76% MeOH and then purified by prepared HPLC using 72% MeOH to afford compound 1 (19 mg), compound 2 (46 mg) and compound 3 (25 mg).

3.4. Antifungal assay

The microorganisms used in the antifungal assay A. flavus (GIM3.18) were maintained at the Guangdong Institute of Microbiology. A. flavus were cultivated on PDA medium. Compounds (1–3) were evaluated for their activities against A. flavus by using filter diffusion assay. The sterile paper disk of 6 mm diameter impregnated with the compound. The mycelia actively grown on PDA plates were dissolved in sterile water to be used to inoculate to the agar plate (concentration was 0.5 × 10⁵–2.8 × 10⁵ CFU/ml). Ketoconazole was used as positive control. The impregnated disks were placed on the medium suitably spaced apart and the plates were incubated at 28 °C for 48 h. The diameter (mm) of growth inhibition halos caused by the compound was examined.

Conflicts of interest

All authors have none to declare.

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References