

Supporting Information

Supplementary Material

S1. Determination of the Purity of Isolated Phellamurin by Quantitative ¹H-NMR

The purity of compound **1** (phellamurin), isolated from the leaves of *Phellodendron amurense*, was determined by quantitative ¹H-NMR (qNMR) using an internal standard method. Accurately weighed phellamurin (approximately 5 mg) and DSS-*d*₆ (approximately 2 mg; purity 92.4%, Lot No. ELG6825; FUJIFILM Wako Pure Chemical Corporation) were dissolved in dimethyl sulfoxide-*d*₆; 1000 μL. After complete dissolution, the solution was transferred to a 5-mm NMR tube and subjected to qNMR analysis. Three independently prepared samples were obtained by separately weighing phellamurin and DSS-*d*₆, and each sample was measured three times by qNMR.

The ¹H-qNMR spectrum was recorded on an AVANCE NEO 400 system equipped with a PI HR-BBO400S1-BBF/H/D-5.0-Z SP probe. The measurement was performed at 25°C without sample spinning. The P_PROTON_IG protocol with ¹³C decoupling was used, and the pulse program was zgig. The spectral width was 20.485 ppm (8196.72 Hz), and the center of the observation range was set at 5.000 ppm. The number of scans was 32. The relaxation delay (D1) and acquisition time (AQ) were 60 s and 2.4999025 s, respectively, giving a pulse repetition time of approximately 62.5 s. A 90° pulse was used, and the 90° pulse width was 8.300 μs. No dummy scans were applied, and the receiver gain was set to 101.

For quantification, a well-resolved singlet proton signal of phellamurin at δ 6.32 ppm, corresponding to 1 proton, was selected as the quantitative signal, and its integral value was normalized to 1.00. The singlet signal of DSS-*d*₆ at δ 0.00 ppm, corresponding to 9 protons, was used as the internal standard signal. No interfering signals were observed in the integrated regions used for quantification.

The purity of phellamurin was calculated from the ratio of the integrated signal area of phellamurin to that of the internal standard, taking into account the number of protons contributing to each signal, the weighed amounts of phellamurin and DSS-*d*₆, the molecular weights of both compounds, and the certified purity of DSS-*d*₆.

$$\text{Purity (\%)} = [(I_x / N_x) / (I_{std} / N_{std})] \times [(M_x / m_x) / (M_{std} / m_{std})] \times P_{std} \times 100$$

where I_x and I_{std} are the integrated signal areas of phellamurin and DSS-*d*₆, respectively; N_x and N_{std} are the numbers of protons corresponding to the selected signals; M_x and M_{std} are the molecular weights

of phellamurin and DSS- d_6 , respectively; m_x and m_{std} are the accurately weighed masses of phellamurin and DSS- d_6 , respectively; and P_{std} is the certified purity of DSS- d_6 expressed as a decimal (0.924). The mean purity of isolated phellamurin was determined to be $91.7 \pm 1.7\%$ by qNMR. Therefore, the isolated phellamurin was considered to have sufficient purity for the pharmacological evaluation described in this study.

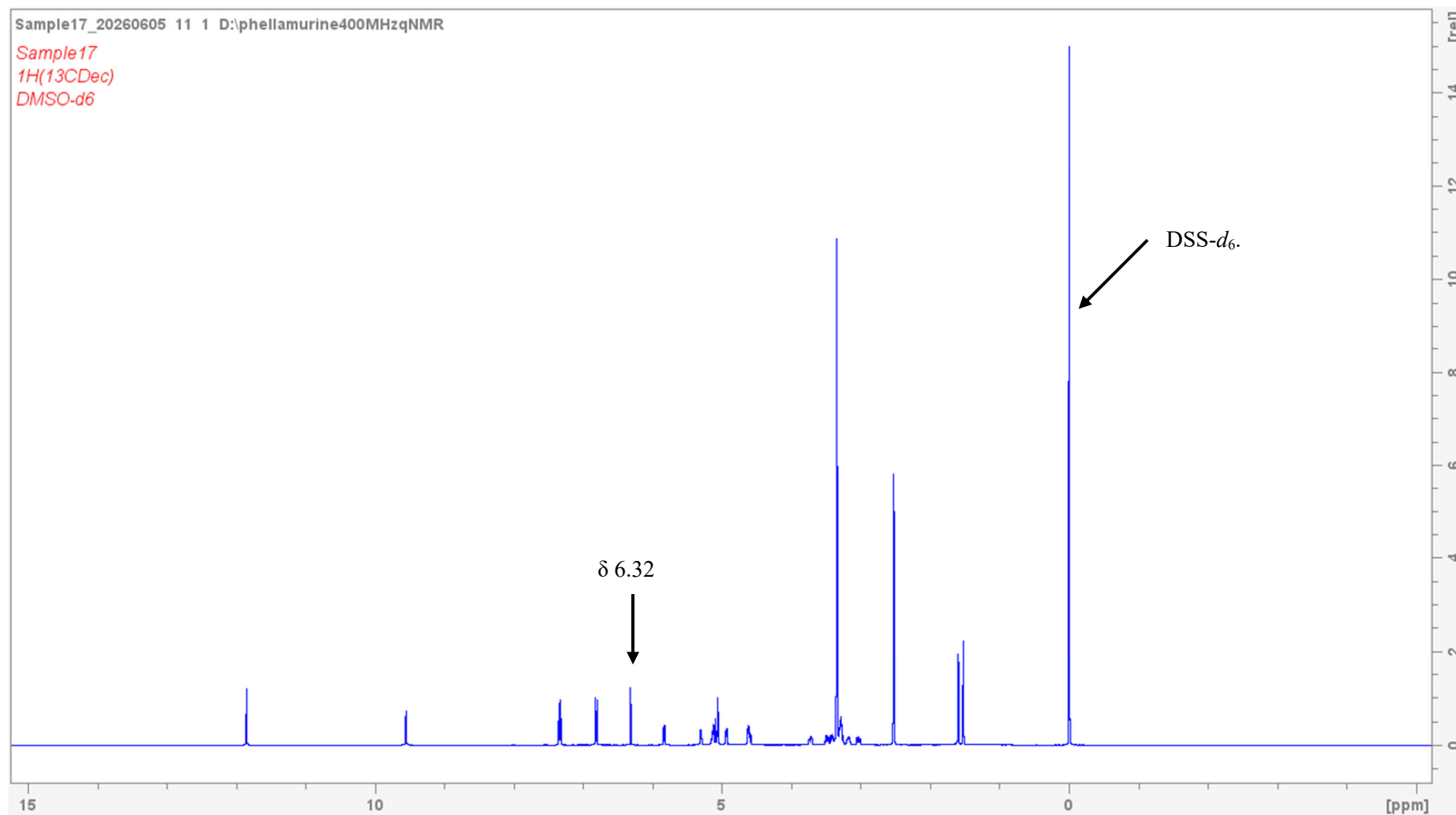


Fig. S1. ¹H-qNMR spectrum of isolated phellamurin. ¹H-qNMR spectrum of isolated phellamurin. The singlet signal at δ 6.32 ppm was used for quantification of phellamurin, and the singlet signal at δ 0.00 ppm was assigned to DSS-d₆. ¹H-qNMR spectrum of isolated phellamurin. The singlet signal at δ 6.32 ppm was used for quantification of phellamurin, and the singlet signal at δ 0.00 ppm was assigned to DSS-d₆.

Table S1. Sample preparation and acquisition parameters for ¹H-qNMR analysis of phellamurin.

Parameter	Condition
Instrument	AVANCE NEO 400 system
Probe	PI HR-BBO400S1-BBF/H/D-5.0-Z SP
Temperature	25°C
Sample spinning	Off
Protocol	P_PROTON_IG with ¹³ C decoupling
Pulse program	zgig
Spectral width	20.485 ppm (8196.72 Hz)
Center of observation range	5.000 ppm
Number of scans	32
Dummy scans	0
Relaxation delay (D1)	60 s
Acquisition time (AQ)	2.4999025 s
Pulse repetition time	Approximately 62.5 s
Pulse angle	90°
90° pulse width	8.300 μs
Receiver gain	101
Internal standard	DSS- <i>d</i> ₆
Internal standard purity	92.4% (Lot No. ELG6825; FUJIFILM Wako Pure Chemical Corporation)
Approximate weighed amount of phellamurin	Approximately 5 mg
Approximate weighed amount of DSS- <i>d</i> ₆	Approximately 2 mg
Phellamurin signal for quantification	δ 6.32 ppm (s, 1H; integral normalized to 1.00)
Internal standard signal for quantification	δ 0.00 ppm (s, 9H)

Sample preparation and acquisition parameters for ¹H-qNMR analysis of phellamurin.