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A New Aporphine Alkaloid from *Litsea glutinosa* to Attenuate Palmitate Induced Viability in MIN6 Cells

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Abstract: A new alkaloid called Litsine E (**1**) and three known aporphine alkaloids (**2-4**) were isolated from *Litsea glutinosa*. The structure of the new compound was established using spectroscopic techniques such as HMBC, HSQC, COSY, NOESY, and HRESIMS. Electronic circular dichroism (ECD) calculations were used to estimate its absolute configuration. Subsequently, the newly identified compound was evaluated for its potential to attenuate palmitate-induced viability, demonstrating a significant increase in viability in MIN6 cells.

Keywords: *Litsea glutinosa*; aporphine alkaloids; palmitate-induced viability.

1. Plant Source

The barks of *Litsea glutinosa* were harvested from Wenchang City, Tong Gu Ling in Hainan Province. The species was identified by Professor Yuguang Fan and, the voucher specimen (FHMU7692) was deposited in Herbarium of Hainan Medical University.

2. Previous Studies

L. glutinosa (Lauraceae) is a medium-sized, evergreen tree and is extensively found in subtropical South America, North America, and tropical and subarctic Asia. It grows mostly in the provinces of Yunnan, Guangdong, and Hainan in China. *L. glutinosa* has significant importance as a medicinal plant in China. Its leaves and roots are utilized for various purposes in medicine, including clearing dampness and heat, reducing swelling and poison, stopping bleeding, and relieving pain [1]. The plant exhibits a rich diversity of chemical compositions. Currently, more than 50 components, including alkaloids [2-3], flavonoids [4], and lipids [5-6], have been isolated and structurally characterized. Among these, alkaloids stand out as characteristic components. For example, a series of alkaloids were obtained from leaves through neutral alumina column chromatography [2]. Studies

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have consistently demonstrated that medicinal plants serve as valuable sources for drug development. Aqueous extracts of *L. glutinosa* have been formulated into anti-inflammatory and antibacterial agents [7-9].

3. Present Study

In the current study, a series of chromatographic techniques were employed to successfully isolate a new aporphine alkaloid from *L. glutinosa*. The determination of its structure was achieved through a combination of spectroscopic methods. Furthermore, its biological effect in attenuating palmitate-induced cell death viability was assessed in MIN6 cells.

The root bark was dried in a shaded area for one month (20.0 kg) and then broken up and extracted using ethanol under reflux for two hours each time. After that, the solvent was extracted from the crude extract using vacuum evaporation, which was carried until the smell of ethanol was eliminated. After adjusting the crude extract's pH to 2-3 with 1% H_2SO_4, it was extracted six times using ethyl acetate to eliminate liposoluble components. After that, the pH was adjusted to 10-11. This was accomplished by adding 1% sodium hydroxide solution. Following five repeated distributions of the extract in ethyl acetate, 68 g of the extract was isolated following concentration. After an aliquot of the whole alkaloid was subjected to column chromatography, it was eluted using a gradient of CH_2Cl_2-C_3H_6O (100:0 to 2:1, v/v). For every 875 milliliters, the eluting solvent was recovered and concentrated under reduced pressure, resulting in the TLC analysis-based collection of five fractions (FA-FE). Three primary fractions (FB1-FB3) were obtained by further purifying fraction B using column chromatography on a Sephadex LH-20 column and CH_3OH elution. Fraction FB1 was purified using semi-preparative HPLC and yielded compounds **1** (8.3 mg) and **2** (7.8 mg) when eluted with CH_3CN-H_2O (0.5% HCOOH and 0.1% $C_6H_{15}N$) (22:78). Fraction FB2 was also purified by semi-preparative HPLC under similar conditions, affording compounds **3** (8.6 mg) and **4** (4.6 mg).

Compounds **1-4** were isolated and purified from *L. glutinosa* using various spectroscopic techniques. Their structures were identified as Litsine E (**1**), Boldine (**2**) [10], Isoboldine (**3**) [11], and Launobine (**4**) [12]. Since compounds **1** and **4** were extracted from *L. glutinosa* for the first time, these results increased our understanding of the phytochemical components of *L. glutinosa*.

*Litsine E (**1**)*: Amorphous powder; $[\alpha]D^{20}$ +33.0 (*c* 0.1, MeOH); UV(MeOH) λ_{max} (logε) 226.0 (5.97), 270.8 (6.05), 310.8(6.11) nm; ^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 6.64 (1H, s, H-3), 2.52 (1H, br d, *J* = 15.6 Hz, H-4β), 2.73 (1H,m, H-4α), 2.20 (1H, m, H-5β), 2.91 (1H, m, H-5α), 3.01 (1H, m, H-6a), 2.20 (1H, m, H-7β), 3.22 (1H, br d, *J* = 13.2 Hz, H-7α), 6.90 (1H, d, *J* = 8.0 Hz, H-9), 6.80 (1H, d, *J* = 8.0 Hz, H-8), 3.65 (3H, s, 10-OCH_3), 6.04 and 5.90 (2H, s, -O-CH_2-O-), 7.11 (2H, d, *J* = 8.2 Hz, H-2', 6'), 6.72 (2H, d, *J* = 8.2 Hz, H-3',5'), 3.23 (1H, br d, *J* = 13.2 Hz, H-7'β), 4.11(1H, br d, *J* = 13.2 Hz, H-7'α); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 142.9 (C-1), 114.6 (C-1a), 129.0 (C-1b), 146.5 (C-2), 107.3 (C-3), 126.5 (C-3a), 29.3 (C-4), 48.5 (C-5), 60.5 (C-6a), 35.2 (C-7), 123.6 (C-7a), 116.2 (C-8), 122.8 (C-9), 149.7 (C-11), 145.7 (C-10), 128.7 (C-11a), 60.2 (10-OCH_3), 100.5 (-O-CH_2-O-), 129.5 (C-1'), 130.4 (C-2', 6'), 115.4 (C-3', 5'), 156.8 (C-4'), and 57.9 (C-7'). HRESIMS: *m/z* 418.1652 $[M + H]^+$, (calcd. *m/z*: 418.1654 for $C_{25}H_{24}NO_5$).

*Boldine (**2**)*: amorphous powder;^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 7.84 (1H, s, H-11), 6.72 (1H, s, H-8), 6.50 (1H, s, H-3), 3.77 (3H, s, 10-OCH_3), 3.55 (3H, s, 1-OCH_3), 2.94 (1H, m, H-7α), 2.91 (H, m,H-4β), 2.89 (H,m, H-5α), 2.83 (1H, d, *J*= 13.7 Hz, H-6a), 2.41 (3H, s, *N*-CH_3), 2.33 (1H, t, *J*= 11.8 Hz, H-5β), 2.22 (1H, t, *J*= 13.7 Hz, H-7β); ^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) = 149.3 (C-2), 146.1 (C-10), 145.9 (C-9), 142.7 (C-1), 129.5 (C-7a), 128.7 (C-3a), 126.3 (C-la), 125.3 (C-lb), 122.8 (C-11a), 115.3 (C-8), 114.1 (C-3), 112.0 (C-11), 62.3 (C-6a), 52.7 (C-5), 59.3 (1-OCH_3), 55.7 (10-OCH_3), 45.3 (*N*-CH_3), 33.6 (C-7), 28.4 (C-4) [10].

*Isoboldine (**3**)*: amorphous powder;^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 6.59 (1H, s, H-3), 2.94(1H, m, H-4α), 2.56 (1H, m, H-4β), 2.93 (1H, m, H-5α), 2.32(1H, m, H-5β), 2.80 (1H, dd, *J*= 13.6,

3.9 Hz, H-6a), 2.92 (1H, m, H-7α), 2.24 (1H, m,H-7β), 6.70 (1H, s, H-8), 7. 96 (1H, s, H-11), 3.79 (3H, s, 2-OCH3), 3.74 (3H, s, 10-OCH_3), 2.39 (3H, s, *N*-CH_3);^{13}C NMR (DMSO-d_6, 150 MHz): δ (ppm) =146.6 (C-2), 145.4 (C-9), 145.5 (C-10), 140.8 (C-1), 129.3 (C-7a), 126.9 (C-3a), 123.5 (C-11c), 123.2 (C-11a), 120.0 (C-11b), 115.1 (C-8), 113.7 (C-11), 109.4 (C-3), 62.5 (C-6a), 56.0 (10-OCH_3), 55.9 (10-OCH3), 53.0 (C-5), 43.8 (*N*-CH_3), 33.8 (C-7), 28.6 (C-4) [11].

Launobine ***(4)***: amorphous powder;^{1}H NMR (DMSO-d_6, 600 MHz): δ (ppm) = 6.83 (1H, d, *J*= 8.0 Hz, H-9), 6.78 (1H, d, *J*= 8.0 Hz, H-8), 6.67 (1H, s, H-3), 6.03,5.89 (2H, d, *J*= 8.6 Hz, -OCH_2O-), 3.65 (3H, s, -OCH_3), 2.82 (1H, m, H-4β), 3.18 (1H, m, H-4α), 3.27 (1H, m, H-5β), 3.56 (1H, m, H-5α), 2.62 (1H, m, H-7β), 2.73 (1H, m, H-7α); ^{13}C NMR (DMSO-d_6, 150 MHz):δ (ppm) = 149.2 (C-10), 146.3 (C-2), 145.4 (C-11), 142.4 (C-1), 129.3 (C-7a), 127.7 (C-3b), 126.0 (C-3a), 123.3 (C-8), 122.2 (C-11a), 115.7 (C-1a), 113.6 (C-9), 107.4 (C-3), 99.9 (-OCH_2O-), 59.7 (-OCH_3), 53.5 (C-6a), 41.9 (C-5), 36.5 (C-7), 28.3 (C-4) [12].

Compound **1** was separated as an amorphous powder. Positive HRESIMS was used to determine its chemical formula (*m/z* 418.1652 $[M + H]^+$, calculated to be $C_{25}H_{24}NO_5$ 418.1654). One methylenedioxy group at δ = 6.04 and 5.90 (s, O-CH_2-O) and one methoxy group at δ = 3.65 (s, 10-OCH_3) were found in the ^{1}H NMR spectrum. Signals at δ = 7.11 (d, *J* = 8.2 Hz, H-2', 6'), 6.72 (d, *J* = 8.2 Hz, H-3',5'), 6.90 (d, *J* = 8.0 Hz, H-9), 6.80 (d, *J* = 8.0 Hz, H-8), and 6.64 (s, H-3) were found for different groups of protons from the benzene ring in the aromatic area. One methine signal was detected at δ = 3.01 (m, H-6a) in the aliphatic region, and four methylene groups were detected at δ = 2.52 (m, H-4β), 2.73 (m, H-4α), 2.20 (m, H-5β), 2.91 (m, H-5α), 2.20 (m, H-7β), 3.22 (m, H-7α), 3.23(m, H-7'β), 4.11(br d, *J* = 13.5 Hz, H-7'α). Twenty-five carbon resonances were detected in the ^{13}C NMR spectrum, showing that at δ = 100.5, there is one methylenedioxy group present; two carbons are present in the benzene ring at δ= 123.6, 116.2, 122.8, 149.7, 145.7, 128.7, 129.5, 130.4, 115.4, 156.8, 57.9; one tetrahydroisoquinoline ring is present at δ = 142.9, 146.5, 107.3, 29.3, 48.5, 114.6, 129.0, 126.5, 60.5; a single methoxyl group is present at δ = 60.2; and a single methylene group is found at δ = 35.2. The above NMR signals are typical of an aporphine alkaloid [13–14]. The information presented leads one to the conclusion that compound **1** shares the same carbon skeleton as cassythine, as seen in Figure 1.

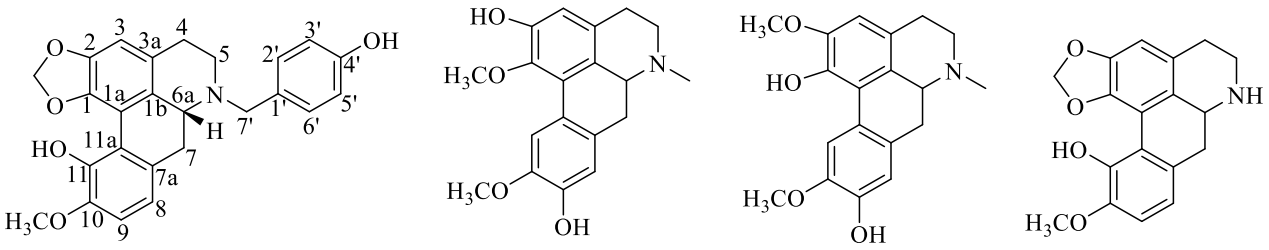
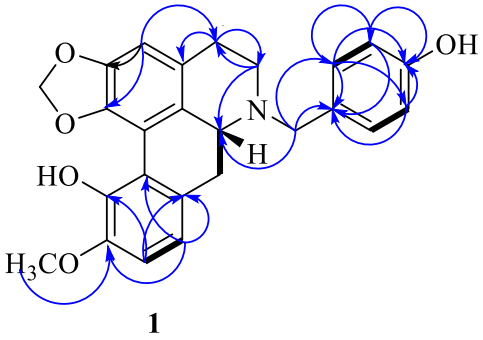


Figure 1. Structures of compounds **1-4** isolated from *L. glutinosa*

Four existing units were determined, according to the ^{1}H-^{1}H COSY associations found between H-8 and H-9, as well as between H-2', H-6' and H-3', H-5', and between H-7 and H-6a, as shown in Figure 2. In the HMBC spectrum, correlations were observed between 10-OCH_3 and C-10, as well as from H-8 to C-7a, C-11a, and C-10, establishing the structural linkage from C-7 to the phenyl ring. Furthermore, correlations were detected from H-9 to C-7a, C-11, and C-10, confirming confirmed the above moieties. Moreover, HMBC correlations from H-4 to C-3a and C-5 as well as from H-5 to C-6a, C-4, C-3a, and C-7' were noted, which established connections from C-4 to C-7. Additionally, correlations of H-3 with C-1, C-2, and C-4 confirmed the absence of substituent groups at positions C-3, 4, and 5. HMBC correlations from H-3 to C-3a and C-4 indicated the linkage from C-4 to the

benzene ring. These 2D NMR data confirmed that the skeleton of **1** was cassythine. Apart from these, the HMBC spectrum exhibited correlations from H-7' to C-1', C-2' C-6a, and C-5, as well as from H-2' and 6' to C-1', C-4', C-3', and C-7'. Moreover, H-3', 5' correlated with C-1', and C-4', confirming the presence of a benzyl group with a hydroxyl group at C-4' and a connection between C-7' and the *N* atom [15]. The location of 10-OCH_3 was further confirmed in the NOESY spectrum by the observed association between H-9 and 10-OCH_3. Based on the above analysis, the structure of the new compound was established, as shown in Figure 2.



1

Figure 2. HMBC (arrow) and ^{1}H-^{1}H-COSY (bold) data for **1**

By comparing the experimental and calculated circular dichroism curves depicted in Figure 3, the absolute configuration was established to be 6*R* [16-17]. Further confirmation of this result came from the specific rotation of **1** ([α]D^{20} +33.0). Finally, compound **1** was recognized as a new alkaloid and given the name Litsine E, as shown in Figure 1.

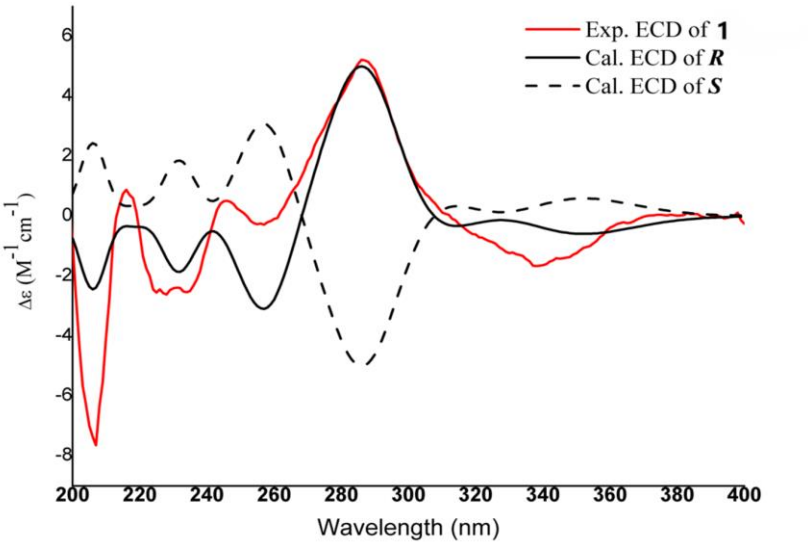


Figure 3. The experimental and calculated ECD spectra of **1**

Cell Viability Assay: Using prior research techniques as a guide [18-21], the capability of compound **1** to prevent palmitate-induced MIN6 cell death was tested. The research method is provided in the supporting information. Compound **1** considerably reduced the decline in viability induced by palmitate in MIN6 cells, as shown in Figure 4.

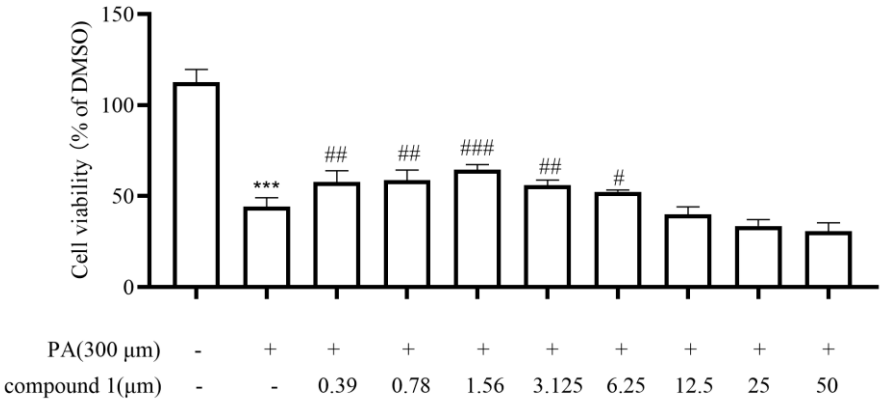


Figure 4. Viability of MIN6 cells treated with compound **1**
All data points represent the means ± SDs, *p < 0.05, **p < 0.01, ***p < 0.001 vs. DMSO; #p < 0.05, ##p < 0.01, ###p < 0.001 vs. PA.

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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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