**Leucas mollissima**, a Source of Bioactive Compounds with Antimalarial and Antimycobacterium Activities

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**Abstract**

A phytochemical investigation of the acetone extract from the aerial parts of *Leucas mollissima* afforded one new (-)-epi-marmelo lactone, (2S, 4R, 6S)-2,6-dimethyl-6-hydroxy-7-ene-4-olide (1), along with five known compounds, schensianol A (2), vanillin (3), β-hydroxy propiovanillone (4), lanost-9(11),25-diene-3β,24β-diol (5), and lanost-9(11),23E(24)-diene-3β,25-diol (6). Similarly, an investigation of the methanol extract of the aerial parts of *L. mollissima* resulted in the isolation of three known compounds, (+)-syringaresinol (7), anisofolin A (8), and apigenin 7-O-β-D-glucosyl-6″-O-β-D-(E-coumaroyl) glucose (9). Structure elucidation of the isolated compounds was carried out using detailed analysis of 1D and 2D nuclear magnetic resonance. All compounds were evaluated for antimalarial activity against *Plasmodium falciparum* (3D7) and for antimycobacterium activity against *Mycobacterium tuberculosis* H37Ra and *Mycobacterium bovis*. Compound 8 was found to have promising antimalarial activity (IC50 4.39 ± 0.25 µM), promising antimycobacterium activity (IC50 4.50 ± 0.75 µM (3.31 µg/mL)) against *M. tuberculosis* H37Ra and *M. bovis*, respectively, by comparing their NMR data with data from a previously reported article in which it was isolated.

**Key words**

*Leucas mollissima* · Lamiaceae · antimalarial · antimycobacterium · phytochemicals

**Supporting information** available online at http://www.thieme-connect.de/products

The genus *Leucas* from the family Lamiaceae comprises about 80 species [1]. In India, 43 species are available [2], of which 21 are found in the state of Maharashtra [3]. Plants of the genus *Leucas* have been widely employed by traditional healers to cure many disease conditions, which suggests that this genus has potential for the discovery of new drugs or lead molecules [1]. *Leucas mollissima* Wall. is distributed in India in the western peninsular, subtropical Himalayan region, and in the states of West Bengal and Orissa [4]. The juice from the leaf of this herb is applied externally to treat ailments relating to headache, while the decoction has been used orally to treat diabetes mellitus and liver diseases such as hepatitis [5]. In our continuing efforts to isolate bioactive compounds from plants found in the Western Ghats of Maharashtra for the development of new drugs active against infectious diseases such as malaria and tuberculosis, we report herein the isolation and structure elucidation of compound 1, (-)-epi-marmelo lactone, a new natural product, along with eight known compounds (2–9) (Fig. 2). Compounds 8 and 9 were evaluated for antimalarial activity against *Plasmodium falciparum* (3D7) and antimycobacterium activity against *Mycobacterium tuberculosis* H37Ra and *Mycobacterium bovis*. Compound 1 was isolated as yellow gum. The molecular formula was determined as C10H16O3 from HR-ESI-MS (Fig. 1, Supporting Information), which showed a pseudo-molecular peak at 207.0991 [M + Na]+ indicating three indices of hydrogen deficiency. This was supported by 13 C NMR (Table 1) spectral data. The 1H NMR spectrum (Table 1) showed one singlet methyl at δH 1.35, one doublet methyl at δH 1.30 (J = 7.3 Hz), two methines multiplets at δH 2.69 and 4.76, olefinic methines at δH 5.94 (dd, J = 17, 10 Hz), and methylene protons at δH 5.16 (d, J = 10 Hz) and 5.34 (d, J = 17 Hz). The 13C NMR (Table 1) and DEPT-135 (Fig. 4S, Supporting Information) spectra showed the presence of two methyls, three methylenes, three methines, and two quaternary carbons. Methylene at δC 5.16–5.34 (δC 112.9) and methine at δC 5.94 (δC 143.9) indicated the presence of a double bond as an olefinic end group, and one quaternary carbon at δC 179.4 showed the presence of a lactone carbonyl carbon. These data indicated 1 to be a monocyclic compound belonging to the lactone class. The structure of 1 was assigned by 2D NMR as follows: A proton at δH 2.69 (δC 33.8, H-2) and protons at δH 2.11 and 2.05 (δC 36.5, H-3) showed a heteronuclear multiple bond correlation (HMBC; Fig. 6S, Supporting Information) with a carbonyl at δC 179.4 (C-1) indicating that there was a lactone ring with one methine and one methylene. Protons at δH 1.98 and 1.80 (δC 46.9, H-5) showed an HMBC correlation with a carbon at δC 75.8 (δC 47.6, C-4) and with a quaternary carbon at δC 72.6 (C-6). Protons at δH 5.94 (δC 143.9, H-17), 5.34, and 5.16 (δC 112.9, H-8–8) showed an HMBC correlation with a carbon at δC 72.6 (C-6). Similarly, the proton at δH 1.35 (δC 28.7, H-10) showed an HMBC correlation with an unsaturated carbon at δC 143.9 (δC 5.94, C-7). These observations confirmed the presence of a side chain with an olefinic end group. The key HMBC correlations are shown in (Fig. 2). Correlation spectroscopy (COSY; Fig. 7S, Supporting Information) correlations observed between δH 1.30 (H-9–9) and 2.69 (H-2), δH 2.69 (H-2) and 2.11 (H-3), δH 2.11 (H-3) and 4.76 (H-4), and δH 4.76 (H-4) and 1.80–1.98 (H-5) supported the structure of 1 to be a lactone with a side chain (Fig. 2). The nuclear Overhauser effect spectroscopy (NOESY; Fig. 8S, Supporting Information) correlations observed between δH 2.69 (H-2) and δH 1.35 (H-10), δH 1.30 (H-9–9) and δH 2.11 (H-3), and δH 1.30 (H-9–9) and δH 4.76 (H-4) led us to assign the stereochemistry by placing methyl at the 2 position, β orientating, and the side chain at the 4 position, α orientating, relatively (Fig. 2). Compound 1 was found to have a negative specific rotation ([α]D20 ~ 81.90). Thus, 1 was found to be an epimer of the previously isolated and structurally similar marmelo lactone from the fruit of *Cydonia oblonga* Mill. (Rosaceae) [6], and hence identified as a new natural product, (2S, 4R, 6S)-2,6-dimethyl-6-hydroxy-7-ene-4-olide, belonging to the class of (-)-epi-marmelo lactones.

Compound 2 was identified as schensianol A by comparing its NMR data from a previously reported article in which it was isolated from *Euonymus schensianus* Maxim. (Celastraceae) [7]. Compounds 3 and 4 were identified as vanillin and β-Hydroxy propiovanillone, respectively, by comparing their NMR data with...
**Fig. 1** Compounds isolated from *L. mollissima*.

**Table 1**

<table>
<thead>
<tr>
<th>Carbon</th>
<th>$^1$C ($\delta_c$)</th>
<th>$^1$H ($\delta_h$)</th>
<th>HMBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>179.4</td>
<td>-</td>
<td>$H_1-2, H_2-3$</td>
</tr>
<tr>
<td>2</td>
<td>33.8</td>
<td>2.69 (1 H, m)</td>
<td>$H_2-3, H_3-9$</td>
</tr>
<tr>
<td>3</td>
<td>36.5</td>
<td>2.05 (1 H, m), 2.11 (1 H, m)</td>
<td>$H_3-9$</td>
</tr>
<tr>
<td>4</td>
<td>75.8</td>
<td>4.76 (1 H, m)</td>
<td>$H_2-5$</td>
</tr>
<tr>
<td>5</td>
<td>46.9</td>
<td>1.80 (1 H, m), 1.98 (1 H, m)</td>
<td>$H_2-10$</td>
</tr>
<tr>
<td>6</td>
<td>72.6</td>
<td>-</td>
<td>$H_2-5, H_3-10, H_4-7, H_4-8$</td>
</tr>
<tr>
<td>7</td>
<td>143.9</td>
<td>5.94 (1 H, dd, $J = 10, 17$ Hz)</td>
<td>$H_5-7$</td>
</tr>
<tr>
<td>8</td>
<td>112.9</td>
<td>5.16 (1 H, d, $J = 10$ Hz), 5.43 (1 H, d, $J = 17$ Hz)</td>
<td>$H_5-7$</td>
</tr>
<tr>
<td>9</td>
<td>15.8</td>
<td>1.30 (3 H, d, $J = 7.3$ Hz)</td>
<td>$H_6-2$</td>
</tr>
<tr>
<td>10</td>
<td>28.7</td>
<td>1.35 (3 H, s)</td>
<td></td>
</tr>
</tbody>
</table>

$m$: multiplet; $s$: singlet; $d$: doublet; $dd$: doublet of doublet

**Fig. 2** Key HMBC ($\rightarrow$), NOESY ($\leftrightarrow$), and COSY ($\leftarrow$) correlations of compound 1.
Table 2  In vitro antimalarial activity of compounds 8 and 9.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (µM) or (*µg/mL)</th>
<th>Average % growth inhibition (n = 3) (± standard deviation)</th>
<th>IC50 (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>10</td>
<td>102.2 ± 1.11</td>
<td>4.39 ± 0.25</td>
</tr>
<tr>
<td>9</td>
<td>10</td>
<td>35.29 ± 7.95</td>
<td>ND</td>
</tr>
<tr>
<td>LMM</td>
<td>1*</td>
<td>32.24 ± 3.62</td>
<td>ND</td>
</tr>
<tr>
<td>ATQ</td>
<td>1</td>
<td>100 ± 4.59</td>
<td>0.0082</td>
</tr>
</tbody>
</table>

ATQ = atovaquone (standard antimalarial compound); ND = not determined

those available in the literature [8, 9]. Compound 5 was identified as a lanost-9(11),25-diene-3β,24β-diol and compound 6 was identified to be a lanost-9(11),23E(24)-diene-3β,25-diol by comparison of literature NMR data and mass spectra reported for compounds isolated from *Mulgedium tataricum* (L.) DC. (Asteraceae) [10, 11]. Compound 7 was identified as (+) syringaresinol by comparing its NMR data with those available in literature [12]. Compound 8 was identified as apigenin 7-O-β-D-(3″-6″-p-E-dicoumaryl)-glucoside, Anisofolin A, by comparing its spectral data with those available in literature [13–15]. Compound 9 was identified as apigenin 7-O-β-D-(3″-6″-p-E-dicoumaryl)-glucoside by comparing its spectral data with the literature [16, 17].

Material and Methods

**General experimental procedures, chemicals, and biochemicals:** Optical rotations were measured using a JASCO P-1020 polarimeter. The 1H and 13C NMR spectra were recorded on a Bruker Avance III Ultra Shield NMR instrument (proton operating field strength: 400 MHz) at 25°C. LC-ESI-MS was recorded on a Waters Acquity LC-MS instrument. HR-ESI-MS using an Autocom-}

References

2. Khanam M, Hassan MA. A critical study of the genus *Leucas* R. Br. (Lam-}
3. Singh NP, Lakshminarasimhan P, Karkikeyan S, Prasanna PV. Flora of Mahara-

Supporting information

HR-ESI-MS, 1H, 13C, DEPT, and 2D NMR data of compound 1, the dose-response curve for compound 8, isolation of compounds 1–9, and biological screening protocols and NMR and other charac-

Conflict of Interest

The authors declare no conflict of interest.
9 Lou JR, Jiang HE, Zhao YX, Zhao J, Qian JF. Components of the heartwood of from an ancient tomb. Chem Nat Compd 2008; 44: 6–9