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, (2*S*, 4*R*, 6*S*)-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-3*β*,24*β*-diol (**5**), and lanost-9(11),23*E*(24)-diene-3*β*,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(− 6″-p-E-coumaroyl)-glucoside (**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 (IC_{50} 4.39 ± 0.25 µM), promising antimycobacterium activity [IC_{50} 4.50 ± 0.75 µM (3.31 µg/mL)] against *M. tuberculosis* H37Ra and at 100 µg/mL, showed 55.6% inhibition of *M. bovis*. Compound **9** showed moderate inhibition of *P. falciparum* growth (35% inhibition at 10 µM) with respect to the positive control atovaquone and 67.4% inhibition against *M. bovis* at 100 µg/mL with respect to the positive control rifampicin.

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. 1**). 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 $C_{10}H_{16}O_3$ from HR-ESI-MS (**Fig. 1 S**, 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 δ_H 1.35, one doublet methyl at δ_H 1.30 (J = 7.3 Hz), two methines multiplates at δ_H 2.69 and 4.76, olefinic methines at δ_H 5.94 (dd, J = 17, 10 Hz), and methylene protons at δ_H 5.16 (d, J = 10 Hz) and 5.34 (d, J = 17 Hz). The ^{13}C NMR (**Table 1**) and DEPT-135 (**Fig. 4 S**, Supporting Information) spectra showed the presence of two methyls, three methylenes, three methines, and two quaternary carbons. Methylene at δ_H 5.16–5.34 (δ_C 112.9) and methine at δ_H 5.94 (δ_C 143.9) indicated the presence of a double bond as an olefinic end group, and one quaternary carbon at δ_C 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 δ_H 2.69 (δ_C 33.8, H-2) and protons at δ_H 2.11 and 2.05 (δ_C 36.5, H_2-3) showed a heteronuclear multiple bond correlation (HMBC; **Fig. 6 S,** Supporting Information) with a carbonyl at δ_C 179.4 (C-1) indicating that there was a lactone ring with one methine and one methylene. Protons at δ_H 1.98 and 1.80 (δ_C 46.9, H_2-5) showed an HMBC correlation with a carbon at δ_C 75.8 (δ_H 4.76, C-4) and with a quaternary carbon at δ_C 72.6 (C-6). Protons at δ_H 5.94 (δ_C 143.9, H_1-7), 5.34, and 5.16 (δ_C 112.9, H_2-8) showed an HMBC correlation with a carbon at δ_C 72.6 (C-6). Similarly, the proton at δ_H 1.35 (δ_C 28.7, H_3-10) showed an HMBC correlation with an unsaturated carbon at δ_C 143.9 (δ_H 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. 7 S,** Supporting Information) correlations observed between δ_H 1.30 (H_3-9) and 2.69 (H-2), δ_H 2.69 (H-2) and 2.11 (H_2-3), δ_H 2.11 (H_2-3) and 4.76 (H-4), and δ_H 4.76 (H-4) and 1.80–1.98 (H_2-5) supported the structure of **1** to be a lactone with a side chain (**Fig. 2**). The nuclear Overhauser effect spectroscopy (NOESY; **Fig. 8 S**, Supporting Information) correlations observed between δ_H 2.69 (H-2) and δ_H 1.35 (H_3-10), δ_H 1.30 (H_3-9) and δ_H 2.11 (H_2-3), and δ_H 1.30 (H_3-9) and δ_H 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 ($[\alpha]_D^{26}$: − 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, (2*S*, 4*R*, 6*S*)-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

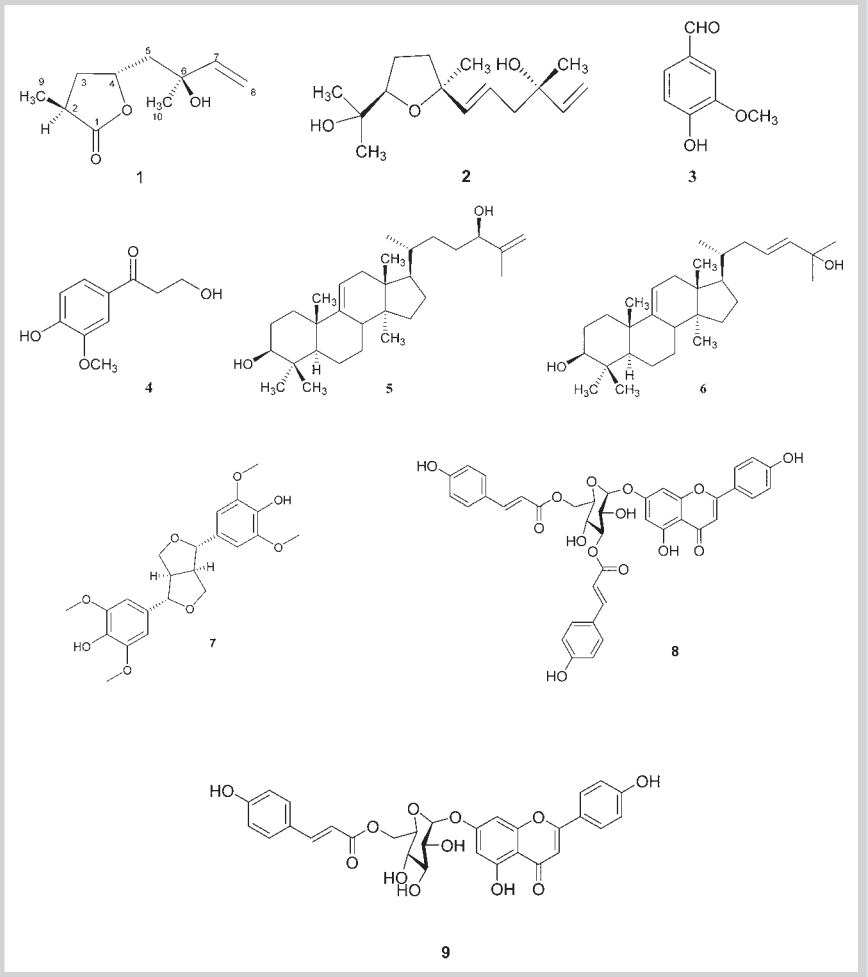

Fig. 1 Compounds isolated from *L. mollissima*.

Carbon	^{13}C (δ_C)	^{1}H (δ_H)	HMBC
1	179.4	–	H_1-2, H_2-3
2	33.8	2.69 (1 H, m)	H_2-3, H_3-9
3	36.5	2.05 (1 H, m), 2.11 (1 H, m)	H_3-9
4	75.8	4.76 (1 H, m)	H_2-5
5	46.9	1.80 (1 H, m), 1.98 (1 H, m)	H_3-10
6	72.6	–	H_2-5, H_3-10, H_1-7, H_2-8
7	143.9	5.94 (1 H, dd, *J* = 10, 17 Hz)	H_3-10
8	112.9	5.16 (1 H, d, *J* = 10 Hz), 5.34 (1 H, d, *J* = 17 Hz)	H_1-7
9	15.8	1.30 (3 H, d, *J* = 7.3 Hz)	H_1-2
10	28.7	1.35 (3 H, s)	

m: multiplate; s: singlet; d: doublet; dd: doublet of doublet

Table 1 ^{1}H (chloroform-*d*, 400 MHz), ^{13}C NMR (chloroform-*d*, 100 MHz), and HMBC data of compound **1**.

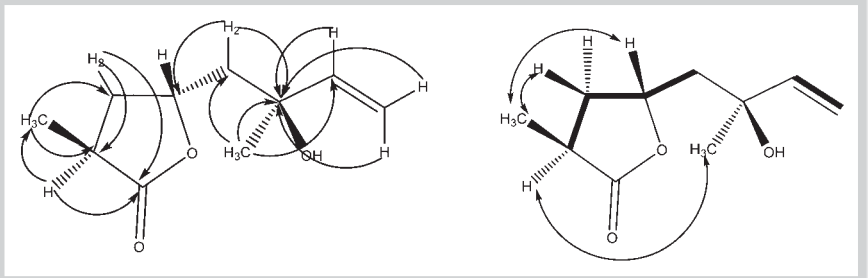

Fig. 2 Key HMBC (→), NOESY (↔), and COSY (—) correlations of compound **1**.

Table 2 *In vitro* antimalarial activity of compounds **8** and **9**.

Compound	Concentration (μM) or (*μg/mL)	Average % growth inhibition (n = 3) (± standard deviation)	IC_{50} (μM)
8	10	102.2 ± 1.11	4.39 ± 0.25
9	10	35.29 ± 7.95	ND
LMM	1*	32.24 ± 3.62	ND
ATQ	1	100 ± 4.59	0.0082

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-3*β*,24*β*-diol and compound **6** was identified to be a lanost-9(11),23*E*(24)-diene-3*β*,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 the literature [12]. Compound **8** was identified as apigenin 7-*O*-*β*-D(-3″,6″-p-E-dicoumaroyl)-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-(-6″-p-E-coumaroyl)-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 ^{1}H and ^{13}C 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 with a Waters Acquity LC-MS instrument. HR-ESI-MS using an Autoconcept mass spectrometer. Column chromatography was performed using silica gel, mesh 230–400 (Thomas Baker, Ltd.), and preparative thin-layer chromatography plates supplied by Merck Ltd. A Spectramax Plus 384 plate reader was used. Rifampicin and MTT were purchased from Sigma-Aldrich. Britelite plus reagent was purchased from Perkin Elmer. *M. tuberculosis* H37Ra (ATCC 25177) was obtained from MTCC, Chandigarh, India. *M. bovis* (ATCC 35745) was obtained from AstraZeneca, Bangalore, India. SybrGreen I nucleic acid stain was purchased from Life Technologies.

Plant material: L. mollissima, were collected from the mulshi area of Western Ghats, Pune, India on January, 2012 in full flowering season, shade dried, and pulverized. A herbarium voucher of this plant has been deposited in the Botanical Survey of India, Western Circle, Pune (Deposition No. SPJ-4).

Extraction and isolation: Pulverized aerial parts (1.09 kg) were extracted with acetone (3 L × 3 × 14 h) at room temperature. The acetone solubles were filtered and concentrated under reduced pressure to yield a greenish acetone extract, LMA (13.6 g, 1.24% based on dry weight of plant). The residual plant material was extracted with methanol (3 L × 3 × 14 h) at room temperature. The methanol solubles were filtered and concentrated under reduced pressure to yield a brownish methanol extract, LMM (47.5 g, 4.35%, based on dry weight of plant). The isolation of compounds **1**–**6** from the acetone extract and **7**–**9** from the methanol extract is provided in Supporting Information.

*(−)epi-marmelo lactone (**1**):* Gum; $[\alpha]_D^{26}$: − 81.90 (*c* 0.3% in $CHCl_3$); ^{1}H NMR (chloroform-*d*, 400 MHz) δ_H and ^{13}C NMR (chloroform-*d*, 100 MHz) δc are shown in **Table 1**; HR-ESI-MS: *m/z* $[M + Na]^+$ 207.0991 (calculated for $C_{10}H_{16}O_3$, 184.23).

Biological screenings: Antimalarial screening: A primary screening for compounds **1**–**9** was done as per standard protocols [18] at the 10 μM concentration, and for the crude mixture, a 1 μg/mL concentration was used. Protocol details are given in Supporting Information. Compound **8** was found to have significant antimalarial activity and was capable of completely inhibiting parasite growth at the 10 μM concentration. The IC_{50} was found to be 4.39 ± 0.25 μM compared to the positive control atovaquone, as shown in **Table 2**. However, compound **9** had only a moderate effect on parasite growth.

Antimycobacterial screening: Compounds **1**–**9** were evaluated for their *in vitro* inhibition effect against *M. tuberculosis* H37Ra (ATCC No. 25177) using the XTT Reduction Menadione Assay protocol and *M. bovis* (ATCC No. 35745) using the Nitrate Reductase assay protocol [19–21]. Protocol details are given in Supporting Information. Compound **8** was found to be active with an IC_{50} of 4.50 ± 0.75 μM (3.31 μg/mL) against *M. tuberculosis* H37Ra compared to the positive control rifampicin, with an IC_{50} of 0.0019 ± 0.0003 μg/mL. Compounds **8** and **9** at the 100 μg/mL concentration showed growth inhibition of 55.6% and 67.4%, respectively, against *M. bovis*.

Supporting information

HR-ESI-MS, ^{1}H, ^{13}C, 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 characterization data for compounds **2**–**9** are available as Supporting Information.

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Conflict of Interest

The authors declare no conflict of interest.

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