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Research Article

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A new pentacyclic triterpenoid from *Syzygium alternifolium*

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ABSTRACT

*A new pentacyclic triterpenoid, 3β-O-(E)-caffeoyl methyl oleanolate (**1**) has been isolated from the fruits of Syzygium alternifolium together with three known flavonoids, 5-hydroxy -7,4′-dimethoxy-6,8-di-C-methylflavone (**2**), kaempferol-3-O-β-D-glucopyranoside (**3**) and kaempferol-3-O-α-L-rhamnopyranoside (**4**). The structure of the new pentacyclic triterpenoid was elucidated by extensive 1D and 2D NMR techniques including ^{1}H-^{1}HCOSY, HSQC, HMBC and NOESY experiments.*

Keywords: *Syzygium alternifolium*, Myrtaceae, Roots, Pentacyclic triterpenoid, Flavonoids

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INTRODUCTION

The genus *Syzygium* which belongs to the family Myrtaceae comprises 500 species, of which 40 species occur in tropical India [1]. The genus Myrtaceae is a rich source for *C*-methylated flavonoids [2,3] and terpenoid constituents [4,5]. A few plants of this genus are extensively used in traditional medicine as antimicrobial, anti-inflammatory, antidiabetic, antiulcer, antianalagesic and antipyretic agents [6-11].

Syzygium alternifolium (Wight.) Walp. (Syn. *Eugenia alternifolia*) is an endemic and endangered deciduous evergreen tree [12] found in Seshachalam hill ranges of Andhra Pradesh, South India. As there is no record of any phytochemical work on the fruits of this species, we have investigated this part of the plant and report herein the isolation and structural elucidation of a new pentacyclic triterpenoid, 3*β*-*O*-(*E*)-caffeoyl methyl oleanolate (**1**), besides with three known flavonoids, 5-hydroxy-7,4′-dimethoxy-6,8-di-*C*-methylflavone (**2**), kaempferol-3-*O*-β-D-glucopyranoside (**3**) and kaempferol-3-*O*-α-L-rhamnopyranoside (**4**).

EXPERIMENTAL SECTION

General Experimental Procedures

Melting points were determined using a Kofler hot stage apparatus and are uncorrected. UV spectra were measured in MeOH on a Shimadzu UV-1800 spectrophotometer. IR spectra were recorded on a Bruker Alpha Eco ATR-FTIR spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 600 and 400 spectrometers operating at 600.19 and 400.13, and 150.93, 100.61, 75.47 MHz, respectively using DMSO with TMS as internal standard. ^{1}H-^{1}H COSY, HSQC, HMBC and NOESY spectra were obtained using standard pulse sequences. ESI-TOFMS was recorded in positive mode on an API Q-STAR PULSA of Applied Biosystem. Column chromatography (CC) was performed on silica gel (Acme) finer than 200 mesh (0.08 mm). TLC was performed on silica gel 60 F_{254} precoated plates (Merck).

Plant Material

The fruits of the *S. alternifolium* were collected from Seshachalam hill ranges of Andhra Pradesh, South India in June 2012 and identified by Dr. K. Madhava Chetty, Assistant Professor and Plant taxonomist, Department of Botany, Sri Venkateswara University, Tirupati, India where a voucher specimen (DG-117) has been deposited.

Extraction and isolation

Shade dried and powdered fruits (2.2 kg) of *S. alternifolium* was successively extracted with *n*-hexane (3 x 4L) and MeOH (3 x 4L) at room temperature. The concentrated MeOH extract (78 g) was segregated into *n*-hexane (2 x 2L), $CHCl_3$ (2 x 2L) and EtOAc (2 x 2L) soluble fractions. The $CHCl_3$ soluble fraction (24 g) on purification over a silica gel column using *n*-hexane-EtOAc step gradient (7:3 and 1:1) yielded **1** (14 mg) and **2** (16 mg). The EtOAc soluble fraction (42 g) was purified over a silica gel column using *n*-hexane-EtOAc step gradient to afforded **3** (15 mg) and **4** (17 mg) from 1:1 and 3:7 eluates, respectively.

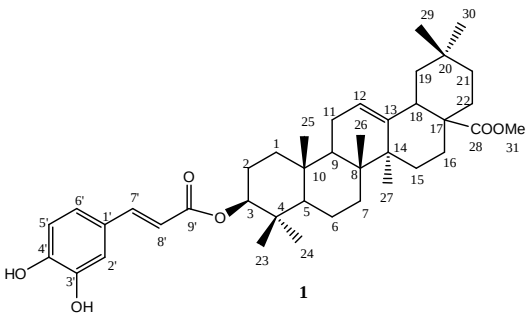
RESULTS AND DISCUSSION

Compound (**1**), isolated as white amorphous solid, gave positive reaction to the Libermann-Burchard and $FeCl_3$ reagents, characteristic of a triterpenoid and presence of phenolic hydroxyl group, respectively [13]. It showed $[M+H]^+$ ion peak at m/z 633.4124. in its positive ESI-TOFMS, consistent with the molecular formula $C_{40}H_{56}O_6$. This was corroborated by ^{13}C NMR spectrum which showed signals for all the 40 carbons present in the molecule. The IR spectrum showed characteristic bands at 3280 (OH), 1696, 1668 (>C=O), 1607, 1521, 1463 (Ar-C=C) and 1255,1188 (C-O).

The ^1H NMR spectrum of **1** showed seven tertiary methyl groups in the region δ 0.73-1.11 and a broad triplet at δ 5.16 corresponding to C-12 olefinic proton, typical of Δ^{12}-oleane skeleton [14,15]. This was confirmed by the ^{13}C NMR spectrum which showed signals in the region δ 15.0-32.8, and at δ 121.4 and 143.8 corresponding to seven tertiary methyl carbons, and carbons 12 and 13 of the Δ^{12}-oleane skeleton, respectively. In addition, it showed signals for ten methylene protons (δ 0.96-1.93), four methine protons (δ 4.49, 2.74, 1.55 and 0.96) and a methoxyl group at δ 3.36. The proton signal at δ 4.49 (dd, J = 11.3, 4.12) was assigned to an oxymethine proton at C-3, as it showed cross peaks with both Me-23 and Me-24 in its HMBC spectrum.

The ^1H NMR spectrum of **1** further showed a typical ABX spin system in the aromatic ring at δ 6.74 (1H, d, J = 8.12 Hz), 6.96 (1H, dd, J = 8.12, 2.0 Hz) and 7.02 (1H, d, J = 2.0 Hz) together with trans coupled (J = 15.8 Hz) protons at δ 6.20 (H-8′) and 7.43 (H-7′) characteristic of a caffeoyl moiety [16] in **1**. The placement of the caffeoyl moiety was confirmed by the cross peak of the oxymethine axial proton at δ 4.49 (H-3) with the carbonyl carbon signal at δ 166.2 (C-9′) in its HMBC spectrum (Fig. 1), indicating that the caffeoyl moiety was etherified with C-3 position of oleanolic acid [16]. The methoxyl singlet at δ 3.36 which showed long range correlation with the carbonyl carbon (C-28) at δ 178.5 in the HMBC spectrum indicates the presence of a carboxy methyl group at C-17 position. The assignments of seven tertiary methyl, ten methylene groups and the remaining ^1H and ^{13}C signals were further confirmed by HMBC, HSQC and NOESY experiments.

Thus from the foregoing spectral studies the structure of compound **1** was established as, 3*β*-*O*-(*E*)-caffeoyl methyl oleanolate.



1

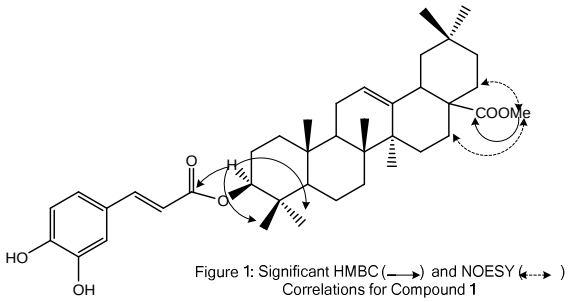


Figure 1: Significant HMBC (⟶) and NOESY (⟷) Correlations for Compound **1**

3*β*-*O*-(*E*)-Caffeoyl methyl oleanolate (1)
White amorphous solid ($CHCl_3$), UV λ_{max} nm (MeOH) (log ε): 211 (4.58), 265 (4.24), 323 (4.40); IR ν_{max} (KBr) cm^{-1}: 3278 (-OH), 2939, 2854, 1696, 1668 (>C=O), 1607, 1521, 1464 (Ar-C=C), 1372, 1301, 1255, 1188 (C-O), 1109, 1018, 967, 852, 806, 738, 640; ^{1}H NMR (400 MHz, DMSO-d_6) : δ 7.43 (1H, d, *J*=15.80, H-7′), 7.02 (1H, d, *J*=2.0, H-2′), 6.96 (1H, dd, *J*=8.12, 2.0, H-6′), 6.74 (1H, d, *J* = 8.12, H-5′), 6.20 (1H, d, *J*=15.80, H-8′), 5.16 (1H, brt, *J* = 8.12, H-12), 4.49 (1H, dd, *J*=11.38, 4.12 Hz, H-3), 3.36 (3H, s, OMe-31), 2.74 (1H, dd, *J*=13.66 & 3.72Hz, H-18), 1.93 (2H, m, H_a-11,16), 1.83 (1H, m, H_b-11), 1.63 (4H, m, H_a-2,15, 19 & 22), 1.55 (3H, m, H_a-1, H_b-2 & H-9), 1.42 (4H, m, H_a-6, H_a-7, H_b-16 & H_b-22), 1.31 (2H, m, H_b-6, H_a-21), 1.24 (1H, d, *J*=12.04 Hz, H_b-7), 1.13 (1H, m, H_b-21), 1.11 (3H, s, Me-27), 1.05 (2H, d, *J*=13.84 Hz, H_b-1 & H_b-19), 0.96 (2H, m, H-5, H_b-15), 0.91 (3H, s, Me-25), 0.88 (3H, s, Me-26), 0.87 (6H, s, Me-23 & 29), 0.83 (3H, s, Me-30), 0.73 (3H, s, Me-24); ^{13}C NMR (75 MHz, DMSO-d_6) : δ 178.5 (C-28), 166.2 (C-9′), 148.2 (C-4′), 145.5 (C-3′), 144.7 (C-7′), 143.8 (C-13), 125.5 (C-1′), 121.4 (C-12), 121.2 (C-6′), 115.6 (C-5′), 114.7 (C-2′), 114.4 (C-8′), 79.7 (C-3), 54.6 (C-5), 51.7 (C-31), 46.8 (C-9), 45.6 (C-17), 45.4 (C-19), 41.3 (C-14), 40.7 (C-18), 40.3 (C-8), 37.5 (C-4), 37.4 (C-1), 36.5 (C-10), 33.3 (C-21), 32.8 (C-29), 32.2 (C-7), 32.0 (C-22), 30.3 (C-20), 27.8 (C-23), 27.2 (C-15), 25.5 (C-27), 23.3 (C-30), 23.2 (C-2), 22.9 (C-11), 22.6 (C-16), 17.8 (C-6), 16.7 (C-24 & C-26), 15.0 (C-25); ESI-TOFMS (positive ion mode) *m/z* (rel.int.): 633.4124 $[M+H]^+$ (calcd. for $C_{40}H_{56}O_6$+H, 633.414).

By comparison of the spectral data with literature values, the known compounds were identified as 5-hydroxy-7,4′-dimethoxy-6,8-di-*C*-methylflavone (**2**) [17], kaempferol-3-*O*-β-D-glucopyranoside (**3**) [18] and kaempferol-3-*O*-α-L-rhamnopyranoside (**4**) [19] by comparison of the spectral data with literature values.

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