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Two rare antioxidant and anti-inflammatory oleanenes from loop root Asiatic mangrove *Rhizophora mucronata*

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ABSTRACT

Two oleanenes, olean-18(19)-en-3β-yl-(3,6-dimethyl-3*E*,6*Z*-dienoate) and (13α)-27-*frido*-olean-14(15)-en-(17α)-furanyl-3β-ol representing a class of rare natural pentacyclic triterpenoids were isolated from the chloroform extract of Asiatic mangrove, *Rhizophora mucronata* Lam. (Family: Rhizophoraceae). The furanyl oleanene exhibited significantly greater antioxidative activities (IC_{50} 0.73–0.76 mg/mL), than prenylated oleanene (IC_{50} 0.84–0.96 mg/mL) ($P < 0.05$). No significant differences in anti-5-lipoxygenase activities of these compounds with the synthetic drug ibuprofen was discernable (IC_{50} 0.8–0.9 mg/mL), whilst furanyl oleanene demonstrated significantly greater anti-cyclooxygenase-2 (IC_{50} 0.84 mg/mL) and anti-5-lipoxygenase activities (IC_{50} 0.78 mg/mL) over prenylated oleanene (IC_{50} > 0.90 mg/mL). These compounds exhibited lesser activity against cyclooxygenase-1 than cyclooxygenase-2 isoform, and therefore, their selectivity indices remained significantly greater (anti-cyclooxygenase-1_{IC50}/anti-cyclooxygenase-2_{IC50} > 1) than the aspirin (0.02) and ibuprofen (0.44). The lipophilic and steric molecular descriptors were found to occupy a prominent role in determining the bioactivities of the compounds. These previously undescribed oleanenes might serve as potential antioxidative and anti-inflammatory lead molecules in medicinal formulations and food industries.

1. Introduction

Mangrove plant extracts have been found to be rich sources of proven activities against human, animal and plant pathogens, and were used in folklore medicines (Bandaranayake, 2002). These plants possess specially modified morphological structures and physiological mechanisms to deter the adverse states in their harsh surroundings, such as salinity and anoxic soil conditions. These group of plants was reported to harbor valuable chemical compounds of medicinal importance (Nebula et al., 2013; Joel and Bhimba, 2010). *Rhizophora mucronata* Lam. (family Rhizophoraceae) is a true mangrove and is extensively distributed along the coastal region of India. It is a well known medicinal plant (Khare, 2007), and has been popularly used as traditional medicine in the treatment of diarrhea, dysentery, blood in urine, fever, angina, and diabetes (Kusuma et al., 2011). Medicinal properties like anti-diabetic, antioxidant, antimicrobial and anticholinesterase activities of the crude solvent extracts derived from *R. mucronata* have been reported in the earlier literature (Gaffar et al., 2011; Imdadul et al., 2011; Joel and Bhimba, 2010; Ravikumar and Gnanadesigan, 2012; Suganthy and Devi, 2015). The previous studies on *R. mucronata* revealed that presence of prenylated terpenoids, carbohydrate, polysaccharides, carotenoids, tannins, inositols, lipids, gibberellins, anthocyanidins, alkaloids, flavonoids, proteins, saponins, minerals, polyphenols, and procyanidins (Bandaranayake 2002; Raola and Chakraborty, 2016).

Among various naturally derived secondary metabolites, triterpenoids were considered as the multi-target paradigm, with potential and larger group of bioactive pharmacophores, and greater than 15000 compounds belonging to dammarane-euphane, lanostane, oleanane, hopane, ursane, etc were reported in literature (Li et al., 2013). Triterpenoids were reported to possess analgesic, anti-inflammatory, immunomodulatory, anticancer, and antimicrobial activities. Among various groups of triterpenoids, the oleananes were found to possess a wide range of potential bioactivities against various diseases. Oleanane triterpenoids was found their place in the prevention and therapy of breast cancer (Parikh et al., 2014). Oleanolic and α-boswellic acids isolated from *Sambucus chinensis* and *Boswellia serrata*, respectively are prominent

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examples of bioactive triterpenoids with potential anti-inflammatory and antitumor activities (Dzubak et al., 2005). Anti-inflammatory activities of oleanene and ursene group of triterpenoids have been reported in detail (Gupta et al., 1969). Notably, a close relation between the concentration of free radicals in the metabolic system *vis-a-vis* recruitment of pro-inflammatory mediators was reported in previous literature (Balkwill and Mantovani, 2012), and the anti-inflammatory properties of the triterpenoids were ascribed to their ability to inhibit pro-inflammatory enzymes, such as 5-lipoxygenase (Dzubak et al., 2005).

Anti-diabetic, antioxidant, and antimicrobial activities of the crude solvent extracts from *R. mucronata* have been reported in the earlier work (Gaffar et al., 2011; Imdadul et al., 2011), although there were few studies related to the characterization of bioactive triterpenoids from the mangrove species. In the course of a search for bioactive agents from Loop-root Asiatic mangrove, *R. mucronata*, we presented the isolation and structure elucidation of previously undescribed oleanane triterpenoids, as well as an evaluation of their *in vitro* antioxidative and anti-inflammatory activities were described. Structure-activity relationship analysis was used to correlate different physicochemical parameters that significantly contribute towards the target bioactivities of the title compounds.

2. Results and discussion

2.1. Spectral analyses of oleanane triterpenoids from R. mucronata

The chloroform-soluble fraction of the leaves of *R. mucronata* Lam. was fractionated by repeated column chromatography over silica gel to afford two previously undescribed oleanenes, olean-18(19)-en-3β-yl-(3,6-dimethyl-3,6-dienoate) (**1**) and (13α)-27-*frido*-olean-14(15)-en-(17α)-furanyl-3β-ol (**2**) representing a class of rare natural pentacyclic triterpenoids bearing *cis*-fused C/D rings. These compounds were the first examples of *frido*-oleanen-furanyl and 3β-dimethyldienoate substituted oleanene triterpenoids.

Compound **1**, a derivative of prenylated olean-18-ene triterpenoid, was isolated as a yellow crystalline solid. The ^{1}H NMR spectrum (Table 1) displayed eight singlets at δ 0.73 (assigned as H-27), 0.85 (H-24), 0.87 (H-23), 0.93 (H-30), 0.95 (H-29), 0.97 (H-26), 1.04 (H-28) and 1.10 (H-25), each with three proton integrals, and were situated at the quaternary carbons. These assignments were characteristic for an olean-18-ene triterpenoid germanicol (Awan et al., 2013; Hassan et al., 2012; Raola and Chakraborty, 2016; Yang et al., 2006). An additional two methyl signals were detected as singlets at δ 1.14 (H-39) and 1.68 (H-40) related to the carbons at δ 26.96 (C-39) and δ 19.31 (C-40), respectively, which were assigned to be situated in the side chain. The structural assignments were carried out by comparing the NMR data of **1** with those of previously reported olean-18-ene triterpenoids (Raola and Chakraborty, 2016; Yang et al., 2006). The deshielded resonance of H-3 (δ 4.51, dd, J = 10.5, 5.51 Hz) in the ^{1}H NMR spectrum of **1** suggested that the C-3 hydroxyl group has been acylated, and therefore, an acyl substitution at C-3 was apparent. This was further validated by the intense HMBC correlation between H-3 (δ 4.51) and the C-31 carbonyl carbon at δ 173.53 (Raola and Chakraborty, 2016).

The IR spectrum revealed the vicinity of a carbonyl (1739.85 cm^{-1}) and olefinic (1558.54 cm^{-1}) groups. Its molecular formula, $C_{40}H_{64}O_2$, was deduced from the HR-ESIMS (m/z 577.4992 $[M+H]^+$; D 0.0 amu) and ^{13}C NMR spectroscopic data, showing nine indices of hydrogen deficiency. The ^{13}C NMR chemical shifts displayed the characteristic double bond at δ 142.62 (assigned to C-18), δ 129.75 (C-19) alongside the olefinic proton signal at δ 4.87 (H-19). These chemical shifts were observed to be similar to those of the reported olean-18-ene (Osorio et al., 2012) displaying olefinic signature peaks (Raola and Chakraborty, 2016; Yang et al., 2006). In the ^{1}H-^{1}H COSY spectrum couplings were apparent between δ 5.34 (H-34)/δ 2.80, 2.77 (H-35) and δ 5.19 (H-37)/δ 1.87 (H-38) (Fig. 1A), which supported the vicinity of dimethyloctadienoate moiety. Auxiliary olefinic bond at δ 145.16 (assigned to C-36) was affirmed by the strong HMBC correlations of δ 1.14 (H-39)/C-36; δ 2.80 (H-35) and δ 5.19 (H-37) with C-36. It is intriguing to note that the side chain 33, 36-dimethylocta-33, 36-dienoate moiety has been shaped due to the two isoprene units orchestrated in a “head to tail” style. The signature peak at δ 4.51 has been relegated to H-3 (1H, dd), whereas the chemical shift encountered a downfield shift from the typical value of δ 3.20, potentially because of the vicinity of the >C═O group (assigned to C-31) at its region. This attachment was affirmed by the HMBC correlations between δ 4.51 (assigned to H-3) and δ 173.53 (assigned to carbonyl carbon). A large vicinal coupling constant of H-3 ($J_{2ax,\ 3}$ = 10.5 Hz) demonstrated the equatorial configuration of the hydroxyl groups at this position. The geometric isomerisms of the olefinic proton at δ 5.30 (H-34) had a large coupling constant (J = 12.01 Hz), which uncovered the *E* configuration of the olefinic bond, though the olefinic proton at δ 5.19 (relegated to H-37) recorded a lesser coupling constant (J = 3.78 Hz), demonstrating the *Z* configuration for C-36 olefinic bond. The stereochemistry of the chiral centre at δ 80.51 (assigned to C-3) was set up from the NOESY spectra, though the proton at δ 4.51 (dd, J = 10.5, 5.51, H-3) demonstrated common NOE relationships with δ 0.83 (assigned to H-5) and δ 0.87 (assigned to H-23), which was at the α-face of the molecule (axial configuration) (Fig. 1B) (Osorio et al., 2012). The spatial arrangement of the angular methyl groups at δ 0.85 (H_3-24), 1.08 (H_3-25), 0.97 (H_3-26), 1.02 (H_3-28), 0.93 (H_3-30), 1.14 (H_3-39) were β-disposed and 0.87 (H_3-23), 0.73 (H_3-27), 0.95 (H_3-29), 1.68 (H_3-40) were at the α-side of the reference plane of the compound. These attributions were identical to the assignments of germanicol (Awan et al., 2013; Hassan et al., 2012; Kaennakam et al., 2013; Li et al., 2014; Osorio et al., 2012; Yang et al., 2006).

The ring frameworks of B, C, D and E have been built up by ^{1}H-^{1}H-COSY correlations between δ 1.53,1.36 (H-6)/δ 1.34,1.49 (H-7); δ 1.29 (H-9)/δ 1.55 (H-11)/δ 1.77,1.50 (H-12)/δ 2.00 (H-13); δ 2.01,1.79 (H-15)/δ 1.44,1.23 (H-16) and δ 1.48,1.10 (H-21)/δ 1.76,1.72 (H-22) (Fig. 1A), and comparison with the literature values (Osorio et al., 2012; Raola and Chakraborty, 2016). The designations of the rest proton and carbon signals of D and E rings were accomplished by the HSQC and HMBC correlations (Table 1) thereby affirming the pentacyclic structure. Based on these spectroscopic results, the structure of compound **1** was determined to be olean-18(19)-en-3β-yl-(3, 6-dimethyl-3*E*, 6*Z*-dienoate).

Molecular formula $C_{40}H_{64}O_2$ of **1** was established with the help of mass spectral data and molecular ion peak appeared at m/z 577. In EIMS, fragmentation peak appeared at m/z 409 [M^+-167] indicating the presence of a dimethylocta-33, 36-dienoic moiety. The base peak at m/z 189 was due to C-ring cleavage from the parent molecule at m/z 409. The appearance of a base peak at m/z 189 was in accordance with the literature values of oleanane triterpenoid (Madureira et al., 2004). The mass fragmentation pattern for oleanene derivatives was shown in Fig. 3A. The molecular ion peak at m/z 577 (**1a**, $C_{40}H_{64}O_2^+$, $[M]^+$) appeared to undergo elimination of 3,6-dimethylocta-3,6-dienoic acid (**1c**, m/z 168) to yield olean-2(3), 18(19)-dien (**1b**) (m/z 408), which underwent Retro-Diels-Alder mechanism to afford a fragment with m/z 326 (tetradecahydro-hexamethylchrysene). The ion at m/z 190 (hexahydro-tetramethylnaphthalene) was from the ion at m/z 246 (dodecahydro-tetramethylphenanthrene) by Retro-Diels-Alder mechanism. The former yielded ion at m/z 138 (decahydronaphthalene). The fragment ions at m/z 298 and m/z 108 (1, 4-dimethylcyclohexa-1, 3-diene) also substantiated the structure of the title compound. The

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