Two rare antioxidative prenylated terpenoids from
loop-root Asiatic mangrove Rhizophora mucronata (Family
Rhizophoraceae) and their activity against pro-inflammatory
cyclooxygenases and lipoxidase

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ABSTRACT
Two new biogenic prenylated terpenoids were isolated from the methanol
extract of Rhizophora mucronata. The extended C20 sesquiterpenoid
with prenylated guaiane framework was characterised as (4E, 8Z)-3,3a, 6, 7-tetrahydro-3, 9-dimethyl-5-(6-methylheptan-2-yl) cycloocta[b] furan-2-(9aH)-one (1). (35E)-1,2,3,5,6,6-icosahydro-4,4,8b,10,14,17,20,20-octamethylpicen-3-yl-34,35-dimethyloct-31-enoate (2) represents
the first example of naturally occurring C40 prenylated oleanane-type
triterpenoid, whereas one 4,5-dimethyloct-5-enoate side chain remains
attached at C-3 position of the oleanane framework formed by the E-ring
closure of C30 saccharide moiety. The structures of the compounds were
elucidated using NMR and mass spectrometric analysis. Compound 1 was
found to have significantly greater antioxidant activities (IC50 ~ 0.75 mg/
ml) compared to 2 (IC50 > 0.80 mg/mL). No significant differences in
anti-cyclooxygenase-2 of these compounds were discernable (IC50
0.8 – 0.9 mg/mL), whilst compound 1 showed greater anti-5-lipoxidase
activities (IC50 ~ 0.8 mg/mL) those that of 2 (IC50 0.96 mg/mL). Bioactivities
of the prenylated terpenoids were inversely proportional to lipophilic
and bulk descriptors.

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1. Introduction

Mangrove plants are rich source of secondary metabolites such as steroids, alkaloids, phenolics, triterpenes, saponins, tannins and flavonoids, which were reported to have toxicological, pharmacological and ecological importance. These species have greater capacities of salt tolerance as they adapted to live in anoxic conditions in the coastal ecosystems (Nebula et al. 2013). *Rhizophora mucronata* Lam. (family Rhizophoraceae) is one of the predominantly available mangrove plants grown in the coastal regions of India. It has been used as traditional medicine in the treatment of diabetes, diarrhoea, dysentery, blood in urine, fever, angina, diabetes and the Indo-Chinese use, and the roots for angina and haemorrhage were well documented. In India, bark used for diabetes and old leaves used as decoction at childbirth (Kusuma et al. 2011), and was reported for bioactive triterpenoids, such as 4-methoxy cinnamoyl-15-hydroxy-β-amyрин, adian-5-en 3-ol, lupeol (Rohini & Das 2010), pentacyclic triterpenoids from *R. stylosa* (Li et al. 2008) and secolabdane diterpenoids from *R. mucronata* (Anjaneyulu & Rao 2001). Earlier *in vitro* studies revealed that extract of *R. mucronata* demonstrates the significant anti-inflammatory and anti-arthritic activities due to the presence of active principles, such as triterpenoids, alkaloids, polyphenolic content and flavonoids (Kumari et al. 2015). Polysaccharide extracts from the leaves were reported for anti-HIV activity (Premanathan et al. 1999). However, there are no reports of the occurrence of naturally occurring antioxidative and anti-inflammatory prenylated type sesqui- and triterpenoids from this mangrove species. The present study was aimed to isolate and characterise two rare biogenic prenylated terpenoids from the methanol extract of *R. mucronata* and their evaluation for antioxidative and anti-inflammatory properties by various *in vitro* models. These terpenoids included one new prenylated guaiane sesquiterpenoid with an uncommon five-membered lactone ring, (4E,8Z)-3,3a,6,7-tetrahydro-3,9-dimethyl-5-(6-methylheptan-2-yl) cycloocta[b]furan-2(9aH)-one (1) and prenylated oleanane-type triterpenoid, designated as (35E)-1,2,3,5,6,6-icosahydro-4,4,8b,10,14,17,20,20-octamethylpicen-3-yl-34,35-dimethyloct-31-enoate (2). Structure–activity relationship analysis was used to correlate different physicochemical parameters that significantly contribute towards the target bioactivities of the prenylated terpenoids. This study established the potential of prenylated terpenoids as potential lead molecules for use in pharmaceutical and functional food industries.

2. Results and discussion

2.1. Chromatographic purification and spectral analysis of secondary metabolites from *R. mucronata*

Compound 1, a new derivative of the prenylated C₂₀ guaiane sesquiterpenoid, was isolated as greenish oil upon repeated chromatography using neutral alumina as adsorbent. Its mass spectrum exhibited a molecular ion peak at m/e 304 (HRESIMS m/e 304.2638 [M + H]+), which in combination with its ¹H and ¹³C NMR data indicated the elemental composition as C₂₀H₃₂O₂ with 5° of unsaturation. The ¹³C NMR of 1 in combination with DEPT 135 recorded five each of methyl and methylene signals, along with seven methine carbons (Figure S1). Couplings were apparent between δ 2.29 (assigned as H-2)/δ 1.64 (H-3)/δ 5.11 (H-4); δ 2.01 (H-6)/δ 2.06 (H-7)/δ 5.15 (H-8) and δ 4.50 (H-10)/δ 1.64 (H-3) in the ¹H–¹H COSY spectrum of 1, which support the presence of 1,3-olide (lactone) moiety with two double bonds at C4 and C9 positions of the prenylated guaiane framework skeleton (Figure 1(A), Figure S2). The methine proton (gives doublet) at δ 4.50 is characteristic of the junction point of the two rings, which
was similar to the literature value $\delta 4.49$ (Luyen et al. 2013). The $^1$H–$^1$H COSY correlations between $\delta 1.97$ (assigned to H-11)/$\delta 1.52$ (H-12)/$\delta 0.98$ (H-13)/$\delta 1.37$ (H-14)/$\delta 1.29$ (H-15) and $\delta 0.87$ (H-16), $\delta 0.90$ (H-20) support the 2-methylheptane skeleton (Figure 1(A)). The $^1$H–$^1$H and $^{13}$C–$^1$H connectivities apparent in the $^1$H–$^1$H COSY and HMBC spectra, respectively, indicate that three of the five unsaturations in 1 were due to the double bond, and the others were due to two rings. In the HMBC spectrum, it was observed that $\delta 2.29$ (H-2)/C-1, 3, 20; $\delta 5.11$ (H-4)/C-3; $\delta 2.06$ (H-7)/C-8, 6; and $\delta 1.69$ (H-18)/C-9, 8 were correlated with each other (Table S1, Figure 1(A)). In addition, a methine proton $\delta 5.15$ (H-8) was coupled to the olefinic tertiary carbon $\delta 135.19$ (C-9) and methine proton at $\delta 4.50$ (H-10) connected to C-9. This indicated that these protons (H-8 and H-10) were connected to the olefinic tertiary carbon atoms. 3° of unsaturation along with the $^1$H and $^{13}$C NMR spectra suggested that 1 was a prenylated guaiane sesquiterpenoid. The characteristic olefinic (C=C) and carbonyl (–C=O) groups IR stretching vibrations were represented by the 1652 and 1733 cm$^{-1}$ absorption bands, respectively, which further reinforced the attributed structure. The mass spectrum showed a base peak at $m/z$ 191 ($C_{12}H_{15}O^-$) by loss of $m/z$ 113 ($C_8H_{17}^-$) assigned to the 2-methyl heptane moiety (Figure S3). The low field methine signal (DEPT$_{135}$) at $\delta$ 80.51 was in agreement with that to a carbon signal adjacent to a lactone group (Han et al. 2010). This was also supported by the relatively downfield shift of the proton signal appeared at $\delta$ 4.50 (assigned to H-10) and methine multiplet at $\delta$ 2.29 (assigned to C-2), which was referred to a possible oxygenation in its vicinity. The position of the carbonyl group at C-1 ($\delta$ 173.67) was further confirmed by the long-range C–H correlations in the HMBC spectra. The protons at $\delta$ 4.50
(t, J = 4.8 Hz) and δ 2.29 (m, 1H) exhibited HMBC correlation with the carbon signal at δ 173.67 (assigned to C-1). The relative stereochemistries of the chiral centres of 1, particularly that of C-10 carrying the methine proton at δ 4.50 (d, 1H), were deduced from the NOESY spectrum of the compound and the J-values. NOE correlations between the protons at δ 4.50 (Hβ-10)/δ 2.29 (Hβ-2) indicated the close proximity of these groups and their β-disposition, whereas methyl group at C-17 was α-orientation (Han et al. 2010, Luyen et al. 2013). Further couplings were observed between the protons at δ 1.64 (H-3)/δ 1.97 (Hα-11) and δ 1.29 (Hα-15), thus indicating that these groups must be equatorial and on the α-side of the molecule.

Compound 2, a new derivative of the naturally occurring C40 prenylated oleanane-type triterpenoid, was isolated as yellow amorphous powder upon repeated column chromatography using silica gel as adsorbent. Its mass spectrum exhibited a molecular ion peak at m/e 579 (HRESIMS m/e 579.5264 [M + H]+; D 0.0 amu), which in combination with its 1H and 13C NMR data indicated the elemental composition as C40H66O2. It satisfied 8° of unsaturation in which 3° of unsaturation were due to the double bonds, whilst 5° of unsaturation were from the ring systems. The 1H NMR spectrum showed signals for eight methyl groups, which were positioned at the quaternary carbons as singlets at δ 0.73, 0.83, 0.85, 0.89, 0.93, 0.95, 0.97, 1.02 and 1.08 (Table S1). These assignments were found to be close with olean-18-ene as reported in the literature (Taye et al. 2015). An additional methyl signal was detected as a singlet at δ 1.12, corresponding to the carbon at δ 26.04 (C-39), which was assigned to be located in the side chain. The signal at δ 4.50 has been assigned to be due to H-3 (1H, t), and the chemical shift experienced a downfield shift from the typical value of δ 3.60, possibly due to the presence of the electronegative group (assigned to o–C=O) at its vicinity. This attachment was confirmed by the HMBC correlations between δ 4.50 (assigned to H-3) and δ 173.68 (assigned to carbonyl carbon). In the 1H–1H COSY spectrum, couplings were apparent between the protons at δ 1.73, 1.04 (assigned to be as H-1)/δ 2.03, 1.61 (H-2)/δ 4.50 (H-3), which along with the HMBC correlations between the proton at δ 4.50 (H-3) and the carbon at δ 27.53 (assigned to C-2)/δ 39.82 (C-4) supported the presence of substituted cyclohexane ring A with a possible oxygenation (assigned to O–C=O) at the C-3 position (Figure 1(B)). The 13C NMR chemical shifts exhibited the signal for the characteristic double bond at δ 148.67 (assigned to C-18), δ 129.77 (C-19) along with the olefinic proton signal at δ 4.87 (H-19). These chemical shifts were found to be similar to those of the reported olean-18-ene (Osorio et al. 2012) exhibiting olefinic signature peaks (Yang et al. 2006). The ring systems B, C, D and E have been established by strong 1H–1H–COSY correlations between δ 1.53, 1.36 (H-6)/δ 1.34, 1.49 (H-7); δ 1.29 (H-9)/δ 1.73, 1.04 (H-1)/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) based on the literature value (Osorio et al. 2012). In the 1H–1H COSY spectrum, couplings were apparent between δ 0.89(H-38)/δ 1.88, 1.63(H-37)/δ 5.18(H-36) and δ 1.94(H-34)/δ 2.27, 1.64(H-33)/δ 2.31(H-32), which support the presence of 4, 5-dimethylpent-5-enolate network (Figure S5). Auxiliary olefinic bond at δ 145.2 (assigned to C-35) was confirmed by the strong HMBC correlations between δ 1.14 (H-39)/C-35, δ 1.94 (H-34) and δ 5.18 (H-36) with δ 145.2 (C-35). Spatial arrangement of the angular methyl groups between the ring junctions was identified from the NOE spectra. The stereochemistry at δ 80.59 (assigned to C-3) was established by coupling constants of the proton at δ 4.50 (dd, J = 5.52,7.36 H-3) has mutual NOE correlations with δ 0.88 (assigned as H-40) and δ 0.84 (H-5) which was at the α-face of the molecule (axial configuration) (Osorio et al., 2012) and has no NOE interactions with the protons at δ 1.08
(H-25) and δ 0.97 (H-26) and δ 1.02 (H-28), thereby indicating that these groups must be equatorial and on the β-side of the molecule. The relative stereochemistries of the chiral centres at δ 55.59 (assigned to C-5), 51.14 (C-9) were α-side and 39.1 (C-13), and 47.56 (C-34) were in β-side orientation deduced from the NOESY spectrum of the compound. Its mass spectrum exhibited a molecular ion peak at m/e 578, which appeared to undergo elimination of 4, 5-dimethyloct-5-enoate (m/e 169) to yield octamethylpicene at m/e 409 (Figure s6). The latter appeared to undergo C-ring cleavage to yield the base peak at m/e 189. The appearance of base peak at m/e 189 was in accordance with the literature values of oleanane-type triterpenoid (Madureira et al. 2004).

### 2.2. Antioxidative and anti-inflammatory activities

Compound 1 with the extended C20 guaiane sesquiterpenoid recorded greater antioxidant activity as determined by in vitro DPPH and ABTS radical scavenging properties (IC$_{50}$ 0.73–0.76 mg/mL) than 2 (IC$_{50}$ 0.85–0.89 mg/mL) with C40 prenylated oleanane triterpenoid framework. Likewise, compound 1 exhibited greater anti-inflammatory property as described by the anti-COX-2 and anti-5-LOX activities (IC$_{50}$ 0.87 and 0.85 mg/mL, respectively) than those exhibited by 2 (IC$_{50}$ 0.92 and 0.96 mg/mL, respectively) (Table 1). Both compounds 1 and 2 exhibited lesser activity against COX-1 isoform (IC$_{50}$ 1.77 and 1.86 mg/mL, respectively) and their selectivity indices remained significantly greater (anti-COX-2 IC$_{50}$ to anti-COX-1 IC$_{50}$, 0.4–0.5). No significant differences in anti-cyclooxygenase-2 of these compounds were discernable (IC$_{50}$ 0.8–0.9 mg/mL), whilst compound 1 showed greater anti-5-lipoxidase activities (IC$_{50}$ ~ 0.8 mg/mL) than those of 2 (IC$_{50}$ 0.96 mg/mL). The physicochemical parameters such as polarisability, steric and hydrophobic descriptors (lipophilicity, partition coefficients) were reported to have predominant roles to influence the biological activities (Cinq-Mars et al. 2008). The ability of any molecule to penetrate biological membranes is a primary factor in controlling the interaction of compounds with biological systems and is dependent on lipophilicity factors as determined by the partition coefficient between 1-octanol and water (log $P_{\text{ow}}$). Although no significant differences in the polarisability depicting the electronic descriptor ($32–38 \times 10^{-24} \text{cm}^3$) in 1 and 2 were apparent, the anti-inflammatory activity (in IC$_{50}$) of the former was lesser (0.85–0.87 mg/mL) than the latter (0.92–0.96 mg/mL) due to the greater hydrophobic values of 2 (log $P_{\text{ow}}$ 9.29) those that recorded in 1 (log $P_{\text{ow}}$ 5.22). The larger size (MR > 150 cm$^3$/mol) and greater lipophilic descriptor values of 2 might prevent the access to the catalytic cleft of 5-LOX and COX-2 resulting in lesser anti-inflammatory activity than 1. This leads demonstrated in the present study will be significant in explaining the

### Table 1. In vitro bioactivities (antioxidative and anti-inflammatory)* of 1 and 2 isolated from $R$. mucronata.

| Compounds | DPPH scavenging activity | ABTS scavenging activity | Anti-COX-1 activity | Anti-COX-2 activity | IC$_{50}$ anti-COX-2/COX-1 | Anti-5-LOX activity |
|-----------|--------------------------|--------------------------|---------------------|---------------------|---------------------------|---------------------|
| 1         | 0.76±0.00$^{a}$          | 0.73±0.00$^{a}$          | 1.77±0.02$^{a}$    | 0.87±0.03$^{a}$    | 0.49±0.03$^{a}$          | 0.85±0.06$^{a}$    |
| 2         | 0.89±0.02$^{b}$          | 0.85±0.03$^{b}$          | 1.86±0.04$^{b}$    | 0.92±0.05$^{a}$    | 0.50±0.04$^{b}$          | 0.96±0.11$^{b}$    |

*The bioactivities were expressed as IC$_{50}$ values (mg/mL).

$^{a,b}$Column-wise values with different superscripts indicate significant difference ($p < 0.05$).

Note: Results were expressed as mean ± SD ($n=3$).
pharmacophore-fit in the macromolecular receptor site and exploring the primary site and mode of action of this class of the substituted terpenoid compounds. Sesquiterpenoids with free radical scavenging properties were isolated from the marine macroalga *Ulva fasciata* (Chakraborty & Paulraj 2010). Previous study revealed the antioxidant and antimicrobial properties of different solvent extracts *R. mucronata* (Imdadul et al. 2011). The anti-inflammatory sesquiterpenes from *Annona reticulata* L. bark (Chavan et al. 2012), antioxidant sesquiterpenes from *Cedrus deodara* (Roxb.) Loud. (Chaudhary et al. 2015), new oleanane-type triterpene saponins from *Glycyrrhiza glabra* (Wei et al. 2014) and anti-inflammatory novel lindenane sesquiterpenes were isolated from *Chloranthus fortunei* (Zhang et al. 2012). An olean triterpene 3β-estearioxy-olean-12-ene was isolated from the medicinal plant *Acacia brachypoda* exhibited anti-inflammatory properties (Da Rocha et al. 2015).

3. Experimental

3.1. Chemicals and instrumentation

Fourier transform infra-red spectrometer spectra of the compounds under KBr pellets were recorded in a Thermo Nicolet, Avatar 370 in the IR range between 4000 and 400 cm⁻¹. UV spectra were obtained on a Varian Cary 50 UV–vis spectrometer (Varian Cary, USA). The GC-MS analysis was performed in electronic impact ionisation mode in a PerkinElmer Clarus 680 GC-MS fitted with an Elite 5 MS nonpolar, bonded phase capillary column (50 m × 0.22 mm i.d. × 0.25 μm film thicknesses). Helium (He) was used as the carrier gas, and the flow rate used was 1 mL/min. The temperature was programmed initially at 50 °C for 2 min., then increased with a rate of 10 °C min⁻¹ to 180 °C and kept for 2 min, and raised at 4 °C/min to 280 °C and held for 15 min. ESI-MS spectra were acquired on a liquid chromatography–mass spectrometry system (Applied Biosystems QTrap 2000, Applied Biosystems, Darmstadt, Germany). ¹H and ¹³C NMR spectra were recorded on a Bruker AVANCE III 500 MHz (AV 500) spectrometer (Bruker, Germany) in CDCl₃ as aprotic solvent at ambient temperature with TMS as the internal standard (δ 0 ppm). All the reagents and solvents used in this study were of analytical grade and purchased from E-Merck.

3.2. Plant material and preparation of crude extracts

The mangrove used in this study was *R. mucronata* (Family Rhizophoraceae) freshly collected from the Vallarpadam area of Kochi (Kerala State of India) located between south west coast of India (9°59′24.0″ North and 76°15′18.0″ East). A voucher specimen (No. CMFRI/MoES/DFS/AC 1144) was deposited in Marine Biodiversity Museum at CMFRI. The samples (1000 g) were washed in running water and transported to the laboratory before being shade dried (35 ± 3 °C) for 36 h. The shade-dried leaves were powdered (400 g) and extracted with aqueous methanol (50–60 °C, 3 h) followed by partitioning with *n*-hexane (500 mL × 3). The *n*-hexane fraction was filtered (Whatman No. 1 filter paper) through anhydrous Na₂SO₄, and the pooled filtrate was concentrated (50 °C) in rotary vacuum evaporator (Heidolph Instruments, Germany) to furnish the *n*-hexane extract (24 g).
3.3. Chromatographic purification of substituted terpenoid derivatives from *R. mucronata*

An aliquot of the *n*-hexane extract (18 g) of *R. mucronata* was slurried in silica gel (4 g, 60–120 mesh) and loaded into a glass column (90 cm × 4 cm) packed with silica gel (60–120 mesh, 50 g) as adsorbent before being subjected to vacuum liquid chromatography. The column was initially eluted with *n*-hexane, and polarity was gradually increased by addition of EtOAc (*n*-hexane: EtOAc 99:1 to 30:70, v/v) to furnish 20 fractions of 20 ml each, which were reduced to eight groups (RM₁–RM₈) after TLC analysis (*n*-hexane: EtOAc, 9:1, v/v). The fraction 2 (RM₂, 514.3 mg) obtained by eluting with *n*-hexane: EtOAc (4:1, v/v) was found to be a mixture, which was flash chromatographed (Biotage AB sP1-B1a, Biotage AB, Uppsala, Sweden) on a silica gel column (Biotage, 230–400 mesh, 12 g; Sweden, Biotage No. 25+M 0489-1) at a collection UV wavelength at 236 nm using a step gradient of EtOAc/*n*-hexane (0–10% EtOAc) to afford 120 fractions (9 ml each). Based on analytical TLC, the fractions with similar patterns were pooled together to afford eight pooled fractions (72 ml, RM₉–RM₁₆). The fraction RM₃, which was found to be a mixture, was further fractionated by flash chromatography using 70% EtOAc/*n*-hexane followed by 10% MeOH/CHCl₃ to afford 90 fractions (9 ml each). Based on analytical TLC, the fractions with similar patterns were pooled together to afford eight pooled fractions (RM₂₂–RM₂₉). The fraction RM₂₄ was further fractionated over preparatory TLC on silica gel GF₂₅₄ using CHCl₃/MeOH (9:1, v/v) to afford compound 1 (50.5 mg) as major component. Evaporation of solvents from the fractions followed by TLC over silica gel GF₂₅₄ (particle size 15 mm) using 20% EtOAc/*n*-hexane supported the purity.

3.3.1. (4E, 8Z)-3,3a,6,7-tetrahydro-3,9-dimethyl-5-(6-methylheptan-2-yl)cycloocta[b]furan-2(9aH)-one (1)

Greenish oil; UV (MeOH) **λ**<sub>max</sub> (log ε): 238 nm (2.82), 262 nm (2.40); TLC (Si gel GF₂₅₄ 15 mm; 1% MeOH/CHCl₃, v/v) **R**<sub>f</sub>: 0.80; **R**<sub>t</sub>: 12.22 min.; IR ν<sub>max</sub> (KBr) cm<sup>−1</sup> (**ν** = stretching, **δ** = bending, **ρ** = rocking vibrations): 724.2 (C–H **ρ**), 813.99,1377.22 (C–H **ρ**), 1464.02 (C–H **δ**), 1652 (C=C **ν**), 1733.1 (C–Co–C **ν**), 2926.11 (alkane C–H **ν**); 1H NMR (500 MHz, CdCl₃) **δ** 5.15 (t, **J** = 6.7 Hz, 1H), 5.11 (d,1H), 4.50 (d,1H), 2.29 (m,1H), 2.06 (q,2H), 2.01 (t,2H), 1.97 (m,1H), 1.69 (s,3H), 1.64 (t,1H), 1.60 (dd,3H), 1.52 (m,1H), 1.37 (m,3H), 1.29 (m,1H), 1.26 (s,3H), 0.98 (m,2H), 0.90 (d,1H), 0.87 (d,1H);<sup>13</sup>C NMR (125 MHz, CDCl₃) **δ** 173.67 (C-1), 135.19 (C-9), 131.22 (C-5), 125.02 (C-8), 124.27 (C-4), 80.57 (C-10), 39.73 (C-11), 34.87 (C-2), 32.21 (C-6), 31.93 (C-7), 29.7 (C-19), 29.36 (C-14), 27.98 (C-12), 26.4 (C-18), 25.69 (C-3), 22.69 (C-15), 17.68 (C-20), 16.56 (C-13), 16.01 (C-17), 14.11 (C-16). **1**–**H**–**H**–COSY, and HMBC data, see Table S1; HRMS (ESI) m/z Calcd for C<sub>20</sub>H<sub>33</sub>O<sub>2</sub> 304.2420, found 304.2638 [M + H]<sup>+</sup>.

3.3.2. (35E)-1,2,3,5,6,6-icosahydro-4,4,8b,10,14,17,20,20-octamethylpicen-3-yl-34,35-dimethyloct-3-enoate (2)

Yellow amorphous powder; m.p. 166 °C; UV (MeOH) **λ**<sub>max</sub> (log ε): 245 nm (3.26); TLC (Si gel GF₂₅₄ 15 mm; 5% MeOH/CHCl₃, v/v) **R**<sub>f</sub>: 0.65; **R**<sub>t</sub>: 17.20; IR ν<sub>max</sub> (KBr) cm<sup>−1</sup>: 721.40 (C–H **ρ**), 965.40 (C–H **δ**), 1029.06 (C–O **ν**), 1376.26 (C–H **ρ**), 1560.46 (C–C **ν**), 1735.03 (C–O **ν**), 2925.15,
2953.12 (C–H ν); ¹H NMR (500 MHz, CDCl₃): δ 5.18 (t, 1H), 4.50 (t, 1H), 2.31 (t, 1H), 2.28–2.27 (m, 2H), 2.03–2.00 (m, 3H), 1.94 (t, 2H), 1.88 (m, 1H), 1.79–1.76 (m, 3H), 1.73–1.72 (t, 2H), 1.64–1.61 (m, 3H), 1.55–1.50 (m, 3H), 1.49–1.48 (m, 2H), 1.44 (m, 1H), 1.36 (m, 1H), 1.34 (t, 1H), 1.29 (t, 1H), 1.23 (m, 2H), 1.14 (s, 3H), 1.10 (m, 1H), 1.08 (s, 3H), 1.04 (t, 1H), 1.02 (s, 3H), 0.97 (s, 3H), 0.95 (s, 3H), 0.93 (s, 3H), 0.89 (d, 3H), 0.88 (d, 3H), 0.87 (s, 3H), 0.85 (s, 3H), 0.84 (t, 3H), 0.73 (s, 3H); ¹³C NMR (125 MHz, CDCl₃): δ 173.68 (C-31), 145.2 (C-35), 142.67 (C-2H), 129.77 (C-19), 121.65 (C-36), 80.59 (C-3), 55.59 (C-5), 51.14 (C-9), 47.56 (C-34), 43.34 (C-14), 41.73 (C-8), 39.82 (C-4), 39.1 (C-13), 38.62 (C-1), 38.32 (C-33), 37.87 (C-22), 37.76 (C-16), 37.38 (C-10), 34.91 (C-32), 34.54 (C-7), 33.56 (C-21), 33.35 (C-17), 33.27 (C-40), 32.61 (C-20), 31.34 (C-29), 29.37 (C-30), 28.07 (C-15), 28.06 (C-23), 27.53 (C-2), 26.19 (C-12), 26.04 (C-39), 25.19 (C-28), 23.46 (C-37), 21.1 (C-11), 18.1 (C-6), 16.78 (C-26), 16.1 (C-25), 15.56 (C-24), 14.56 (C-27), 14.11 (C-38). ¹H–¹H COSY, and HMBC data, see Table S1; HRMs (ESI) m/e Calcd for C₄₀H₆₇O₂ 579.5152, found 579.5264 [M + H]⁺.

3.4. Antioxidant and anti-inflammatory activities

Antioxidant activities were measured using the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Lim et al. 2007) and 2, 2′-azino-bis-3 ethylbenzothiazoline-6-sulphonic acid diammonium salt (ABTS) (Vijayabaskar & Shiyamala 2012) radical scavenging activity. In vitro anti-inflammatory activities were carried out using cyclooxygenase-2 (COX-2) (Larsen et al. 1996) and 5-lipoxygenase (5-LOX) enzyme inhibition assay (Baylac & Racine 2003).

3.5. Structure–activity relationship analysis

The structure–antioxidant activity relationship analysis was carried out using different physicochemical parameters. The structural descriptors were calculated or taken from ACD Chemsketch (version 8.0) and ChemDraw Ultra 8.0 databases: steric (molar volume, MV; parachor, Pr; molar refractivity, MR), hydrophobic (logarithmic scale of the octanol-water partition coefficient, log Pₒₜₜ); and electronic descriptor variables (topological polar surface area, tPSA; polarisability, Pl).

3.6. Statistical analysis

Statistical evaluation was carried out with the Statistical Program for Social Sciences 13.0 (SPSS Inc, Chicago, USA, ver. 13.0). Analysis was carried out in triplicate, and the means of all parameters were examined for significance by analysis of variance (ANOVA). The level of significance for all analyses was p ≤ 0.05.

4. Conclusion

Bioactivity-guided chromatographic fractionation of the n-hexane fraction obtained from the aqueous methanol extract of the leaves of mangrove R. mucronata afforded two terpenoids: one with an extended guaiane sesquiterpenoid and the other containing C₄₀ prenylated oleanane-type triterpenoid framework with potential antioxidative and anti-inflammatory activities. The bioactivities of the C₂₀ prenylated sesquiterpenoid (1) were greater
than the oleanane triterpenoid (2). The antioxidant activities of these compounds were inversely proportional to the bulk and lipophilicity parameters.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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