First report of two new antioxidative meroterpeno 2H-pyranoids from short-necked yellow-foot clam *Paphia malabarica* (family: Veneridae) with bioactivity against pro-inflammatory cyclooxygenases and lipoxygenase

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**ABSTRACT**

Two new meroterpeno 2H-pyranoids were isolated from the EtOAc:MeOH extract of yellow-foot clam *Paphia malabarica*. The structures of these newly reported compounds were elucidated based on spectroscopic interpretations. This is the first report of biogenic 2H-pyrans bearing decadienyl and allyloxy-(isopentanyl)-cyclohexene skeletons from marine biota. The extended C18 sesquiterpenoid with prenylated irregular farnesene framework was characterised as 2-((E)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-2H-pyran (1). The compound 2, 1′-((10E)-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrhydro-2′,2′-dimethyl-2H-pyran represents the first example of naturally occurring C21 prenylated bisabolene-type meroterpenoid, whereas tetrhydro-2′,2′-dimethyl-2H-pyran remains attached at C-2′ position of rearranged bisabolene framework formed by allyloxy linkage. The antioxidant activities (DPPH/ABTS+) of 1 and 2 were comparable (IC50 < 1.0 mg/mL) with α-tocopherol. In addition, these compounds exhibited greater activity against cyclooxygenase-2 than COX-1, and the selectivity indices were significantly lesser (~1.1). No significant differences in anti-5-lipoxygenase activity of 1 and 2 (IC50 1.02–1.06 mg/mL) than ibuprofen (IC50 0.93 mg/mL) indicated the potential anti-inflammatory properties of title compounds.

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1. Introduction

Pyran derivatives, an important category of organic compounds, which proved to constitute an important class of heterocycle, were found to occur in marine organisms, and attracted a great deal of interest due to their pharmacological potentials (Cueto et al. 1998a; Ciavatta et al. 2011). The occurrence of pyranoids in biological system and their role as precursors in the production of pharmacologically active metabolites as anti-fungal, anti-coagulant, anti-inflammatory, antimicrobial and anti-cancer compounds were reported from natural and synthetic origin (Arora & Mathur 1963; Goel & Ram 2009; Nemouchi et al. 2012; Rajguru et al. 2013). The pyranoid cladiellane diterpenes were isolated from marine mollusk, *Tritoniopsis elegans* (Ciavatta et al. 2011) and naturally occurring 1-(6-butyl-3, 4-dihydro-2H-pyran-2yl)-pentanone was reported from a marine invertebrate, *Neosadocus maximus* as their self-defence agents (Rocha et al. 2011). The bioactive pyranoid diterpenes were isolated from soft coral, *Lobophytum pauciflorum* (Govindam et al. 2012). Polyoxygenated marine monoterpenes pantopyranoids A–C, and pantoisofuranoids A–C, oxane derivative monoterpenes and monoterpenoids with tetrahydrofuran ring have been isolated from the Antarctic marine alga *Pantoneura plocamioides* (Cueto & Darias 1996; Cueto et al. 1998b). 6-pentyl-2H-pyran-2-one and its analogs showed antibacterial properties (Parker et al. 1997) and furanoid monoterpenes, furoplocamioids A–C from the marine red alga *Plocamium cartilagineum* were earlier reported (Darias et al. 2001).

The short-necked yellow-foot clam *Paphia malabarica* (family, Veneridea) is a benthic filter feeding bivalve, and is distributed mainly in the estuarine habitats on the southern coasts of India. As part of our ongoing programme, we aimed at the isolation of biologically active compounds from crude EtOAc:MeOH extract (1:1 v/v) of *P. malabarica*, collected from the south-west coast of Arabian Sea, showed promising antioxidative and anti-inflammatory activities by various in vitro assays. Its chemical constituents have not been reported previously, suggesting *P. malabarica* would be an attractive source for chemical investigation. In view of this, *P. malabarica* was chosen as a research target, and two unprecedented meroterpeno 2H-pyranoids were obtained, 2-((E)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-2H-pyran (1) and 1′-((10E)-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrahydro-2′,2′-dimethyl-2H-pyran (2). The structures were elucidated with the help of detailed spectroscopic analysis and the isolated compounds were tested against several pro-inflammatory enzymes (COX-1, 2 and 5-LOX) as well as antioxidant activities (DPPH/ABTS+ radical scavenging assays) to evaluate their potential biological activities, and the results obtained are described herein. It is also noteworthy that the compounds 1–2 represent the first example of naturally occurring meroterpeno 2H-pyranoids featuring unique decadienyl and allyloxy-(isopentanyl)-cyclohexene skeletons possessing rare C_{18} and C_{21} prenylated irregular bisabolene frameworks.

2. Results and discussion

2.1. General

Two (1–2) new meroterpeno 2H-pyranoids characterised as 2-((E)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-2H-pyran (1) and 1′-((10E)-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrahydro-2′,2′-dimethyl-2H-pyran (2) have been isolated from the EtOAc:MeOH (1:1, v/v) extract of the freeze dried powder of the edible part separated from *P. malabarica*. These
compounds are new marine natural products that possess a hitherto unknown pyranyl regiochemistry, with C$_{18}$ sesquiterpenoid with prenylated irregular farnesene framework as in compound 1. The compound 2 incorporate unusual naturally occurring C$_{21}$ prenylated rearranged bisabolene type meroterpenoid with the allyloxy linkage coordinating between the C$_{14}$ meroterpenes and substituted terahydropyran network.

2.2. Spectral analyses of compounds from *P. malabarica*

The compound 1, 2-((E)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-2H-pyran, a new C$_{18}$ sesquiterpenoid with prenylated irregular framework, was isolated as yellow amorphous powder upon repeated chromatographic purifications over silica gel columns and preparatory TLC plates. The detailed $^1$H, $^{13}$C NMR and mass spectral analysis confirmed the structure (Table S1, Figures S1–S2). The title compound exhibited a molecular ion peak at $m/e$ 262 (HRESIMS $m/e$ 262.1604 ([M]$^+$), along with $^1$H and $^{13}$C NMR spectra, signifying the elemental composition as C$_{18}$H$_{30}$O with 4° of unsaturation associated with three double bonds and a ring system. The $^1$H--$^1$H COSY couplings from $\delta^H$ 4.68 (assigned to H-10)/$\delta^H$ 1.58, 1.60 (H-11)/$\delta^H$ 2.02, 2.00 (H-12) were apparent (Table S1, Figures 1(A) and S4). The downfield methine (–CH) at $\delta^H$ 4.68 was found to be a triplet, HSQC with $\delta^C$ 108.3 was attributed to the part of a pyran ring system (Figure S5). The quaternary carbon at C-13 ($\delta^C$ 112.37) recognised as the junction point of pyran ring system and the propyl moiety. However, the lower chemical shift value of quaternary carbon predicted that it might be adjacent to highly electron withdrawing group with high downfield shift at $\delta^C$ 123.34/$\delta^H$ 6.96. The higher chemical shift of this alkenic proton was due to the neighboring oxygen atom and found to be a singlet proton, but not related to an aromatic ring. The HMBC relation from $\delta^H$ 6.96 (assigned as H-14) to $\delta^C$ 108.30 (C-10), 112.37 (C-13); $\delta^H$ 4.68 (H-10) to $\delta^C$ 112.37 (C-13); $\delta^H$ 1.58 (H-11) to $\delta^C$ 29.71 (C-12); and $\delta^H$ 2.02 (H-12) to $\delta^C$ 108.30 (C-10) established the 2H-pyran ring system (Table S1, Figure S6). The signal at $\delta^C$ 112.37 (assigned to C-13) attached to a propyl side chain was evident from the HMBC relations from the protons of propyl moiety to the pyran ring (Figure 1(A)). The two methylenes at $\delta^C$ 37.11 (C-5a), $\delta^C$ 22.7 (C-5b) and methyl at $\delta^C$
14.12 (C-5c) were in good agreement with the reported 2H-pyranoid compound, 1-(6-butyl-3, 4-dihydro-2H-pyran-2-yl)-pentanone (Rocha et al. 2011). The long range couplings from $\delta_1^{H}$ 2.02 (H-12) to $\delta_3^{C}$ 37.11 (C-15)/$\delta_4^{C}$ 22.70 (C-16); $\delta_5^{H}$ 1.37 (H-16) to $\delta_6^{C}$ 29.71 (C-12); and $\delta_7^{H}$ 6.96 (H-14) to $\delta_8^{C}$ 37.11 (C-15) confirmed the attachment to pyran moiety and $^1H$–$^1H$ COSY correlations between $\delta_1^{H}$ 2.03 (H-15)/$\delta_9^{H}$ 1.37 (H-16)/$\delta_{10}^{H}$ 0.88 (H-17) established the propyl side chain. The proton at $\delta_9^{H}$ 4.68 (H-10) exhibited HMBC relations with $\delta_3^{C}$ 129.91 (C-9) implied the attachment to the alkenic quaternary carbon of deca-1,8-dien-10-yl side chain. The signal at $\delta_3^{C}$ 129.91 was found to be due to the quaternary carbon, and was attached to an alkenic proton, $\delta_9^{H}$ 5.35 (corresponding to the $^{13}$C NMR signal at $\delta_3^{C}$ 130.38 at C-8 position) (Wang et al. 2010). The singlet proton at $\delta_5^{H}$ 1.44 (H-18) was attached to $\delta_6^{C}$ 30.41 exhibiting HMBC relations to $\delta_3^{C}$ 129.91 (C-9), $\delta_4^{C}$ 130.38 (C-8), $\delta_5^{C}$ 31.94 (C-7) and $\delta_6^{C}$ 108.30 (C-10). It was found that the group of carbons from C-1 to C-9 were linearly aligned as assigned by the $^1H$–$^1H$ COSY correlation analyses. The $^1H$–$^1H$ COSY correlations from $\delta_1^{H}$ 4.94/$\delta_2^{H}$ 5.10 (H-1)/$\delta_3^{H}$ 5.81 (H-2)/$\delta_4^{H}$ 2.06 (H-3)/$\delta_5^{H}$ 1.36 (H-4)/$\delta_6^{H}$ 1.26 (H-5)/$\delta_7^{H}$ 1.30 (H-6)/$\delta_8^{H}$ 1.97 (H-7)/$\delta_{9}^{H}$ 5.35 (H-8) and long range HBMC correlations from $\delta_1^{H}$ 1.97 (H-7) to $\delta_3^{C}$ 130.38 (C-8)/$\delta_{4}^{C}$ 129.91 (C-9)/$\delta_{5}^{C}$ 108.30 (C-10); $\delta_2^{H}$ 1.26 (H-5) to $\delta_3^{C}$ 33.83 (C-3)/$\delta_6^{C}$ 29.37 (C-4)/$\delta_7^{C}$ 28.97 (C-6); $\delta_4^{H}$ 2.06 (H-3) to $\delta_{5}^{C}$ 139.27 (C-2)/$\delta_{6}^{C}$ 114.06 (C-1); $\delta_7^{H}$ 5.81 (H-2) to $\delta_{4}^{C}$ 114.06 (C-3), and $\delta_{8}^{H}$ 5.01/4.94 (H-1) to $\delta_{5}^{C}$ 139.27 (C-2)/$\delta_{6}^{C}$ 33.83 (C-3) unambiguously confirmed the presence of side straight chain of 1 (Figure 1A). The >CH$_2$ group at $\delta_1^{H}$ 5.01 and $\delta_2^{H}$ 4.94 were found to be considerably downfield due to its terminal position and the presence of highly downfielded alkene (–CH=, $\delta_1^{H}$ 5.81). The geometrical arrangement of these alkenic protons was confirmed from their J values, which were 5.81 ($J = 10.21, 6.99$ Hz), 5.01 ($J = 14.52$ Hz) and 4.94 ($J = 10.21$ Hz), thus established the trans ($E$) configuration comparable with a related compound, lobatriene, identified from soft coral, (Govindam et al. 2012). The $^{13}$C NMR spectrum of this compound in combination with DEPT indicated the presence of a total of 18 carbons, which enclosed two CH$_3$, ten CH$_2$, and four CH groups. The relative stereochimeristics of the chiral centre of 1, particularly that of C-10 carrying the methine proton, $\delta$ 4.68 (1H, t) was deduced from the NOESY spectrum (Figure S7) and their J-values. NOE correlations between the protons, $\delta$ 1.60 (H-11)/$\delta$ 4.98 (H-1)/$\delta$ 5.81 (H-2)/$\delta$ 2.00 (H-12)/$\delta$ 4.68 (H-10), $\delta$ 5.35 (H-8) indicated the close proximity of these groups and their $\alpha$-disposition (Figure S17). Further NOE couplings were observed between the protons at $\delta$ 1.58 (H-11), $\delta$ 2.02 (H-12)/$\delta$ 5.01 (H-1)/$\delta$ 1.43 (H-18)/$\delta$ 6.96 (H-14) which indicated that these groups are on the same side of the plane of the molecule, and disposed in $\beta$-orientation. Notably, the geometric isomerism of the olefinic protons $\delta$ 4.94 and $\delta$ 5.01 (H-1) had a large coupling constant ($J = 10.2$ and 14.5 Hz, respectively) which showed the E configuration of the olefinic bond. Additionally, the large coupling constant of 10.2 Hz (each) between the pertinent olefinic protons at 5.81 (related to H-2$^a$) and $\delta$ 5.35 (assigned to H-8$^b$, bearing C8–C9 double bond) revealed that they are disposed in the same plane of geometry; all these effects are in accord with the J values observed and indicate the stereochemistry. The protons at $\delta_1^{H}$ 1.44 (CH$_3$, s, C-18) was found to be situated at $\beta$ position because of NOE relationship with $\delta_1^{H}$ 6.96 (H$^a$-14, s), and has no NOE associations with the protons at $\delta_1^{H}$ 4.94 (H$^a$-1) and $\delta_1^{H}$ 4.68 (H$^a$-10) which suggested the cis-orientation for the methyl groups at C-18 and olefinic proton at C-14, and that these groups must be disposed on the $\beta$-side. The H-10 should be trans-orientation with the methyl protons at C-18 and olefinic proton at C-14 since there was no cross peak could be detected between H-14 and H-10 in NOESY experiment. The IR spectrum revealed the presence of olefinic (C=C) and alkyl (C–H) bending vibrations that were represented by the 1661 and 1455/965 cm$^{-1}$ absorption
bands, respectively. The absorption bands at 2932/2860 cm\(^{-1}\) indicated C–H stretching vibration, whereas those at 1372, 1242, 1187, 1108 and 1042 cm\(^{-1}\) showed the C–O stretch, thereby substantiated the structure of prenylated sesquiterpeno pyranoid framework. The mass spectrum exhibited the molecular ion peak at \(m/e\) 262, which appeared to undergo elimination of methyl radical to yield a radical ion fragment at \(m/e\) 247 (1a, attributed to dihydro-2-(nona-1,8-dienyl)-13-propyl-2\(H\)-pyran). The latter appeared to undergo fragmentation by eliminating \(\text{CH}_3\) and \(\text{C}_2\text{H}_3\) radicals to obtain fragment peaks at \(m/e\) 233 (1d, 13-ethyl-dihydro-2-(nona-1,8-dienyl)-2\(H\)-pyran) and \(m/e\) 220 (1b, 2-(hept-1-ethyl)-9,10-dihydro-11-propyl-2\(H\)-pyran), respectively. Fragmentation of the ion at \(m/e\) 220 (1b) was perceived to be accompanied by the loss of a C-3 fragment resulting in an ion at \(m/e\) 179 (1c), which on subsequent rearrangement yielded the fragments at \(m/e\) 110 (1f, 5-methylhex-5-en-2-ol) and 97 (1g, dihydro-5-methyl-2\(H\)-pyran). The latter eliminated \(\text{CH}_2\text{O}\) radical to yield \(m/e\) 66 (1j, penta-1,3-diene). The fragment peak at \(m/e\) 85 (assigned to tetrahydro-2\(H\)-pyran) was found to be the base peak (Figure S8).

The compound 2, 1\'-(10\(E\))-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrahydro-2,2\,\'dimethyl-2\(H\)-pyran, a naturally occurring \(\text{C}_{31}\) prenylated bisabolene type meroterpenoid, was isolated as a amorphous powder on repeated chromatographic purifications over silica gel. The molecular ion peak was recorded at HRESIMS \(m/e\) 320.2715 ([M\(^+\)]) and \(^1\text{H}\)–\(^13\text{C}\) spectral details established the elemental composition as \(\text{C}_{21}\text{H}_{36}\text{O}_2\) with four double bond equivalence enclosing two double bonds and two cyclic system. The singlet at \(\delta\) 4.59 (assigned to C-1') attributed to the methine (–CH) group, in which the upfield shift of the corresponding carbon (\(\delta\) 68.39) was due to the electronegative –O– moiety (Table S1, Figures S3, S9 and S10). This carbon (C-1') was also attached to an oxygenated side chain as apparent from the HMBC correlations. The long range couplings from \(\delta\) \(H\) 4.59 (H-1`) to \(\delta\) \(C\) 25.74 (C-4`), \(\delta\) \(C\) 39.07 (C-2`), \(\delta\) \(C\) 30.25 (C-2'a) and \(\delta\) \(C\) 30.09 (C-2'b) proved that it was a part of pyran moiety. The \(^1\text{H}\)–\(^1\text{H}\) COSY revealed that the presence of four spin systems, H-3' to H-6' (\(\delta\) \(H\) 1.63, 1.66 (H-3')/\(\delta\) \(H\) 1.55, 1.56 (H-4')/\(\delta\) \(H\) 3.64 (H-5')) in the pyran ring, H-11 to H-12 (\(\delta\) \(H\) 4.12, 4.14 (H-12)/\(\delta\) \(H\) 5.35 (H-11)) in the allyloxy chain, H-5 to H-9 (\(\delta\) \(H\) 2.02 (H-5)/\(\delta\) \(H\) 2.35 (H-6)/\(\delta\) \(H\) 1.57, 1.59 (H-7)/\(\delta\) \(H\) 1.99, 2.00 (H-8)/\(\delta\) \(H\) 5.34 (H-9)) including H-6 to H-10 (\(\delta\) \(H\) 2.35 (H-6)/\(\delta\) \(H\) 5.38 (H-10)) in the cyclohexenyl ring and from H-1 to H-3 consisting of H-3/\(\delta\) \(H\) 0.89 (H-13)/\(\delta\) 1.43 (H-14), \(\delta\) 0.87 (H-1)/\(\delta\) 1.42 (2-H)/\(\delta\) 2.31 (H-13)) in the isopentanyl side chain (Table S1, Figures 1(B) and S12). Two intense singlet protons due to methyl (–CH\(_3\)) at \(\delta\) \(H\) 1.48 and \(\delta\) \(H\) 1.46 showed HSQC correlation with \(\delta\) \(C\) 30.25 (C-2'a) and \(\delta\) \(C\) 30.09 (C-2'b), respectively, which were attached to a quaternary carbon at \(\delta\) \(C\) 39.07 (Figure S13). This was apparent from the HMBC correlations, \(\delta\) \(H\) 1.46/1.48 (C-2'a/2'b) to \(\delta\) \(C\) 63.11 (C-5'), \(\delta\) \(C\) 39.07 (C-2'), which proved the attachment of dimethyl groups to the pyran ring (Table S1, Figure S14). The HMBC relations between \(\delta\) \(H\) 1.55 (H-4') to \(\delta\) \(C\) 29.62 (C-3'), \(\delta\) \(C\) 30.25 (C-2'a), \(\delta\) \(C\) 30.09 (C-2'b) and \(\delta\) \(H\) 3.64 (H-5') to \(\delta\) \(C\) 25.74 (C-4') established the pyran moiety (Table S1, Figure 1(B)). The attachment of carbon atom in the allyloxy side chain to pyran network was evident. The methine (–CH) groups, \(\delta\) \(H\) 5.38/\(\delta\) \(C\) 139.27 and \(\delta\) \(H\) 5.35/\(\delta\) \(C\) 127.67 at H-10 and H-11, respectively with large coupling constants \(J=9.18\) and 8.58, respectively) revealed its trans (\(E\)) geometry (assigned to C10=C11). The cyclohexenyl ring system enclosed –C=CH moiety in which the olefinic quaternary carbon (–C=) registered higher chemical shift of \(\delta\) 143.18 (C-4) compared to the methine (–CH; \(\delta\) 124.47) and a pentanyl group attached to the quaternary carbon attributed to C-12. This cyclic system exhibited HMBCs from \(\delta\) \(H\) 5.38 (H-10) to \(\delta\) \(C\) 31.93 (C-5), \(\delta\) \(H\) 2.35 (H-6) to \(\delta\) \(C\) 29.37 (C-7). The spectral data for quaternary olefinic carbon at \(\delta\) \(C\) 143.18 (C-4) and the methine
at \( \delta_c 68.39 \) (C-1') were comparable with 1-(6-butyl-3, 4-dihydro-2H-pyran-2-yl)-pentanone (Rocha et al. 2011). The HMBC couplings from \( \delta_1^i 0.89 \) (H-14) to \( \delta_c 22.69 \) (C-13), \( \delta_1^i 1.43 \) (H-13) to \( \delta_c 34.38 \) (C-3)/\( \delta_c 143.18 \) (C-4)/\( \delta_1^i 31.93 \) (C-5), \( \delta_h^i 1.42 \) (H-2) to \( \delta_c 34.38 \) (C-3)/\( \delta_c 143.18 \) (C-4), \( \delta_h^i 0.87 \) (H-1) to \( \delta_c 34.38 \) (C-3)/\( \delta_c 22.69 \) (C-2) confirmed the attachment of isopentany moiety to the cyclohexenyl ring system (Figure 1(B)). The \(^{13}\)C NMR and DEPT spectra identified the presence of 21 carbon atoms, in which four \( \text{CH}_3 \), nine \( \text{CH}_2 \), six \( \text{CH} \) were accounted for in the compound (Table S1, Figures S10 and S11). The relative stereochemistries of compound 2, mainly at C-1' and C-6 protons, \( \delta_h^i 4.59 \) (1H) and \( \delta_h^i 2.35 \) (1H) were confirmed from the NOE spectrum (Figure S17). The NOE couplings between \( \delta_h^i 1.55 \) (H-4')/\( \delta_h^i 4.59 \) (H-1'), \( \delta_h^i 2.39 \) (H-6), \( \delta_h^i 2.00 \) (H-8) along with \( \delta_h^i 2.35 \) (H-6)/\( \delta_h^i 4.12 \) (H-12) demonstrated that these protons are in the same plane of geometry and \( \alpha \)-disposed (Figure S15). Further the NOE relations among the protons H-8 (\( \delta_h^i 1.99 \)), H-3' (\( \delta_h^i 1.66 \)), H-12 (\( \delta_h^i 4.14 \)) and H-2'b (\( \delta_h^i 1.46 \), \( \text{CH}_3 \)) showed that they are disposed in the identical plane of geometry, and are disposed at \( \beta \)-orientation. The methyl protons at \( \delta_h^i 2.02 \) (H-2'b (\( \delta_h^i 1.46 \) was found to be \( \beta \) disposed with the reference plane due to the NOE correlations with \( \delta_h^i 4.14 \) (H\( ^\beta \)-12), and not with the protons at \( \delta_h^i 4.59 \) (H\( ^\alpha \)-1') and \( \delta_h^i 2.39 \) (H\( ^\alpha \)-6). This further explained the trans-orientation of the methine and methyl groups at C-6 and C-2'b positions of 2. The distinctive IR absorptions at 2932 and 2860 cm\(^{-1} \) were due to the C–H alkane stretching, whereas the olefinic (C=C) stretching and =C–H bending were represented by the 1661 and 965 cm\(^{-1} \) absorption bands, respectively. The distinctive absorption at 1456 cm\(^{-1} \) indicated bending vibration due to C–H groups. The fourier-transform infrared (FTIR) absorption bands at 1372, 1242, 1187, 1108 and 1042 cm\(^{-1} \) substantiated the C–O stretching vibration. The molecular ion peak at \( m/e ^{320} \) ([M]\(^+\)) appeared to undergo elimination of one –C\(_2\)H\(_5\) group to yield 1'-(10-(3-propylcyclohexyl)propoxy)-tetrahydro-2',2'-dimethyl-2H-pyran (2a, \( m/e 296 \)). The elimination of –C\(_3\)H\(_5\) group from the fragment ion at \( m/e 296 \) yielded the fragment with \( m/e 253 \) (2b, attributed to 1'-((3-cyclohexyl)propoxy)-tetrahydro-2',2'-dimethyl-2H-pyran) and \( m/e 254 \) (2c, 1'-(4-methylloctyloxy)-tetrahydro-2',2'-dimethyl-2H-pyran). The elimination of one –CH\(_3\) group from the fragment ion at \( m/e 254 \) yielded the fragments with \( m/e 238 \) (2d, attributed to 1'-(4-methylenehept-1-enyloxy)-tetrahydro-2',2'-dimethyl-2H-pyran). The elimination of –C\(_3\)H\(_5\) group from the fragment ion at \( m/e 238 \) yielded the fragments with \( m/e 198 \) (2e, attributed to 1'-((pent-2-enyloxy)-tetrahydro-2',2'-dimethyl-2H-pyran) and \( m/e 184 \) (2f, attributed to 1'-(butoxy)-tetrahydro-2',2'-dimethyl-2H-pyran), which on subsequent rearrangement yielded the fragments at \( m/e 142 \) (2h, 1'-(allyloxy)-tetrahydro-2H-pyran), 102 (2j, tetrahydro-2H-pyran-2-ol), 86 (pentan-1-ol) and 73 (2l, but-3-en-1-ol). The fragment peak at \( m/e 85 \) (2k, assigned to tetrahydro-2H-pyran) appeared as base peak of 2 (Figure S16).

### 2.3. Antioxidant and anti-inflammatory activities

The antioxidant activities of 1 and 2 as determined by in vitro DPPH (IC\(_{50}\) 0.76–0.78 mg/mL) and ABTS\(^+\) radical scavenging properties (IC\(_{50}\) 0.92–0.96 mg/mL) were comparable to that of \( \alpha \)-tocopherol (0.6–0.7 mg/mL) used in various commercial preparations. In addition, it was found that compounds exhibited greater activity against cyclooxygenase-2 (COX-2) when compared to COX-1, and therefore, their selectivity indices (anti-COX-1 IC\(_{50}\)/anti-COX-2 IC\(_{50}\)) were significantly lesser (~1.1) than the synthetic anti-inflammatory drug ibuprofen (0.44) (Table 1). No significant differences in anti-5-lipoxidase (5-LOX) activity of 1 and 2 (IC\(_{50}\) 1.02–1.06 mg/mL) than ibuprofen (IC\(_{50}\) 0.93 mg/mL) indicated the potentially selective
The anti-inflammatory properties of the title compounds without any adverse side effects. It is apparent that the synthetic non-steroidal anti-inflammatory drugs, such as ibuprofen, have greater inhibitory effect towards the COX-1 isoform of cyclooxygenase, which is considered to be a constitutive enzyme, and is a vital component of various metabolic functions. Notably, ibuprofen, a commonly used anti-inflammatory and painkiller, recorded greater inhibitory properties towards COX-1 (IC50 0.04 mg/mL) than COX-2 isoform (IC50 0.09 mg/mL) resulting in lesser selectivity index (Table 1). The title compounds purified from *P. malabarica* can be used as potential selective inhibitors of COX-2 with significantly lesser side effect profiles, such as renal and gastric damage than the present therapies by using non-steroidal anti-inflammatory drugs used to combat inflammatory disorders.

To the best of our knowledge, 2-((E)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-2*H*-pyran (1) and 1′-(((10E)-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrahydro-2′,2′-dimethyl-2*H*-pyran (2) represent the first description of meroterpeno 2*H*-pyranoids possessing the C14 sesquiterpenoid with prenylated irregular farnesene framework and C21 prenylated bisabolene-type meroterpenoid with the allyloxy linkage coordinating between the C14 meroterpenes and substituted tetrahydropyran system from a natural source. These unprecedented meroterpeno 2*H*-pyranoids from *P. malabrica* has bioactive potential as natural antioxidant and anti-inflammatory pharmacophore.

### 3. Experimental

#### 3.1. Chemicals and instrumentation

All compounds, reagents and solvents were of spectroscopic/chromatographic/analytical grade and were obtained from Merck (Darmstadt, Germany). FTIR spectra (KBr) recorded in a Perkin-Elmer Series 2000 FTIR spectrophotometer scanning between 4000 and 400 cm\(^{-1}\). GC-MS analyses were performed in electronic impact ionisation mode in a Perkin-Elmer Clarus 680 GC-MS fitted with a Elite 5 MS non-polar, 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 injection volume was 1 μL in a split mode and temperature of the injector was 280 °C. Electron ionisation energy was set at 70 eV. The scan rates were set as 10 spectra and ion source temperature was maintained at 200 °C. ESI-MS
spectra were acquired on a liquid chromatography-mass spectrometry system (Applied Biosystems QTrap 2000, Applied Biosystems, Darmstadt, Germany). 1D and 2D NMR spectra were recorded on a Bruker Avance DPX 500 (500 MHz) spectrometer in CDCl₃ solvent at ambient temperature with TMS as the internal standard (δ 0 ppm). Standard pulse sequences were used for DEPT, ¹H–¹H COSY, NOESY, HSQC and HMBC experiments. All the reagents and solvents used in this study were of analytical grade and purchased from E-Merck.

3.2. Sample collection and preparation of crude extracts
The clam samples, P. malabarica (10 kg) (voucher specimen No. CMFrI/MoES/DFS/AC 368) were freshly collected from Ashtamudi Lake (8°59′ N and 76°36′ E) situated along the south-west coast of India. The edible portion (6 kg) was separated from the cleaned shell-on samples were homogenised and freeze-dried by lyophilisation (Martin Christ alpha 1-4 LD Plus freeze drier, Germany). The dried powder (1.2 kg, yield 20%) was extracted with EtOAc–MeOH (1:1, v/v, 500 mL × 3) at 40 °C followed by shaking (8 h) and sonication (8 h). The extracts were filtered over anhydrous Na₂SO₄ (100 g), before being evaporated in vacuo using a rotary vacuum concentrator (50 °C) (Heidolf, Germany) to afford a dark brown viscous residue, which was considered as the crude extract of P. malabarica (55.0 g, yield on dry basis 4.58%).

3.3. Chromatographic purification and spectral analysis of pure compounds from P. malabarica
The EtOAc:MeOH crude extract of P. malabarica (45.0 g) was slurried with silica gel (4 g, 60–120 mesh), and packed into a column (1000 mm × 40 mm) containing silica (60–120 mesh). The column was initiated by increasing the polarity from 100% n-hexane followed by ethyl acetate and methanol to attain 16 fractions of 25 mL each, which were pooled six different factions (PM₁–PM₆) after TLC analysis. The fraction PM₄ (eluted at 70% EtOAc:n-hexane) was selected for further purification to afford pure bioactive metabolites since the percentage yield (3.53 g, yield 7.84%) and bioactive potentials (antioxidant and anti-inflammatory) were significantly higher for PM₄ fraction compared to other sub-fractions. This fraction was flash chromatographed (Biotage AB SP1-B1A, Uppsala, Sweden) on a silica gel column (Biotage, 230–400 mesh, 12 g, Biotage No. 25 + M) at a collection UV wavelength of 258 nm and a monitor wavelength of 264 nm with a step gradient elution of n-hexane/EtOAc/MeOH to afford 29 fractions (10 mL each), which were pooled to seven fractions (PM₄₁–PM₄₇) based upon analytical TLC. The fractions PM₄₁ and PM₄₃ were found to exhibit higher in antioxidant activity against DPPH radical and higher yield compared to other fractions. The fraction PM₄₁ was further fractionated over PTLC on silica gel GF₄₅₄ using n-hexane:EtOAc (49:1, v/v) to afford the compound 1 (90 mg; 94% purity by C₁₈ HPLC_RP, MeOH:ACN, 3:2 v/v) as major component, which was homogenous by TLC (Si gel GF₂₅₄ 15 mm; 100% n-hexane, Rₜ: 0.75). The fraction PM₄₃ was fractionated over preparatory TLC over silica GF₂₅₄ using n-hexane:EtOAc (22:3, v/v) to afford the compound 2 (118 mg; 97% purity by C₁₈ HPLC_RP, MeOH: ACN, 3:2 v/v) as the main component. Evaporation of solvents from the fractions followed by TLC (silica GF₂₅₄ using 5% EtOAc:n-hexane, Rₜ: 0.43) supported the purity.
3.3.1. 2-((E)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-2H-pyran (1)
Amorphous yellow; m.p. 169.6 °C (decom.); UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)): 265 nm (3.37); TLC (Si gel GF\(_{254}\) 15 mm; 100% \( n \)-hexane) \( R_f \): 0.75; \( R_t \) (HPLC, MeOH:ACN 3:2 v/v): 20.43 min.; IR \( \nu_{\text{max}} \) (KBr) cm\(^{-1}\) (\( \nu \) = stretching, \( \delta \) = bending, \( \rho \) = rocking vibrations): 2955.83, 2921.24, 2852.45 (C–H\( \nu \)), 1641.60 (C=C\( \nu \)), 1462.91 (C–H\( \delta \)), 1376.87, 1259.93, 1200.56, 1098.43, 1034.57 (C–O\( \nu \)), 922.42, 908.77 (C–H), 802.87, 721.66, 636.88 (C–H\( \rho \)). 1H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 6.96 (1H, s), 5.81 (1H, m, \( J = 10.21, 5.52 \) Hz), 5.35 (1H, t, \( J = 10.68, 5.52 \) Hz), 5.01 (1H, m, \( J = 14.52 \) Hz), 4.94 (1H, m, \( J = 10.21 \) Hz), 4.68 (1H, t), 2.06 (2H, t), 2.03 (2H, t), 2.02 (2H, t), 1.97 (2H, t), 1.58 (2H, m), 1.44 (3H, s), 1.37 (2H, m), 1.36 (2H, m), 1.30 (2H, m), 1.26 (2H, m), 0.88 (3H, t); 13C NMR (125 MHz, CDCl\(_3\)) \( \delta \) 139.27, 130.38, 129.91, 123.34, 114.06, 112.37, 108.30, 37.11, 33.83, 31.94, 30.41, 29.71, 29.52, 29.37, 28.97, 26.72, 22.70, 14.12. 1H–1H COSY, and HMBC data, see Table S1; HRESIMS \( m/e \) Calcd for C\(_{18}\)H\(_{30}\)ONa 262.1604, found 262.1612 ([M+]).

3.3.2. 1′-((10E)-10-(10-(pentan-4-yl)-cyclohex-4-enyl)-allyloxy)-tetrahydro-2′, 2′-dimethyl-2H-pyran (2)
Amorphous light green; m.p. 148.7 °C (decom.); UV (MeOH) \( \lambda_{\text{max}} \) (log \( \varepsilon \)): 260 nm (3.46); TLC (Si gel GF\(_{254}\) 15 mm; 5% EtOAc: \( n \)-hexane) \( R_f \): 0.43; \( R_t \) (HPLC, MeOH:ACN 3:2 v/v): 22.92 min.; IR \( \nu_{\text{max}} \) (KBr) cm\(^{-1}\) (\( \nu \) = stretching, \( \delta \) = bending, \( \rho \) = rocking vibrations): 2932.51, 2860.14 (C–H\( \nu \)), 1661.31, 1455.51 (C=C\( \nu \)), 143.18, 1372.35, 1241.84, 1187.08, 1108.18, 1042.27 (C–O\( \nu \)), 965.02 (C–H\( \rho \)), 803.72 (C–H\( \rho \)). 1H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 5.38 (1H, d, \( J = 8.58 \) Hz), 5.35 (1H, t, \( J = 9.18 \) Hz), 5.34 (1H, t, \( J = 9.18 \) Hz), 4.59 (1H, t), 4.14 (1H, d), 4.12 (1H, d), 3.64 (2H, t), 2.35 (2H, t), 2.31 (2H, m), 2.02 (2H, d), 1.99 (2H, t), 1.63 (2H, m), 1.57 (2H, m), 1.55 (2H, m), 1.48 (3H, s), 1.46 (3H, s), 1.43 (2H, m), 1.42 (2H, m), 0.89 (3H, t), 0.87 (3H, t); 13C NMR (125 MHz, CDCl\(_3\)) \( \delta \) 143.18, 139.27, 127.67, 124.47, 68.39, 65.05, 63.11, 39.07, 34.77, 32.84, 31.93, 30.25, 30.09, 29.62, 29.37, 27.23, 25.74, 22.69, 22.69, 14.15, 14.15. 1H–1H COSY, and HMBC data, see Table S1; HRESIMS \( m/e \) Calcd for C\(_{21}\)H\(_{36}\)O\(_2\) 320.2715, found 320.2722 ([M+]).

3.4. Antioxidant and anti-inflammatory activities
The antioxidant properties were evaluated by the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) (Lim et al. 2008) and 2, 2′-azino-bis-3-ethylbenzothiozoline-6-sulfonic acid diammonium salt (ABTS\(^{+}\)) radical scavenging assays (Vijayabaskar & Shiyamala 2012). In vitro anti-inflammatory properties were determined by COX-2 (Larsen et al. 1996) and 5-lipoxygenase (5-LOX) enzyme inhibition assay (Baylac & Racine 2003). The plot of scavenging and pro-inflammatory enzyme inhibitory activities were recorded, and the results were expressed as IC\(_{50}\) (the concentration of samples at which it inhibits/scavenge 50% of enzyme/radical activities and expressed in mg/mL) values.

3.5. Statistical analysis
Statistical evaluation was carried out with the Statistical Program for Social Sciences 13.0 (SPSS Inc, Chicago, USA, ver. 13.0). Analyses were carried out in triplicate, and the means of all parameters were examined for significance by analysis of variance. The level of significance for all analysis was \( p \leq 0.05 \).
4. Conclusions

Bioassay guided chromatographic fractionation of the EtOAc–MeOH extract of *P. malabarica* afforded two unprecedented meroterpeno 2H-pyranoids characterised as 2-((E)-deca-1,8-dien-10-yl)-11,12-dihydro-13-propyl-2H-pyran (1) and 1′-((10E)-10-(10-(pentan-4-yl)cyclohex-4-enyl)-allyloxy)-tetrahydro-2′,2′-dimethyl-2H-pyran (2) with potential antioxidative and anti-inflammatory activities. The antioxidant activities of the title compounds were comparable to that of α-tocopherol used in various commercial preparations. Additionally, the compounds exhibited greater activity against COX-2 than COX-1 isofrom, and therefore, exhibited greater selectivity than the synthetic non-steroidal anti-inflammatory drugs. These meroterpeno 2H-pyranoids isolated from *P. malabrica* can potentially be used as new generation antioxidant and anti-inflammatory lead pharmacophore for use against oxidative stress and inflammation.

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References


