Phytochemistry 144 (2017) 19–32

Contents lists available at ScienceDirect

Phytochemistry

journal homepage: www.elsevier.com/locate/phytochem

# Previously undescribed *frido*oleanenes and oxygenated labdanes from the brown seaweed *Sargassum wightii* and their protein tyrosine phosphatase-1B inhibitory activity

Anusree Maneesh [1, 2], Kajal Chakraborty [*, 2]

*Marine Bioprospecting Section of Marine Biotechnology Division, Central Marine Fisheries Research Institute, Ernakulam North, P.B. No. 1603, Cochin, India*

ARTICLE INFO

*Article history:*
Received 28 April 2017
Received in revised form
10 August 2017
Accepted 12 August 2017

*Keywords:*
*Sargassum wightii*
Sargassaceae
Brown seaweed
*Frido*oleanene triterpenoids
Labdane diterpenoids
Antioxidant activity
Protein tyrosine phosphatase-1B inhibitors
Anti-diabetic activity

ABSTRACT

Previously undescribed *frido*oleanene triterpenoids 2α-hydroxy-(28,29)-*frido*-olean-12(13), 21(22)-dien-20-propyl-21-hex-4′(*Z*)-enoate, 2α-hydroxy-(28,29)-*frido*-olean-12(13), 21(22)-dien-20-prop-2(*E*)-en-21-butanoate and oxygenated labdane diterpenoids 2α-hydroxy-8(17), (12*E*), 14-labdatriene, 3β, 6β, 13α-tri hydroxy 8(17), 12*E*, 14-labdatriene were purified from the ethyl acetate-methanol and dichloromethane fractions of the air-dried thalli of *Sargassum wightii* (Sargassaceae), a brown seaweed collected from the Gulf-of-Mannar of Penninsular India. Inhibitory potential of $\Delta^{12}$ oleanenes towards protein tyrosine phosphatase-1B, the critical regulator of insulin-receptor activity were found to be significantly greater ($IC_{50}$ $0.1 \times 10^{-2}$ and $0.09 \times 10^{-2}$ mg/mL, respectively) than the standard sodium metavanadate ($IC_{50}$ $0.31 \times 10^{-2}$ mg/mL). *Frido*oleanene triterpenoids displayed greater antioxidant activities ($IC_{50DPPH}$ 0.16–0.18 mg/mL) than the commercially available antioxidants, butylated hydroxytoluene and α-tocopherol ($IC_{50DPPH}$ 0.25 and 0.63 mg/mL, respectively). In general, the oxygenated labdane diterpenoids displayed significantly lesser antioxidant and tyrosine phosphatase-1B inhibitory properties than those exhibited by the *frido*oleanenes. Bioactivities of the titled compounds were primarily determined by the electronic and lipophilic parameters and not by the steric descriptors. Molecular docking simulations and kinetic studies were employed to describe the tyrosine phosphatase-1B inhibitory mechanism. The previously undescribed *frido*oleanene triterpenoids might be used as potential anti-hyperglycaemic pharmacophore leads to reduce the risk of elevated postprandial glucose levels.



## 1. Introduction

Terpenoid group of compounds constitute the large and diverse class of natural products with potent biological dimensions characterized by antioxidant, anti-inflammatory, antibacterial, antifungal, antiproliferative, antitumor and antidiabetic properties (Hegazy et al., 2015; Lee et al., 2017). There were reports of various terpenoids with distinctive carbon frameworks from marine organisms (Blunt et al., 2005), in which those from seaweeds were found to possess potential bioactive properties and novel structural diversity. The halogenated sesquiterpenes were reported from the red seaweed *Laurencia dendroidea* (de Oliveira et al., 2015), whereas various diterpenes were previously characterized from the Chlorophytan seaweeds *Caulerpa trifaria* (Handley and Blackman, 2000), and *Ulva fasciata* (Chakraborty et al., 2010) with potential anti-infective properties. Brown seaweeds were found to harbor structurally diverse terpenoids that constitutes a protective approach against herbivorous organisms in marine ecosystem (Paul et al., 2001). Four novel acyclic diterpenes were isolated from the brown seaweed *Bifurcaria bifurcata* collected from the Atlantic coast of Morocco (Culioli et al., 2001). Stypolactone, a diterpenoid of mixed biogenesis has been isolated from the brown seaweed *Stypopodium zonale* (Dorta et al., 2002). Two diterpenoids with a novel skeleton, dictyterpenoids A and B, were isolated from the brown seaweed *Dilophus okamurae* collected from the Japan Sea Coast and displayed antifeedant activity against young abalone (Suzuki et al., 2002). Cytotoxic, antiviral and algicidal activities of the diterpenoids and sesquiterpenoids from brown seaweeds were reported previously (Gupta and Abu-Ghannam, 2011). Antioxidant activities of the oxygenated tetraterpenes in brown seaweeds were

* Corresponding author.
*E-mail address:* kajal_cmfri@yahoo.com (K. Chakraborty).
[1] Department of Chemistry, Mangalore University, Mangalagangothri, 574199, Karnataka State, India.
[2] Equal contribution.

http://dx.doi.org/10.1016/j.phytochem.2017.08.011
0031-9422/

documented in previous literature (Xia et al., 2013). The terpenoid natural product chemistry, particularly from *Sargassum* sp. was found to include meroditerpenoids from seaweed *Sargassum fallax* (Reddy and Urban, 2009) and *Sargassum siliquastrum* (Jung et al., 2008). A series of endothelin antagonists, the meroditerpenoids nahocols and isonahocols were isolated from *Sargassum autumnale* (Tsuchiya et al., 1998).

Development and advancement of type-2 diabetes and its related complexities were mainly deterred by the increment of oxidative stress (Ceriello, 2000). Insulin resistance due to the alleviated signalling from the receptors were found to play a major role in the evolution of type-2 diabetes. The dephosphorylation of the receptor β-subunit at the inter-membrane space was found to be due to the negative regulation of receptors and the leptin signaling system, accounted by the intracellular enzyme, protein tyrosine phosphatase-1B (PTP-1B). The bioactive specialized metabolites capable of inhibiting PTP-1B were found to be potential activators of insulin and thus favourable for the treatment of type-2 diabetes (Goldstein et al., 1998). Naturally derived pentacyclic oleanene triterpenoids were reported to be a class of pharmacologically active specialized products, which were reported to act on glucose metabolism and reduce the risk of postprandial hyperglycemia by the inhibition of α-amylase, α-glucosidase and PTP-1B enzymes (Lee et al., 2017). Oleanane-type triterpene derivatives from the methanol extract of *Astilbe koreana* (Na et al., 2006) and *Sambucus adnata* plants (Sasaki et al., 2011) were reported to inhibit PTP-1B enzyme. Labdane diterpene from *Hedychium spicatum* (Reddy et al., 2009) and *Alpinia nigra* (Ghosh and Rangan, 2015) displayed potential α-glucosidase inhibitory activities.

Current medications used for the treatment of diabetes, such as biguanides, sulfonlyureas, meglitinides and thiazolidinediones were reported to produce adverse gastrointestinal effects, liver toxicity and other pronounced side effects in their long-term clinical use (Tamrakar et al., 2014). Recently, there is a growing awareness to consume seaweeds in daily diet providing health benefits and reduced risk of diabetics and related complications, particularly in the south East Asian countries (Iso, 2011). The present study aimed to isolate four previously undescribed terpenoids, 2α-hydroxy-(28,29)-*frido*-olean-12(13),21(22)-dien-20-propyl-21-hex-4′(*Z*)-enoate (**1**), 2α-hydroxy-(28, 29)-*frido*-olean-12(13), 21(22)-dien-20-prop-2(*E*)-en-21-butanoate (**2**) and oxygenated labdane di terpenoids named, 2α-hydroxy-8(17), 12 *E*, 14-labdatriene (**3**), 3β, 6β, 13α-tri hydroxy 8(17), 12*E*, 14-labdatriene (**4**) from the crude solvent extract of the brown seaweed *Sargassum wightii* Greville ex J. Agardh (Sargassaceae), with the structures that has not been identified or postulated before. Their structures were elucidated by combined spectroscopic analyses, such as two-dimensional nuclear magnetic resonance (NMR) and mass spectroscopic experiments. Antidiabetic activities of these previously undescribed terpenoids were assessed by *in vitro* α-glucosidase, α-amylase and PTP-IB inhibitory activities, whereas antioxidative activities were determined by various *in vitro* radical scavenging assays. Different physicochemical parameters were used to corroborate the structure-activity correlations of the titled compounds. The modes of inhibition of PTP-IB by the isolated compounds (**1**–**4**) were determined by conducting kinetic studies and molecular docking simulations.

## 2. Results and discussion

### 2.1. Spectroscopic characterization of fridooleanene triterpenoids and labdane diterpenoids isolated from S. wightii

The *frido*oleanene triterpenoid 2α-hydroxy-(28,29)-*frido*-olean-12(13), 21(22)-dien-20-propyl-21-hex-4′(*E*)-enoate (**1**) was isolated as a white amorphous powder having molecular formula of $C_{36}H_{56}O_3$ as ascertained by high resolution mass spectroscopy {HR (ESI) MS *m/z* found 537.4231 $[M+H]^+$, calcd for $C_{36}H_{57}O_3$ 537.4229}, showing nine indices of hydrogen deficiency. The $^1$H NMR (Fig. S1) in conjugation with $^{13}$C NMR (Fig. S2) data (Table 1A) revealed the presence of five singlet methyl protons at $\delta_H$ 0.98 (H-23), 1.0 (H-24), 1.26 (H-25), 1.08 (H-26) and 0.69 (H-27), which were in accordance with the literature values reported for tertiary methyl groups of olean-12-ene-triterpenoid framework (Mimaki et al., 2003; Wang et al., 2014). The $^1$H NMR signals corresponding to oxymethine proton at $\delta_H$ 3.53 (H-2) and a typical olean-12-ene vinylic proton at $\delta_H$ 5.37 (H-12) were also apparent, and in accordance with the values reported for the oleanene type triterpene arjunic acid (Ponou et al., 2011) and also with a noroleanene 2α, 3α-dihydroxy-30-noroleana-12,20(29)-dien-28-oic acid (Mimaki et al., 2003). The $^{13}$C NMR spectrum demonstrated a total of 36 carbon signals, comprising those for two oxygen bearing carbons at $\delta_C$ 71.8 (C-2) and 179.24 (C-1′), and the resonances corresponding to quaternary, methine, methylene and methyl carbons were validated by analyzing the distortionless enhancement by polarisation transfer ($DEPT_{135}$) spectrum (Fig. S3). These spectral details and the literature precedents supported **1** to be a substituted pentacyclic hydroxyl triterpenoid (Mimaki et al., 2003; Ponou et al., 2011).

The structural attributions of **1** were confirmed by the analysis of two dimensional-NMR data comprising of proton correlation spectroscopy ($^1$H-$^1$H COSY; Fig. S4), heteronuclear single quantum coherence spectroscopy (HSQC; Fig. S5) and heteronuclear multiple-bond correlation spectroscopy (HMBC; Fig. S6) experiments. The compound was structurally related to arjunic acid (Ponou et al., 2011) except the differences perceived in the NMR signals corresponding to E-ring skelton of the pentacyclic framework, and also in the number of the hydroxyl groups. A distinct peak at $\delta_H$ 3.53 (1H, *m*) in the $^1$H NMR spectrum was accounted for the hydroxyl substitution at C-2 and this assignment was made by the $^1$H-$^1$H COSY cross peaks displayed between H-2/H-3 ($\delta_H$ 3.53/$\delta_H$ 2.2) and H-2/H-1 ($\delta_H$ 3.53/$\delta_H$ 1.65) (Fig. 1A) and also by the intense HMBC correlation between $\delta_H$ 2.2 (H-3)/$\delta_C$ 71.8 (C-2) (Fig. 1B). Presence of oxymethine proton at C-2 was also supported by the previously described literature (Ponou et al., 2011). The existence of −OH proton at $\delta_H$ 3.53 (positioned at C-2 of the A ring) was further validated by $D_2O$ exchange. Two methyl groups at C-4 resonating at $\delta_H$ 1.0 (C-23) and $\delta_H$ 0.98 (C-24) were *geminal* and were assigned according to the strong HMBC correlations between $\delta_H$ 1/$\delta_C$ 39.74 (H-24/C-4), 0.98/39.74 (H-23/C-4), 1/14.2 (H-24/C-23), 0.98/22.22 (H-23/C-24), and also by the previously reported literatures (Awan et al., 2013; Hassan et al., 2012). The angular methyl group at C-17 ($H_3$-28) and one of the *gem*-methyl groups at C-20 ($H_3$-30) in the basic oleanene skelton (Hassan et al., 2012) were found to be shifted to the $sp^3$ hybridized carbons at $\delta_C$ 29.08 (C-29) and $\delta_C$ 25.7 (C-28), respectively in compound **1**, which altogether constituted the attachment of propyl side chain at C-20. This assignment was supported by the strong $^1$H-$^1$H COSY correlation between $\delta_H$ 1.84 (H-20)/$\delta_H$ 1.07 (H-29), $\delta_H$ 1.18 (H-28)/$\delta_H$ 0.88 (H-30) and also by the strong HMBC cross peaks between H-20/C-30 ($\delta_H$ 1.84/δc 14.11), H-20/C-21 ($\delta_H$ 1.84/δc 146.98), H-29/C-20 ($\delta_H$ 1.07/δc 34.79). Intense $^1$H-$^1$H COSY cross peaks between H-12/H-11 ($\delta_H$ 5.37/$\delta_H$ 1.96) and $^1$H-$^{13}$C long range correlation of H-18 ($\delta_H$ 1.5)/C-13 (δc 140), H-11 ($\delta_H$ 1.96)/C-12 (δc 121), H-12 ($\delta_H$ 5.37)/C-14 (δc 42.4) and H-12 ($\delta_H$ 5.37)/C-18 (δc 36.4) was persistent with the presence of an olefinic bond between C-12 (δc 121/$\delta_H$ 5.37) and C-13. This attribution was in accordance with the occurrence of olefinic bond in the C-ring (Luo et al., 2015; Mimaki et al., 2003; Ponou et al., 2011). The location of olefinic bond at C-21 and C-22 was established by the strong HMBC correlations between H-20 ($\delta_H$ 1.84)/C-21 ($\delta_C$ 146.9) and also by the intense $^1$H-$^1$H COSY cross peaks between H-22 ($\delta_H$