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Research Article

Cembrane diterpenes as chemotaxonomical markers for *Sinularia flexibilis*

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Abstract
Chemical extraction of the soft coral *Sinularia flexibilis* collected from Mantanani Island, Kota Belud, Sabah has led to the isolation of ten known cembrane diterpenes. The chemical structures of isolated compounds were elucidated based on ^{1}H-NMR and ^{13}C-NMR spectroscopic data. This is the first record of chemical analysis of *S. flexibilis* from the waters of Malaysia. Identical secondary metabolites isolated from *S. flexibilis* from other regions suggest the use of these cembrane diterpenes as possible chemotaxonomical markers for this genus of soft coral.

Keywords: Soft coral, *Sinularia*, Mantanani Island, Secondary metabolite, Cembrane diterpenes, Chemotaxonomical marker

Introduction
Complex interaction between the marine environment and marine organisms leads to predation, competition for space or habitat, protection from disease or infection and directly imposes pressure to survive among slow-moving, sessile soft bodied marine invertebrates such as soft corals, sea slugs and sponges. Due to impaired physical characteristics and sedentary life, these invertebrates' biosynthesize secondary metabolites to chemically protect themselves from predators and colonizers (Li et al., 2006; Bonnard et al., 2010).

One interesting marine invertebrate are the soft coral of the genus *Sinularia* that is known as a prolific producer of secondary metabolites among marine invertebrates (Yu et al., 2006). A total of 90 species of *Sinularia* have been identified worldwide and close to 50 species have been chemically studied (Chen et al., 2012) to produce terpenoids, prostaglandins, as well as steroids. Over the past 30 years, more than 15,000 novel secondary metabolites have been discovered from these organisms (Li et al., 2006).

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In addition, compounds from soft corals have also been reported to show anti-fungal, anti-bacterial and anti-bleaching potentials in nature, thus proposed as ecological chemicals of importance for these organisms (Chao et al., 2006; Ishii et al., 2010a). In-depth biomedical and pharmaceutical studies have also shown interesting advancement of soft coral metabolites as anti-cancer, anti-microbial, anti-fungal, anti-inflammatory, anti-coagulant, anti-platelet and anti-viral agents (Kamel & Slattery, 2005; Chao et al., 2006; Arepalli et al., 2009; Chen et al., 2010).

To date, numerous publications are currently available on the chemistry of *Sinularia flexibilis* of Australian, Japanese and Indian (Kamel & Slattery, 2005; Chen et al., 2010; Anjeneyulu et al., 1997) and extensive research on *S. flexibilis* from the Formosan waters (Hu et al, 2013a; 2013b; Duh et al., 1998a; 1998b; Chen et al., 2010; Lin et al., 2013; 2009; Lo et al., 2010; 2009). To complement studies conducted in these areas, we initiated an investigation to document the chemical diversity of *S. flexibilis* in the coastal waters of Sabah in addition to the currently available report by Vairappan & company (2012).

This report focuses on the variety of secondary metabolites in one population of *S. flexibilis* collected from the coastal waters of Mantanani Island, Sabah. Chemical extraction, separation and isolation led to the identification of ten compounds (**1**-**10**) that have been previously reported in *S. flexibilis* from Taiwanese waters. Successful isolation and identification of the ten compounds; cembrene A (Herin & Tursch, 1976) (**1**), diepoxycembrene A (Qin, et al., 2008) (**2**), flexilarin B (Lin et al., 2009) (**3**), 5-dehydrosinulariolide (Hu et al., 2013a) (**4**), flexibilide/sinularin (Weinheimer et al., 1977) (**5**), (1*R*,3S,4S,7E,11*E*)-3,4-epoxycembra-7,11,15-triene (Bowden et al., 1981) (**6**) sinulariolide (Tursch et al., 1975) (**7**), 11-epoxysinulariolide (Mori et al., 1983) (**8**), sinulariolide acetate (Tursch et al., 1975) (**9**), dihydrosinuflexolide (Duh et al., 1998b) (**10**) from *S. flexibilis* suggests the possibility of using these cembrane diterpenes as chemotaxanomical markers for this soft coral species. The chemical data of the isolated compounds were compared to those of up to date chemical database such as Science Finder, Marin Lit and other publication. As such, this paper reports the isolation of ten cembrane diterpenes from the Sabahan species of *S. flexibilis* along with its spectroscopic chemical data.

Materials and Methods

Collection

The *Sinularia flexibilis* population was collected from a coral reef at 15 m

depth in the waters of Indian Brother's Reef (Mantanani Island, Kota Belud, Sabah) (6°43'15.89"N 116°20'36.38"E). The samples were photographed underwater, collected by Scuba divers and a representative specimen (SC0013 MI) was deposited at the Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah. The samples were weighed and cut into pieces. Then the samples were brought back to the laboratory under cool conditions (4 °C) and processed according to the procedures described by Ishii et al. (2009a; 2010b).

Extraction and isolation

The specimen was chopped and soaked in methanol (MeOH) at room temperature for approximately four days. The resulting MeOH extract was concentrated *in vacuo* and the concentrate was partitioned between ethyl acetate (EtOAc) and H_2O. The EtOAc layer was further concentrated and partitioned between hexane (Hex) and 90 % MeOH yielding its resulting Hex and 90 % MeOH extracts. The hexane and 90 % MeOH extract was independently fractionated using Silica gel column chromatography with a step gradient of Hex and EtOAc with the ratio 9:1, 8:2, 7:3, 6:4, 1:1 and $CHCl_3$:MeOH:H_2O (65:25:4). The resulting fractions were then further subjected to Preparative Thin Layer Chromatography (PTLC) and High Performance Liquid Chromatography (HPLC) to isolate the ten cembrane diterpenes reported in this paper. Compounds **1** to **10** were subjected to ^{1}H-NMR, ^{13}C-NMR and 2D NMR measurements such as ^{1}H-^{1}H COSY, HSQC, HMBC and NOESY. High-resolution mass spectroscopy data was measured using Shimadzu LC-MS-IT-TOF. Other physical characteristics were obtained as described by Vairappan et al. (2001) and Ishii et al. (2009b).

Results

Approximately 2 kg of *Sinularia flexibilis* live specimen was collected from a population at the Indian Brothers' Reef, Mantanani Island (Figure 1). Upon extraction, a total of 2.58 g of crude Hexane and 1.46 g 90 % MeOH extracts were obtained. Both the hexane and 90 % MeOH crude extracts were profiled using High Performance Thin Layer Chromatography (HPTLC) and High Performance Liquid Chromatography (HPLC) prior to the separation and isolation of its secondary metabolites.

High Performance Thin Layer Chromatography (HPTLC) chromatogram revealed presence of multiple metabolites in both extracts. Figure 2 shows the HPTLC profiles of the respective crude extracts and provides preliminary information on the presence of potential secondary metabolites in both the crude extracts. Upon detailed analysis, hexane crude extract seems to contain four major metabolites (Rf: 0.77, 0.66, 0.49 and 0.34**)** as indicated by the four dark bands

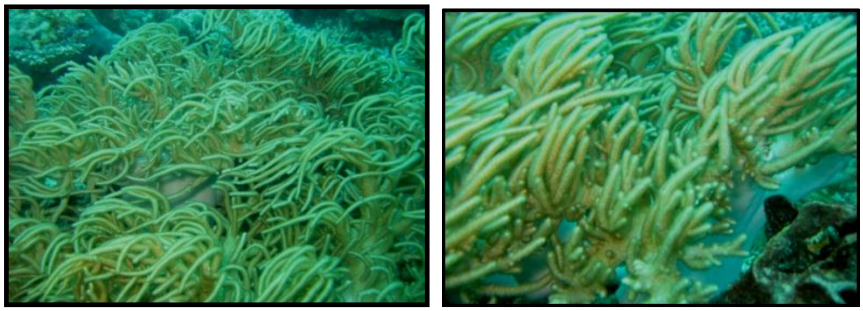

Figure 1. Underwater images of *Sinularia flexibilis*

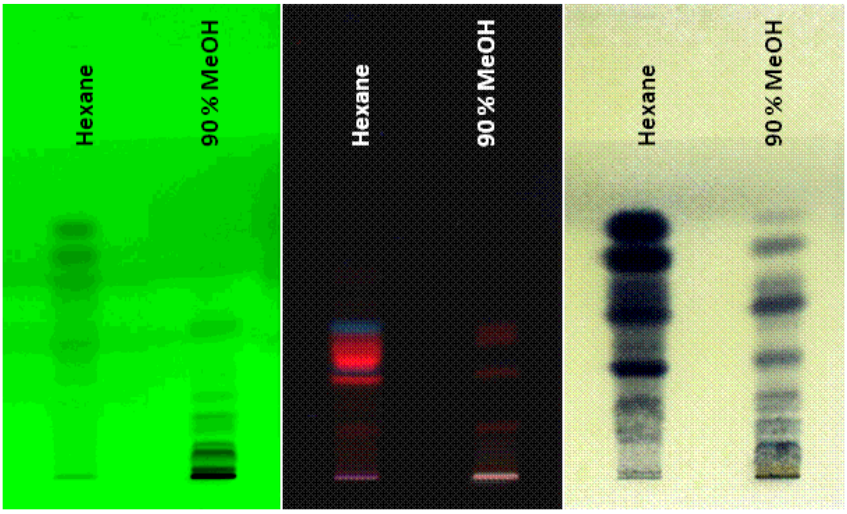

Figure 2. HPTLC Chemical fingerprint of *Sinularia flexibilis* crude extracts. (From left; λ = 254 nm non-derivatized; 366 nm non-derivatized; derivatized)

upon molybdophosphoric acid visualization and heating. Presence of additional minor metabolites was also evident in the extract through the visualization of overlapping bands in the HPTLC profile.

Similarly, 90 % MeOH extract also revealed the presence of dark blue spots upon derivatization with molybdophosphoric acid, and multiple dark bands were observed with lower intensity representing metabolites with lower concentration in the extracts. Detailed comparison between both the profiles suggested the presence of common metabolites in both extracts. Information obtained from this HPTLC profile was used as a reference in deciding the solvent mixtures to fractionate the crude extracts via SiO_2 gel column

chromatography.

Column chromatography fractionation was done using a silica gel absorbent with a step gradient of Hex and EtOAc with the ratio of 9:1 (F1), 8:2 (F2), 7:3 (F3), 6:4 (F4), 1:1 (F5) and $CHCl_3$:MeOH:H_2O (65:25:4) (F6). Resulting fractions were subjected to repetitive Preparative Thin Layer Chromatography separation to isolate 10 different cembrane diterpenes. A total of four compounds were isolated from the hexane extract; cembrene A (**1**), diepoxycembrene A (**2**), flexibilide/sinularin (**3**), (1*R*,3S,4S,7E,11*E*)-3,4-epoxycembra-7,11,15-triene (**4**) while the remaining compounds were isolated from the 90 % MeOH extract; flexilarin B (**5**), 5-dehydrosinulariolide (**6**), sinulariolide (**7**), 11-epoxysinulariolide (**8**), sinulariolide acetate (**9**), dihydrosinuflexolide (**10**). Figure 3 shows the chemical structures of the isolated compounds. Initial structure elucidation was conducted independently and the preliminary structures were compared with information available in Science Finder and Marin Lit. Since all 10 structures were recorded in these databases, their respective publications were downloaded and detailed chemical shift data comparison revealed their data to be similar to those published.

Structure elucidation of the isolated compounds was determined thorough deduction of 1D and 2D-NMR, thus the isolated compounds were suggested to be cembrane type diterpenes. Key characteristics of the isolated compounds are the presence of a basic three methyl substituted 14-membered ring and an iso-propyl unit in most of the compounds. Cyclization of a geranylgeraniol-derived precursor between 1 and 14 generates the 14 membered diterpenoid called cembrene. Members of this genus are known to mainly biosynthesize isopenyl type cembranoid diterpenes as reported in this paper. The absence of this moiety in some compounds is replaced with either α-methylenic lactone or ε- lactone ring, which is a result of structural changes in the position of double bonds, epoxidation, allylic and isopropyl oxidation and carbon cyclization. Spectroscopic chemical data of the isolated compounds are as follows;

Cembrene A (**1**); $C_{20}H_{32}$; whitish powder; $[\alpha]_D^{28}$ -36.0° ($CHCl_3$; 0.23); FT-IR (cm^{-3}); 3075, 2930, 1642, 1383, 891; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.03 (1H, m, H-1), 1.37 (1H, m, H-2), 1.66 (1H, m, H-2), 1.78 (1H, br m, H-3), 1.93 (1H, m, H-3), 5.06 (1H, dd, *J* = 6.19, 6.87 Hz, H-5), 2.12 (1H, m, H-6), 2.05 (1H, m, H-7), 2.07 (1H, m, H-7), 4.98 (1H, dd, *J* = 6.19, 6.19 Hz H-9), 2.16 (1H, dd, *J* = Hz, H-10), 2.26 (1H, m, H-10), 2.13 (1H, m, H-11), 2.17 (1H, m, H-11), 5.19 (1H, dd, *J* = 6.85, 8.27 Hz, H-13), 1.95 (1H, m, H-14), 1.98 (1H, m, H-14), 4.65 (1H,

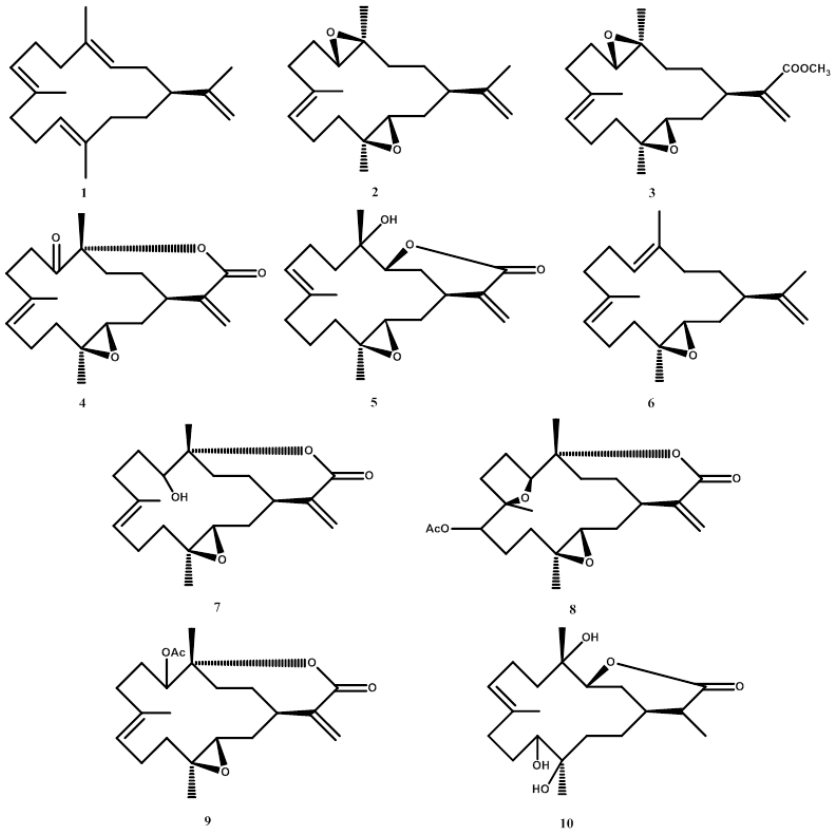

Figure 3. Chemical structures of 10 cembranes from *Sinularia flexibilis*

d, *J* = 2.00 Hz, H-16), 4.71 (1H, d, *J* = 1.09 Hz, H-16), 1.66 (3H, s, H-17), 1.56 (3H, s, H-18), 1.59 (3H, s, H-19), 1.57 (3H, s, H-20); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 46.7 (C-1), 28.9 (C-2), 34.7 (C-3), 134.6 (C-4), 122.5 (C-5), 24.4 (C-6), 40.1 (C-7), 134.1 (C-8), 126.6 (C-9), 25.6 (C-10), 39.6 (C-11), 135.5 (C-12), 124.7 (C-13), 33.1 (C-14), 149.9 (C-15), 110.8 (C-16), 19.9 (C-17), 18.7 (C-18), 15.9 (C-19), 16.2 (C-20). Chemical data matched report by Herin & Tursch, 1976.

Diepoxycembrene A (**2**); $C_{20}H_{32}O_2$; whitish powder; $[\alpha]_D^{28}$ +36.0° ($CHCl_3$; 0.23); FT-IR (cm^{-3}); 3021, 1645, 1252, 885; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.13 (1H, m, H-1), 1.44 (1H, m, H-2), 1.54 (1H, m, H-2), 1.26 (1H, m, H-3), 1.68 (1H, m, H-3), 2.70 (1H, dd, *J* = 3.44, 3.44 Hz, H-5), 1.39 (1H, m, H-6), 2.02 (1H, m, H-6), 2.04 (1H, m, H-7), 2.23 (1H, br m, H-7), 5.16 (1H, dd, *J* = 6.19, 6.19 Hz, H-9), 2.06 (1H, m, H-10), 2.11 (1H, m, H-10), 1.47 (1H, m, H-11), 1.98 (1H, m, H-11), 2.80 (1H, dd, *J* = 4.12, 4.12 Hz, H-13), 1.29 (1H, m, H-14), 1.93 (1H, m, H-14), 4.61 (1H, br s, H-16), 4.75 (1H, brs, H-16), 1.65 (3H, s, H-17), 1.24 (3H, s, H-

18), 1.61 (3H, s, H-19), 1.21 (3H, s, H-20); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 42.4 (C-1), 27.2 (C-2), 33.5 (C-3), 61.4 (C-4), 62.4 (C-5), 25.2 (C-6), 36.6 (C-7), 134.8 (C-8), 127.6 (C-9), 23.3 (C-10), 37.9 (C-11), 60.9 (C-12), 61.8 (C-13), 32.4 (C-14), 147.6 (C-15), 111.4 (C-16), 21.1 (C-17), 18.2 (C-18), 16.3 (C-19), 18.2 (C-20). Chemical data matched report by Qin et al., 2008.

Flexilarin B (**3**); $C_{21}H_{32}O_4$; whitish powder; $[\alpha]_D^{28}$ +79.0° ($CHCl_3$; 0.2); FT-IR (cm^{-3}); 3395, 2976, 2926, 1735; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.86 (1H, m, H-1), 1.57 (1H, m, H-2), 1.74 (1H, m, H-2), 1.18 (1H, m, H-3), 1.66 (1H, m, H-3), 2.66 (1H, d, *J* = 3.44, 3.44 Hz, H-5), 1.38 (1H, m, H-6), 2.09 (1H, m, H-6), 2.11 (1H, m, H-7), 2.25 (1H, br m, H-7), 5.19 (1H, dd, *J* = 5.95, 6.42 Hz, H-9), 2.08 (1H, m, H-10), 2.12 (1H, m, H-10), 1.59 (1H, m, H-11), 1.99 (1H, m, H-11), 2.83 (1H, dd, *J* = 3.44, 4.12 Hz, H-13), 1.34 (1H, m, H-14), 2.06 (1H, m, H-14), 5.49 (1H, br s, H-16), 6.28 (1H, br s, H-16), 1.22 (3H, s, H-18), 1.64 (3H, s, H-19), 1.28 (3H, s, H-20), 3.77 (OCH_3); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 35.9 (C-1), 28.0 (C-2), 33.7 (C-3), 61.6 (C-4), 62.6 (C-5), 25.2 (C-6), 36.7 (C-7), 134.9 (C-8), 125.1 (C-9), 22.8 (C-10), 37.5 (C-11), 60.9 (C-12), 60.8 (C-13), 32.1 (C-14), 143.3 (C-15), 127.2 (C-16), 168.1 (C-17), 17.7 (C-18), 16.0 (C-19), 18.5 (C-20), 52.7 (C-21). Chemical data matched report by Lin et al., 2009.

5-dehydrosinulariolide (**4**); $C_{20}H_{28}O_4$; whitish powder; $[\alpha]_D^{28}$ +93.0° ($CHCl_3$; 0.79); FT-IR (cm^{-3}); 3411, 1713; 1H-NMR ($CDCl_3$, 600 MHz) δ: 1.85 (1H, m, H-1), 2.18 (1H, br m, H-2), 2.27 (1H, m, H-2), 1.92 (1H, m, H-3), 2.44 (1H, ddd, *J* = 6.19, 15.1, 21.3 Hz, H-3), 2.66 (1H, m, H-6), 3.14 (1H, ddd, *J* = 8.94, 19.9, 30.2 Hz, H-6), 1.98 (1H, m, H-7), 2.71 (1H, m, H-7), 5.06 (1H, dd, *J* = 6.87, 6.87 Hz, H-9), 2.16 (1H, dd, *J* = 5.95, 6.42 Hz, H-10), 2.17 (1H, m, H-10), 1.14 (1H, m, H-11), 2.06 (1H, m, H-11), 2.68 (1H, m, H-13), 1.49 (1H, m, H-14), 1.87 (1H, m, H-14), 5.48 (1H, br s, H-16), 6.31 (1H, br s, H-16), 1.47 (3H, s, H-18), 1.62 (3H, s, H-19), 1.17 (3H, s, H-20); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 35.5 (C-1), 31.6 (C-2), 33.9 (C-3), 90.8 (C-4), 209.9 (C-5), 34.3 (C-6), 30.7 (C-7), 135.7 (C-8), 123.4 (C-9), 25.1 (C-10), 38.2 (C-11), 61.3 (C-12), 62.9 (C-13), 33.2 (C-14), 144.3 (C-15), 126.5 (C-16), 168.1 (C-17), 30.4 (C-18), 17.9 (C-19), 16.8 (C-20). Chemical data matched report by Hu et al., 2013a.

Flexibilide/Sinularin (**5**); $C_{20}H_{30}O_4$; whitish powder; $[\alpha]_D^{28}$ -127.0° ($CHCl_3$; 0.23); FT-IR (cm^{-3}); 3600, 1735, 1668; ^{1}H-NMR (CDCl3, 600 MHz) δ: 2.50 (1H, m, H-1), 1.37 (1H, m, H-2), 2.09 (1H, m, H-2), 2.8 (1H, dd, *J* = 4.12, 3.44 Hz, H-3), 1.18 (1H, m, H-5), 2.05 (1H, m, H-5), 1.46 (1H, m, H-6), 2.12 (1H, m, H-6), 2.04 (1H, m, H-7), 2.31 (1H, br m, H-7), 5.23 (1H, dd, *J* = 8.25, 7.56 Hz, H-9), 1.86 (1H, m, H-10), 2.37 (1H, m, H-10), 1.62 (1H, m, H-11), 1.84 (1H, m, H-11), 3.97 (1H,

dd, *J* = 10.31, 10.31 Hz, H-13), 1.50 (1H, m, H-14), 1.91 (1H, m, H-14), 5.69 (1H, d, *J* = 2.06 Hz, H-16), 6.47 (1H, d, *J* = 2.06 Hz, H-16), 1.31 (3H, s, H-18), 1.66 (3H, s, H-19), 1.44 (3H, s, H-20); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 34.4 (C-1), 28.5 (C-2), 63.6 (C-3), 59.6 (C-4), 35.4 (C-5), 26.0 (C-6), 36.6 (C-7), 135.2 (C-8), 126.3 (C-9), 23.3 (C-10), 39.3 (C-11), 74.8 (C-12), 83.4 (C-13), 33.7 (C-14), 140.8 (C-15), 128.6 (C-16), 167.9 (C-17), 16.1 (C-18), 16.1 (C-19), 25.6 (C-20). Chemical data matched report by Weinheimer et al., 1977.

(1*R*,3S,4S,7E,11*E*)-3,4-epoxycembra-7,11,15-triene (**6**); $C_{20}H_{32}O$; whitish powder; $[\alpha]_D^{28}$ +49.0° ($CHCl_3$; 0.22); FT-IR (cm^{-3}); 3070, 2960-2860, 1645, 1450, 1385, 890; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.24 (1H, m, H-1), 1.51 (1H, m, H-2), 1.66 (1H, m, H-2), 1.90 (1H, ddd, *J* = 11.2, 11.2, 23.4 Hz, H-3), 2.03 (1H, m, H-3), 5.08 (1H, m, H-5), 2.16 (2H, m, H-6), 2.01 (1H, m, H-7), 2.15 (1H, m, H-7), 5.09 (1H, m, H-9), 2.06 (1H, m, H-10), 2.18 (1H, m, H-10), 1.29 (1H, m, H-11), 2.04 (1H, m, H-11), 2.83 (1H, dd, *J* = 2.75, 3.44 Hz, H-13), 1.33 (1H, ddd, *J* = 12.3, 12.3, 24.1 Hz, H-14), 1.74 (1H, ddd, *J* = 11.5, 11.5, 24.7 Hz, H-14), 4.62 (1H, br s, H-16), 4.67 (1H, br s, H-16), 1.62 (3H, s, H-17), 1.58 (3H, s, H-18), 1.60 (3H, s, H-19), 1.23 (3H, s, H-20); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 40.9 (C-1), 30.4 (C-2), 35.3 (C-3), 133.9 (C-4), 124.9 (C-5), 25.1 (C-6), 40.2 (C-7), 135.8 (C-8), 124.6 (C-9), 24.4 (C-10), 38.9 (C-11), 61.5 (C-12), 63.9 (C-13), 34.3 (C-14), 149.2 (C-15), 111.3 (C-16), 19.1 (C-17), 17.8 (C-18), 16.5 (C-19), 17.6 (C-20). Chemical data matched report by Bowden et al., 1981.

Sinulariolide (**7**); $C_{20}H_{30}O_4$; whitish powder; $[\alpha]_D^{28}$ +76.0° ($CHCl_3$; 0.7); FT-IR (cm^{-3}); 3460, 1720, 1630; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 2.42 (1H, m, H-1), 1.28 (1H, m, H-2), 2.32 (1H, m, H-2), 1.79 (1H, ddd, *J* = 5.50, 6.19, 6.19 Hz, H-3), 2.29 (1H, m, H-3), 4.06 (1H, dd, *J* = 9.62, 9.62 Hz, H-5), 1.36 (1H, m, H-6), 2.05 (1H, m, H-6), 2.18 (1H, m, H-7), 2.24 (1H, ddd, *J* = 13.7, 13.7, 27.5 Hz, H-7), 5.12 (1H, dd, *J* = 8.94, 8.94 Hz, H-9), 2.15 (1H, m, H-10), 2.36 (1H, m, H-10), 1.23 (1H, m, H-11), 2.17 (1H, m, H-11), 2.92 (1H, dd, *J* = 2.75, 2.75 Hz, H-13), 1.48 (1H, ddd, *J* = 11.7, 11.7, 24.1 Hz, H-14), 1.94 (1H, ddd, *J* = 2.57, 2.75, 2.75 Hz, H-14), 5.41 (1H, br s, H-16), 6.27 (1H, br s, H-16), 1.32 (3H, s, H-18), 1.59 (3H, s, H-19), 1.19 (3H, s, H-20); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 35.8 (C-1), 32.0 (C-2), 32.3 (C-3), 87.8 (C-4), 69.3 (C-5), 27.5 (C-6), 36.5 (C-7), 135.5 (C-8), 127.3 (C-9), 25.6 (C-10), 38.8 (C-11), 61.2 (C-12), 64.6 (C-13), 33.7 (C-14), 145.0 (C-15), 124.9 (C-16), 169.8 (C-17), 23.5 (C-18), 16.4 (C-19), 16.7 (C-20). Chemical data matched report by Tursch et al., 1975.

11-epoxysinulariolide (**8**); $C_{22}H_{32}O_6$; whitish powder; $[\alpha]D^{28}$ +31.0° ($CHCl_3$; 0.5); FT-IR (cm^{-3}); 1735, 1720, 1622, 905; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 3.20 (1H, m,

H-1), 1.32 (1H, m, H-2), 2.56 (1H, m, H-2), 1.86 (1H, ddd, *J* = 5.50, 6.19, 6.19 Hz, H-3), 1.98 (1H, m, H-3), 4.35 (1H, ddd, *J* = 2.06, 5.50, 11.7 Hz, H-5), 1.65 (1H, m, H-6), 2.21 (1H, m, H-6), 1.68 (1H, m, H-7), 1.70 (1H, m, H-7), 5.15 (1H, dd, *J* = 8.94, 8.94 Hz, H-9), 1.74 (1H, m, H-10), 1.84 (1H, m, H-10), 1.98 (1H, m, H-11), 3.37 (1H, dd, *J* = 4.12, 4.12 Hz, H-13), 1.38 (1H, ddd, *J* = 11.0, 11.0, 22.7 Hz, H-14), 2.05 (1H, m, H-14), 5.41 (1H, br s, H-16), 6.24 (1H, br s, H-16), 1.22 (3H, s, H-18), 1.14 (3H, s, H-19), 1.22 (3H, s, H-20), 2.04 (3H, s, H-21); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 36.3 (C-1), 33.8 (C-2), 30.5 (C-3), 88.8 (C-4), 84.1 (C-5), 27.4 (C-6), 35.5 (C-7), 86.0 (C-8), 78.5 (C-9), 27.9 (C-10), 34.9 (C-11), 60.6 (C-12), 61.4 (C-13), 34.2 (C-14), 146.0 (C-15), 124.3 (C-16), 170.2 (C-17), 29.9 (C-18), 17.7 (C-19), 17.0 (C-20), 21.7 (C-21), 171.8 (C-22). Chemical data matched report by Mori et al., 1983.

11-*epi*-sinulariolide acetate (**9**); $C_{22}H_{32}O_5$; whitish powder; $[\alpha]D^{28}$ +76.0° ($CHCl_3$; 0.7); FT-IR (cm^{-3}); 1740, 1720, 1630; ^{1}H-NMR (CDCl3, 600 MHz) δ: 2.98 (1H, m, H-1), 1.28 (1H, m, H-2), 2.07 (1H, m, H-2), 1.87 (1H, m, H-3), 1.98 (1H, m, H-3), 5.70 (1H, dd, *J* = 2.06, 2.06 Hz, H-5), 1.55 (1H, m, H-6), 1.57 (1H, m, H-6), 1.89 (1H, m, H-7), 2.09 (1H, m, H-7), 5.19 (1H, dd, *J* = 5.10, 5.21 Hz, H-9), 2.08 (1H, m, H-10), 2.09 (1H, m, H-10), 1.74 (1H, m, H-11), 2.01 (1H, m, H-11), 2.98 (1H, m, H-13), 1.49 (1H, ddd, *J* = 13.1, 13.1, 26.1 Hz, H-14), 1.95 (1H, m, H-14), 5.45 (1H, br s, H-16), 6.26 (1H, br s, H-16), 1.36 (3H, s, H-18), 1.59 (3H, s, H-19), 1.36 (3H, s, H-20), 2.10 (3H, s, H-21); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 34.5 (C-1), 30.4 (C-2), 33.2 (C-3), 87.4 (C-4), 72.1 (C-5), 27.8 (C-6), 34.7 (C-7), 134.4 (C-8), 127.6 (C-9), 24.1 (C-10), 36.8 (C-11), 61.1 (C-12), 61.7 (C-13), 33.4 (C-14), 144.3 (C-15), 125.3 (C-16), 169.2 (C-17), 24.6 (C-18), 17.7 (C-19), 18.9 (C-20), 21.6 (C-21), 171.4 (C-22). Chemical data matched report by Tursch et al., 1975.

Dihydrosinuflexolide (**10**); $C_{20}H_{32}O_4$; whitish powder; $[\alpha]D^{28}$ -3.8° ($CHCl_3$; 0.06); FT-IR (cm^{-3}); 3404 , 2924, 1732, 1450, 710; ^{1}H-NMR ($CDCl_3$, 600 MHz) δ: 1.35 (1H, m, H-1), 1.34 (1H, m, H-2), 1.98 (1H, m, H-2), 1.26 (1H, m, H-3), 2.05 (1H, m, H-3), 2.85 (1H, dd, *J* = 5.50, 6.19 Hz, H-5), 1.57 (1H, m, H-6), 1.89 (1H, m, H-6), 2.06 (1H, m, H-7), 2.26 (1H, br m, H-7), 5.22 (1H, dd, *J* = 7.56, 7.56 Hz, H-9), 2.03 (1H, m, H-10), 2.29 (1H, br m, H-10), 1.66 (1H, m, H-11), 1.77 (1H, m, H-11), 3.98 (1H, dd, *J* = 8.94. 8.94 Hz, H-13), 1.79 (1H, m, H-14), 2.12 (1H, m, H-15), 1.36 (3H, s, H-16), 1.23 (3H, s, H-18), 1.64 (3H, s, H-19), 1.44 (3H, s, H-20); ^{13}C-NMR ($CDCl_3$, 150 MHz) δ: 37.6 (C-1), 28.4 (C-2), 35.4 (C-3), 59.6 (C-4), 63.6 (C-5), 26.0 (C-6), 36.4 (C-7), 134.5 (C-8), 126.5 (C-9), 23.6 (C-10), 38.7 (C-11), 75.2 (C-12), 84.3 (C-13), 31.9 (C-14), 43.1 (C-15), 18.1 (C-16), 175.5 (C-17), 16.1 (C-18), 17.1 (C-19), 25.8 (C-20). Chemical data matched

report by Duh et al., 1998.

Discussion

Sinularia flexibilis (Quoy and Gaimard) is a common soft coral found in many diverse regions in the Indo-Pacific with reports of chemical studies coming from Japan, Taiwan, Hong Kong, China, India, Philippines and Indonesia. This soft coral species have been chemically examined and is widely known to contain a series of cembranoid diterpenes, which are mostly derivatives of sinulariolide or flexibilide and sinularin, which contain a 14-membered cembrene ring fused to 6 or 7-membered lactone functionality. Similarly, the sample from Sabah was analysed as an effort to establish a pattern to identify chemotaxanomical markers for this species. A total of 10 cembrane diterpenes were isolated from the *S. flexibilis* collected in Mantanani Island and all of the compounds were previously reported from the same species. Cembrene A (**1**) was first reported by Herin & Tursch (1976) from *S. flexibilis*. Apparently, this report was the first for cembrane type metabolites from soft corals. However, the isolation of cembrene A was not only restricted to *S. flexibilis*. It was also reported to be isolated from the soft coral genus *Nephthea* sp by Vanderah et al. (1978). In addition, a recent publication by Mattern et al. (1997) even reported the isolation of cembrene A from the paracloacal gland of the Chinese alligator. This compound is also found in terrestrial sources. The cembrane (1*E*,3*E*,7*E*)-11,12-epoxycembra-1,3,7-triene (**6**) was reported to be isolated by Bowden et al. (1978 & 1983). Bowden (1983) first published on the chemistry of compound **6** from *Sinularia grayi* followed by a second finding in a population of *Lobophytum* sp. interestingly, a recent discovery of this compound was as a major metabolite in *S. flexibilis*. The importance of this metabolite as a chemotaxanomical marker to *S. flexibilis* or the genus is uncertain since similar to cembrene A (**1**), it is found in multiple sources. Compound **6** displayed a minimum inhibitory concentration (MIC) value 30 mg/disc against *Echerichia coli* and *Staphylococcus aureus*.

Next, the diterpene diepoxycembrene A (**2**) from the analysed sample has been reported from the Hainan soft coral *Sinularia* sp (Qin et al., 2008). Therefore this compound is not suitable as a marker for this species of soft coral. Diepoxycembrene A is unique since compounds with two epoxide moieties in their molecules are not common. Biogenetically, this compound is suggested to be derived from cembrene A (**1**). Flexilarin B (**3**) is a compound previously isolated and reported from the Hainan soft coral species *S. flexibilis* (Lin et al., 2009) along with its derivatives flexilarin A - J. To date, the flexilarins have

only been isolated from *S. flexibilis* and therefore can be proposed as a chemotaxanomical marker for this species. Similarly, the compound sinulariolide and its acetate have only been reported from *S. flexibilis*. Sinulariolide (**7**) as reported by Tursch and team in 1975 from the Indonesian species of *S. flexibilis* together with its acetylated derivative, 5-dehydrosinulariolide (**4**). However, 5-dehydrosinulariolide (**4**) was eventually reported from a natural source for the first time from the extract of *S. flexibilis* and reported by Hu et al. (2013a). Sinulariolide (**7**) was again reported from the same species in 1977 from the Australian species of *S. flexibilis* (Weinheinmer et al., 1977) via bioassay guided technique. This discovery makes sinulariolide (**7**) a suitable chemotaxanomical marker for *S. flexibilis*. Bioassay guided isolation of the Australian crude extracts displayed antineoplastic activity against P-388 lymphocytic leukemia cell lines led to the isolation of the compound sinulariolide (**7**) along with sinularin (**5**) which is also isolated from the species collected from Mantanani Island. Sinularin (**5**), which contains δ-lactone, is stereochemically similar to the compound crassin acetate, an antineoplastic, cembranolide present in Caribbean gorgonians. Similar bioassay guided technique was also used to report the isolation of dihydrosinuflexolide.

The *S. flexibilis* collected from Mantanani Island also yielded 11-epoxysinulariolide. This compound was previously reported from the Okinawan species, containing an 8,11-epoxy bridge (Mori et al., 1983). This metabolite is a compound with α-methylenelactone, trisubstituted three membered epoxide, acetoxy group and α, α, α-trisubstituted tetrahydrofuranyl. Along with sinulariolide, cembrene A, flexibilene, 11-dehydrosinulariolide, 11-*epi*-sinulariolide acetate, flexibilide and dihydroflexibilide were isolated. The compound 11-*epi*-sinulariolide acetate was also isolated in the sample collected from Mantanani Island as an output of sinulariolide acetylation by Tursch in 1975. However, in 2013, Hsu & team reported of the isolation of this compound from *S. flexibilis* in its acetate form from the species of Dongsha Atoll. This compound was reported to be potent anti-inflammatory agent, inhibiting gene expression of COX-2 and IL-8 through calcium signaling. This compound's potency as an anti-inflammatory agent was also reported by Lin et al (2013). In addition, since the crude of *Sinularia flexibilis* (Quoy and Gaimard) (Alcyoniidae) showed significant cytotoxicity in A549 (human lung adenocarcinoma), HT-29 (human colon adenocarcinoma), KB (human epidermoid carcinoma) and P-388 (mouse lymphocytic leukemia) cell cultures, Duh et al. (1998b) reported the isolation of dihydrosinuflexolide along with sinuflexolide and sinuflexibilin via bio-assay guided fractionation. The

compound dihydrosinuflexolide (**10**) was successfully isolated from the *S. flexibilis* collected from Mantanani Island.

In general, eight of the ten cembrane diterpenes (except cembrene A and (1*E*,3*E*,7*E*)-11,12-epoxycembra-1,3,7-triene) isolated from the soft coral collected from Mantanani Island were reported only from *S. flexibilis*. These common metabolites being isolated from *S. flexibilis* can be utilised as a chemotaxanomical tool for this species of soft coral. In addition, this investigation also reaffirms that cembranes with isopropyl moieties are unique to *S. flexibilis*. Finally, based on other findings, the cembrane diterpenes from *S. flexibilis* are an important chemotaxanomical marker for this species. In addition, the diversity of these cembranes also provides an excellent array of bioactive candidates for future drug discovery investigations.

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