Pharmacogn. Res.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcogres.com | www.phcog.net

ORIGINAL ARTICLE

New Cytotoxic Cembranoid from Indonesian Soft Coral *Sarcophyton* sp.

Hedi Indra Januar[1,2], Neviaty Putri Zamani[2], Dedi Soedharma[2], Ekowati Chasanah[1]

[1]Department of Biotechnology, Indonesian Research and Development Center for Marine and Fisheries Products Processing and Biotechnology, Jakarta, [2]Department of Marine Science, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Bogor, Indonesia

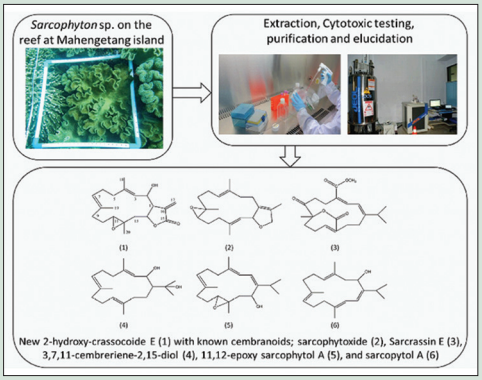
ABSTRACT

Context: *Sarcophyton* is a soft coral species that contains various secondary metabolites with cytotoxic activity. The production of cytotoxic compounds in soft corals is suggested as their allelochemical to win space competition. Therefore, if a particular soft coral species dominates a reef area, it may suggest to contain interesting bioactive compounds. **Aims:** This research aimed to characterize the cytotoxic compounds in dominant soft coral species (*Sarcophyton* sp.) on the reef at the Western side of Mahengetang Island, Indonesia. **Subjects and Methods:** Isolation of cytotoxic compounds through ethanol extracts had been done with preparative high-performance liquid chromatography and bioassay-guided fractionation by MCF-7 (breast) cancer cell lines. The structures of each cytotoxic compounds were elucidated on the basis of mass and nuclear magnetic resonance spectroscopic studies. **Results:** Elucidation through all compounds found a new cembranoid, namely, 2-hydroxy-crassocolide E (1), alongside with 5 known cembranoids; sarcophytoxide (2), sarcrassin E (3), 3,7,11-cembreriene-2,15-diol (4), 11,12-epoxy-Sarcophytol A (5), and sarcophytol A (6). All of these cembranoids were showed to inhibit the growth of MCF-7 (breast) cancer, with 50% inhibition of tumor cell lines growth lower than 30 mg/L. **Conclusions:** Results of this study suggest that a soft coral species which dominate a reef area is a potential source for various bioactive compounds.

Key words: Cembranoid, cytotoxic, *Sarcophyton* sp., soft coral

SUMMARY

Elucidation of cytotoxic compounds from *Sarcophyton* sp. that dominate the reef at Mahengetang Island Indonesia revealed a new compound (2-hydroxy-crassocolide E) alongside with 5 known cembranoid compounds.



Abbreviations Used: SCUBA: Self-contained underwater breathing apparatus; HPLC: High performance liquid chromatography; NMR: Nuclear magnetic resonance; IT-TOF: Ion trap-time of flight; MTT: 3-(4,5-dimethylthiazol-2-yl)-2; 5-diphenyltetrazolinon bromide; DEPT: Distortionless enhancement by polarisation transfer; COSY: Correlation spectroscopy; HMBC: Heteronuclear multiple-bond correlation; NOE: Nuclear overhauser effect; IG50: 50% inhibition growth

Correspondence:
Mr. Hedi Indra Januar,
KS Tubun Petamburan VI Street, Slipi, Jakarta, Indonesia.
E-mail: idjanuar@kkp.go.id
DOI: 10.4103/0974-8490.199779

Access this article online
Website: www.phcogres.com
Quick Response Code:

INTRODUCTION

Soft corals are potential sources that contain various bioactive compounds. Diterpenes, sesquiterpenes, furanoditerpenes, terpenoids, and steroids are major secondary metabolites in soft corals which have shown to display a pharmacological activity.[1] Soft corals are known to produce their bioactive compounds as a chemical/defensive weapon in maintaining or win spatial competition at benthic environment.[2-4] The amount of those bioactive compounds was suggested as a predictor for soft coral invasiveness potential in a coral reef environment.[5] Our previous study has shown a dominant *Sarcophyton* sp. in the Southern part of Panggang Island, Seribu Islands-Indonesia, which detected to contain high amount of cytotoxic sarcophytoxide.[6] Other studies on soft corals and sponges have also found a similar pattern.[7,8] Therefore, if a particular soft coral species dominates a reef area, it may suggest that this organism potentially contains an interesting chemical bioactive compounds.

A dominant cover of soft coral *Sarcophyton* sp. is shown on the reef at the Western part of Mahengetang Island, Indonesia. This condition may suggest to be happened as an impact of natural acidification by huge CO_2 seeps from Banua Wuhu underwater volcano. The domination of *Sarcophyton* sp. in this area is similar to the reef around CO_2 hydrothermal vents on the seashore of Iwo-Tori-shima Islands - Japan.[9] Our preliminary study detected higher cytotoxicity of *Sarcophyton* sp. which was taken from acidification environment on the reef at the Western part of Mahengetang Island compared to normal seawater environment.[10] Therefore, this research aimed to investigate the diversity of bioactive compound in the soft coral *Sarcophyton* sp. at Mahengetang Island, Indonesia. The study shows *Sarcophyton* sp. from this area contains 6 cytotoxic cembranoid compounds; a new 2-Hydroxy-crassocolide

Cite this article as: Januar HI, Zamani NP, Soedharma D, Chasanah E. New cytotoxic cembranoid from indonesian soft coral *Sarcophyton* sp.. Phcog Res 2017;9:65-8.

E cembranoid (1), alongside with known compounds: 11,12-epoxy sarcophytol A (2), sarcopytol A (3), sarcophytoxide (4), 3,7,11-cembreriene-2,15-diol (5), and sarcrassin E (6). This study describes the structural elucidation of 2-Hydroxy-crassocolide E cembranoid and cytotoxic activity of cembranoids from *Sarcophyton* sp. in Mahengetang Island, Indonesia.

SUBJECTS AND METHODS

General methods

Flash vacuum chromatography was performed using Bulk Phenomenex C_{18} (50 μm). Preparative high-performance liquid chromatography (HPLC) was performed using a Shimadzu C_{18} column (250 mm × 21 mm) attached to a Shimadzu Preparative HPLC system with a fraction collector. 1D and 2D-NMR spectra were recorded on Jeol ECS 400 MHz NMR spectrometer, with spectra referenced to ^{1}H and ^{13}C resonances in the deuterated solvents. Accurate mass spectrometric data were analyzed by Shimadzu ion-trap-time-of-flight (IT-TOF) mass spectrometer.

Animal materials

Sarcophyton sp. sample was collected from the Western side of Mahengetang Island, Indonesia, at a depth of 5 m by SCUBA. Soft coral taxonomy was identified by morphological inspection according to Fabricius and Alderslade.[11] Two hundred and fifty grams of sample was immersed in 500 mL ethanol and placed in a cool box containing ice packs for preservation along transportation to the laboratory. The sample was then exhaustively extracted with ethanol, to yield the extract that was used for isolation study. A voucher of sample has been lodged and preserved in Indonesia Research Centre for Marine and Fisheries Product Processing and Biotechnology, Indonesia.

Extraction, isolation, and cytotoxic study

The crude ethanol extract was filtered through a plug of reversed-phase C_{18} silica using methanol and methylene chloride (1:1) as eluent. The solvent on the extract was then removed with freeze dryer and the resultant dry extract was subjected to a preparative reversed-phase C_{18} HPLC (15 mL/min in 60 min, gradient elution from 20% acetonitrile: H_2O to 100%). Fractions were collected every 30 s. Six fractions were found to be active in cytotoxic testing. Cytotoxic activity of each fraction was analyzed against MCF-7 cell lines, based on the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinon bromide (MTT) assay of Zachary.[12] Human breast tumor cell lines (MCF-7) were cultured in RPMI 1640 medium containing 10% fetal bovine serum, 0.5% fungizone, and 2% Penicillin-Streptomycin. The cells were plated, and CO_2 incubated at 10,000 cells/well in 37°C temperature for 24 h. After that, the cell-growth medium was removed from each well and leaved the wells containing only with tumor cell attached. The wells were added with tested cembranoid isolates in a doses ranged from 1000 μg/mL to 1 μg/mL (dissolved in RPMI medium) and further CO_2 incubation for 24 h. Three kinds of controls were made for final absorbance reading in a microplate reader, which were control of tumor cells, control of medium, and control of samples. After 24 h, the solution was removed from each well, and 100 μl of MTT reagent (500 μg/ml) was added into each well. The samples were then further CO_2 incubated. After 4 h of incubation, 100 μl of 10% sodium dodecyl sulfate was added and then the samples were dark-incubated at room temperature (±27°C). After 12 h of incubation, 570 nm absorbance of each well was measured by DYNEX microplate reader and tumor cell lines inhibition percentage was calculated by the absorbance of A (tumor cell control), B (sample), C (sample control), and D (medium control) using the following formula and 50% inhibition of tumor cell lines growth (IG_{50} value) was gained by the regression plot between samples concentration and % inhibition.

$$\%\,\text{inhibition} = \frac{(A-D)-(B-C)}{(A-D)} \times 100\%$$

RESULTS

Preparative HPLC separation with bioassay-guided fractionation revealed six cytotoxic fractions. ^{1}H NMR analyses confirmed that fractions number 21 (compound 1, 9.7 mg, 0.004% wet weight sample), 24 (compound 2, 10.9 mg, 0.004%), 26 (compound 3, 11.2 mg, 0.004%), 28 (compound 4, 5.36 mg, 0.002%), 32 (compound 5, 7.4 mg, 0.003%), and 38 (compound 6, 12.2 mg, 0.005%) are isolate of cembranoid compounds. Analyses into mass and NMR spectroscopic data showed that compounds 2-6 are known cembranoids, which are sarcophytoxide (2), Sarcrassin E (3), 3,7,11-cembreriene-2,15-diol (4), 11,12-epoxy sarcophytol A (5), and sarcopytol A (6) that already characterized by previous studies.[13-17] Further bioassay testing (against human breast tumor cell lines MCF-7) of each compound showed all compounds demonstrated cytotoxic activity with IG_{50} 18.13 ppm (1), 12.22 ppm (2), 24.2 ppm (3), 22.27 ppm (4), 18.88 ppm (5), and 20.041 ppm (6).

Meanwhile, compound 1 was isolated as yellow oil. Molecular mass analysis by IT-TOF spectrometer showed this compound has m/z 333.2216. Deduction into mass and nuclear magnetic resonance spectroscopic data generated the possible structure of 1 as it is shown in Figure 1 (alongside with compounds 2-6). The structure of 1 was elucidated from 2D (COSY, HMBC) and 1D selective NOE NMR data as both are shown in Figure 2. Literature searches for this molecule indicated 1 is a new compound.

Compound 1 ^{1}H ($CDCl_3$): δ 2.88 (1H, m, H-1), 5.42 (1H, m, H-2), 5.16 (1H, d, J = 9.62 Hz, H-3), 2.33 (1H, m, H-5α), 2.63 (1H, m, H-5β), 1.65 (1H, n, H-6α); 2.02 (1H, m, H-6β), 5.22 (1H, t, J = 12.36 Hz, H-7), 1.47 (1H, m, H-9α), 1.93 (1H, m, H-9β), 2.17 (1H, m, H-10α), 2.18 (1H, m, H-10β), 2.97 (1H, m, H-11), 2.17 (1H, m, H-13α), 2.36 (1H, m, H-13β), 4.09 (1H, m, H-14), 5.65 (1H, d, J = 2.29 Hz, H-17α), 6.39 (1H, d, J = 2.75 Hz, H-17β), 1.90 (3H, s, Me-18), 1.67 (3H, s, Me-19), 1.28 (3H, s, Me-20); ^{13}C (CDCl3): δ 47.71 (C-1), 73.86 (C-2), 123.83 (C-3), 142.89 (C-4), 36.31 (C-5), 25.21 (C-6), 129.29 (C-7), 130.17 (C-8), 37.03 (C-9), 22.70 (C-10), 62.92 (C-11), 60.40 (C-12), 44.57 (C-13), 71.75 (C-14), 170.13 (C-15), 138.26 (C-16), 122.12 (C-17), 16.98 (Me-18), 16.59 (Me-19), 17.31 (Me-20). The accurate mass determination (positive ion mode) of compound 1 revealed an ion at m/z 333.2216.

DISCUSSION

Mass spectrometer analysis of compound 1 indicated this compound has a molecular formula of $C_{20}H_{28}O_4$, calculated from $(M+H)^+$ for $C_{20}H_{29}O_4$ (m/z 333.2216). This result suggests the molecule has five double bond equivalents (DBEs) of unsaturation. ^{1}H and ^{13}C NMR data indicated the molecule contained three C=C double bonds (δ_C 123.83, 142.89, 129.29, 130.17, 138.26, and 122.12). 135 DEPT analysis showed one among these double bond carbons is a CH_2 (δ_C 122.12) that attaches to tertiary carbon (δ_C 138.26). Furthermore, DEPT also detected this molecule contained a carbonyl (C=O) double bond (δ_C 170.13), epoxide (δ_C 62.92 and 60.40), and hydroxyl functional group (δ_C 73.86). As there showed two cyclic ring (main 14-cembranoid and epoxide), these deductions lead to another cyclic ring which is a cyclic lactone attaches into the main cembranoid ring (similar to sarcophytoxide) to fulfill the requirement for five DBEs of unsaturation.

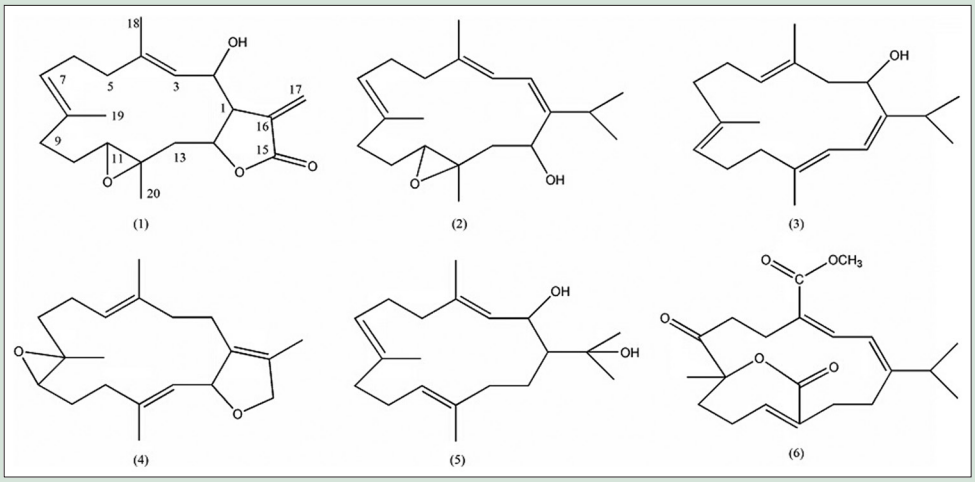


Figure 1: Structures of new compound 2-hydroxy-crassocoide E (1) alongside with known cembranoids; sarcophytoxide (2), Sarcrassin E (3), 3,7,11-cembreriene-2,15-diol (4), 11,12-epoxy sarcophytol A (5), and sarcopytol A (6) isolated from the ethanolic extracts of *Sarcophyton* sp. from Mahengetang Island, Indonesia

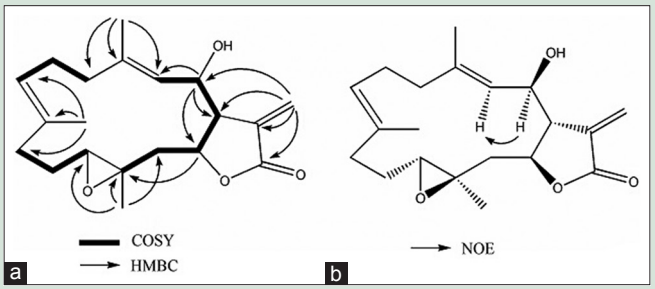


Figure 2: COSY, HMBC (a) and selective 1D-NOE (b) of 2-hydroxy-Crassocolide E

^{1}H-^{1}H COSY spectrum detected three continuous chains from H_2-13 to H-3, via H-14, H-1, and H-2, respectively, from H_2-5 to H-7, via H_2-6, and H_2-9 to H-11, via H_2-10. A long-range ^{1}H-^{13}C HMBC couplings were observed between H_3-18 to C-3, C-4, and C-5; H_3-19 to C-7, C-8, and C-9; and H_3-20 to C-11, C-12, and C-13. From these, it was possible to link all of the main atoms in 14 carbons cembranoid ring. The ring of lactone was generated by the data of ^{1}H-^{13}C couplings between H_2-17 to C-16, C-15, and C-1. Unfortunately, coupling between lactone oxygen bridge (H-14 to C-15) was not observed. Interpretation of lactone ring interpreted with a turnaround, with HMBC couplings between C-14 to H-2 and H-2 to C-15. Furthermore, a long range between C-14 to H_2-17, a strong coupling between H_2-17 to C-15, and spectral data comparison of crassocolides E,[18] confirmed the position of lactone ring in cembranoid structure. The evidence of hydroxyl group is attached in position C-2 is shown by ^{1}H-^{13}C HMBC couplings from H-2 to C-1, C-3, and C-14. Furthermore, it was also detected a long-range ^{1}H-^{13}C coupling from H_2-17 to C-2. All of these leading to the planar structure of 1 as it is shown in Figure 2a. Based on literature, inspection to crassocolide E chemical shift and 1D selective NOE of H-2 (δ_H 5.42) that gave rise to signals corresponding to H-3 (δ_H 5.16), the probable geometry of this compound is shown in Figure 2b. Unfortunately, absolute stereochemistry of this compound could not be determined in this study. Literature searches for this molecule indicated 1 is a new compound as a hydroxyl derivative (at C-2) of crassocolide E.[18]

CONCLUSION

Sarcophyton genus is a soft coral organism that has an ability to produce various cembranoid compounds. This study shows soft coral Sarcophyton in acidified environment produced a new type of cembranoid compound. Therefore, it may suggest soft coral produces specific type of cermbranoids as the results of environment characteristics.

Financial support and sponsorship

This study was supported by the Department of Biotechnology, Indonesian Research and Development Center for Marine and Fisheries Products Processing and Biotechnology, Indonesia.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Rocha J, Peixe L, Gomes NC, Calado R. Cnidarians as a source of new marine bioactive compounds – An overview of the last decade and future steps for bioprospecting. Mar Drugs 2011;9:1860-86.
2. Michalek-Wagner K, Bourne DJ, Bowden BF. The effects of different strains of zooxanthellae on the secondary-metabolite chemistry and development of the soft-coral host *Lobophytum compactum*. Mar Biol 2001;138:753-60.
3. Changyun W, Haiyan L, Changlun S, Yanan W, Liang L, Huashi G. Chemical defensive substances of soft corals and gorgonians. Acta Ecol Sin 2008;28:2320-8.
4. Sotka EE, Forbey J, Horn M, Poore AG, Raubenheimer D, Whalen KE. The emerging role of pharmacology in understanding consumer-prey interactions in marine and freshwater systems. Integr Comp Biol 2009;49:291-313.
5. Lages BG, Fleury BG, Ferreira CE, Pereira RC. Chemical defense of an exotic coral as invasion strategy. J Exp Mar Biol Ecol 2006;328:127-35.
6. Iswani S, Tohir D, Januar HI. Identification of cytotoxic compounds in soft coral *Sarcophyton* sp. from Panggang Island Water, Seribu Islands National Parks. Indones J Pharm Sci 2014;12:238-43.
7. Januar HI, Marraskuranto E, Patantis G, Chasanah E. LC-MS metabolomic analysis of environmental stressors impacts to the metabolites diversity in *Nephthea* sp. Chron Young Sci 2012;2:57-62.
8. Januar HI, Pratitis A, Bramandito A. Will the increasing of anthropogenic pressures reduce the biopotential value of sponges? Scientifica (Cairo) 2015;2015:734385.
9. Inoue S, Kayanne H, Yamamoto S, Kurihara H. Spatial community shift from hard to soft corals in acidified water. Nat Clim Change 2013;3:683-7.
10. Januar HI, Zamani NP, Soedarma D, Chasanah E. Changes in soft coral

Sarcophyton sp. abundance and cytotoxicity at volcanic CO_2 seeps in Indonesia. AIMS Environ Sci 2016;3:239-48.
11. Fabricius K, Alderslade P. Soft Corals and Sea Fans: A Comprehensive Guide to the Tropical Shallow Water Genera of the Central-West Pacific, the Indian Ocean and the Red Sea. Townsville, Qld.: Australian Institute of Marine Science; 2001.
12. Zachary I. Determination of cell number. In: Hughes D, Mehmet H, editors. Cell Proliferation and Apoptosis. Oxford: Bios Scientific Publishers; 2003. p. 13-35.
13. Farag MA, Porzel A, Al-Hammady MA, Hegazy ME, Meyer A, Mohamed TA, *et al.* Soft corals biodiversity in the Egyptian Red Sea: A comparative MS and NMR metabolomics approach of Wild and Aquarium Grown species. J Proteome Res 2016;15:1274-87.
14. Zhang C, Li J, Su J, Liang Y, Yang X, Zheng K, *et al.* Cytotoxic diterpenoids from the soft coral *Sarcophyton crassocaule*. J Nat Prod 2006;69:1476-80.
15. Januar HI, Chasanah E, Motti CA, Tapiolas DM, Liptrot CH, Wright AD. Cytotoxic cembranes from Indonesian specimens of the soft coral *Nephthea* sp. Mar Drugs 2010;8:2142-52.
16. Phan CS, Vairappan CS. Isopropyl (ene)-type cembrane diterpene an important chemotaxonomical marker in Bornean soft coral Genus *Sarcophyton*. J Trop Biol Conserv 2015;12:137-43.
17. Kobayashi M, Nakagawa T, Mitsuhashi H. Marine terpenes and terpenoids. I. Structures of four cembrane-type diterpenes; sarcophytol-A, sarcophytol-A acetate sarcophytol-B, and sarcophytonin-A, from the soft coral, *Sarcophyton glaucum*. Chem Pharm Bull 1979;27:2382-7.
18. Huang HC, Ahmed AF, Su JH, Chao CH, Wu YC, Chiang MY, *et al.* Crassolides A-F, cembranoids with a trans-fused lactone from the soft coral *Sarcophyton crassocaule*. J Nat Prod 2006;69:1554-9.

Hedi Indra Januar

ABOUT AUTHOR

First author is a researcher in Indonesian Research and Development for Marine and Fisheries Product Processing and Biotechnology that currently pursuing a PhD study in Department of Marine Science, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Indonesia. The PhD study is supervised by Neviaty Putri Zamani, Dedi Soedharma, and Ekowati Chasanah. His research area is mainly on marine ecology and marine natural products.