

Screening of Antibacterial and Anticancer Activity of Soft Corals from Togean Islands, Indonesia

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ABSTRACT

Soft corals (*Octocorallia*, *Alcyonaceae*) have been reported to possess diverse biological activities and unique structural chemistry. This study aims to screen the potential antibacterial and anticancer activity of some soft corals collected from Togean Islands, Central Sulawesi, Indonesia. They were *Lobophytum sp*, *Sarcophyton sp*, *Sinularia sp 1*, and *Sinularia sp 2*. All dried coral materials were extracted for 3 x 24 h by maceration method using methanol and then evaporated by rotary evaporator to obtain viscous extracts. The determination of antibacterial activity had been performed by well agar diffusion method against *Staphylococcus aureus* and *Escherichia coli*. Meanwhile, the cytotoxic activity was performed by MTT method, followed by apoptosis annexin V-FTIC assay. Identification for the presence of terpenoids was performed by p-anisaldehyde-sulphuric acid spraying reagent on thin layer chromatography (TLC). *Sinularia sp 2* extract have strongly inhibited *S. aureus* and *E. coli* with the diameter of inhibition of 15.80 mm and 15.73 mm, respectively. Moreover, *Sinularia sp 2* extract possessed also cytotoxic activity against human breast adenocarcinoma (MCF-7) and colorectal carcinoma (HCT-116) with the IC₅₀ of 46.807 and 47.186 µg/mL, respectively. Extract *Sinularia sp 1* was found to have strongest cytotoxicity on human colon colorectal carcinoma (HCT-116) with the IC₅₀ of < 1.505 µg/mL. Annexin V-FTIC assay clearly exhibited that the apoptosis mechanism is proposed by the extracts of *Sinularia sp 1* and *Sinularia sp 2*. Terpenoids were identified on both extracts suggesting for further purification and isolation for the bioactive terpenoid compounds.

Key words: Softcorals, Togean Islands, anti-cancer, antibacterial

INTRODUCTION

Infection diseases caused by bacteria, and resistance of antibiotics and anticancer drugs are still become health problem in the worldwide. There are many studies in this field searching for the new source of antibiotic and anticancer drugs, especially from marine (Afifi *et al.*, 2016). It is because the marine organisms have been reported progressively in the past 50 year that they possessed diverse unique chemical structure of new bioactive compounds (Abou El-Ezz *et al.*, 2013). Among marine organisms, marine invertebrates are the most producers of marine natural product. Soft corals (octocorallia, Alcyonaceae) have been studied well and they become promising sources of new anti bacteria and anticancer agents (Coll, 1992). Most of the reported

compounds are sesquiterpenes, diterpenes, polyhydroxylated steroids, and polyamine compounds (Blunt *et al.*, 2016; Chen *et al.*, 2012).

Indonesian coast is one of the richest biodiversity for marine organisms in the ocean. There are more than 20 publications on the bioactive compounds from Indonesian soft corals (*Cladiella sp*, *Lobophytum sp*, *Sinularia sp*) have been reported during 1997 – 2004. These bioactive compounds showed various pharmacological activities such as antimicrobial, anti-inflammatory and cytotoxic (Putra *et al.*, 2016). Previously, our study on soft corals of *Sarcophyton trocheliophorum* and *lobophytum sp* have found numerous secondary metabolites possessing antibacterial and antitumor activities (Zubair *et al.*, 2016; Al Footy *et al.*,

2016). In continuing our focus research on marine organism, particularly from Indonesian marine, our study now starting on soft corals collected off Togean Islands, located in Tojo Una-una, Central Sulawesi. There are four collected corals : *Lobophytum sp*, *Sarcophyton sp*, *Simularia sp 1*, and *Simularia sp 2*. This study continued by screening assay for antibacterial and cytotoxic activity to find the most potential one that could be used as antibacterial and anticancer drugs in the future. To the best of our knowledge, this is the first report regarding antibacterial and anticancer of soft corals collected from Togean islands, Indonesia

MATERIAL AND METHODS

Material

All samples of soft corals were collected in January 2017 on Togean Island, Tojo Una-una, Indonesia at a depth of 5-10m and identified at UPT. Sumber Daya Hayati, Tadulako University. A voucher sample was deposited at Laboratory of Pharmacognosy and Phytochemistry, Department of Pharmacy, Tadulako University

Extraction

Material of soft corals were washed with water and dried at room temperature. Then, they were minced and repeatedly extracted for 3-5x24h by maceration method with methanol as a solvent at room temperature. Then each obtained extracts were evaporated by using rotary evaporator to reach a viscous extracts. Each of extracts was subjected for further analysis.

Antibacterial Screening

Antibacterial screening was performed against two types of bacteria : *Staphylococcus aureus* and *Escherichia coli* by using agar well diffusion method (Limberger *et al.*, 2001). Briefly, 0.1mL of suspended bacterium in sterile medium (1.5×10^8 CFU/mL) was spread on nutrient agar media. Then 50 μ L of each sample (1000, 500 and 250mg/mL) was poured into the wells (6-mm diameter). All plates were left for 1h at 48°C and then incubated for 24h at 37°C for bacteria. Inhibition zone diameters formed around the well were measured and the

mean diameter of three replicates was calculated. DMSO was used as a negative control and chloramphenicol as a positive control.

Cytotoxic Activity

Cytotoxic activity was applied on human breast adenocarcinoma (MCF-7), human colon colorectal carcinoma (HCT-116), NDHF normal cells and CDD-118 normal cell lines by MTT method as described in our previous study (Zubair *et al.*, 2016). Doxorubicin and fluorouracil were used as a positive standard anticancer drug. The stock samples were diluted with RPMI-1640 medium to desired concentrations of 62.5, 125, 250, 500 and 1000 μ g/mL. The final concentration of dimethylsulphoxide (DMSO) in each sample was 1%v/v. The cancer cells were batch cultured for 10 d, then seeded in 96 well plates of 1×10^4 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24h under 5% CO₂ using a water jacketed carbon dioxide incubator (Cel Culture, Esco Medical ApS, Denmark). The medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample. After 24h and 48h of incubation, cells were added with 10 μ L/well of MTT (5mg/mL) and incubated for 4h in incubator at 37°C in 5% CO₂ humidified atmosphere. The reaction was stopped by 100 μ L dimethylsulfoxide (DMSO). The plate was then incubated for 15min. The absorbance of each well was read at 550nm wavelength in Elisa Reader (Infinite M200 pro NanoQuant, Tecan, Switzerland), using wells without cells as blanks. All experiments were performed in triplicate. The effect of compounds on proliferation of cancer cells was expressed as the % cytoviability, using the following formula:

$$\% \text{ Cytoviability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of untreated cells (negative control)}} \times 100\%$$

The IC₅₀ calculation was done statistically by probit analysis using SPSS 17.0 (SPSS, Inc, Chicago IL, USA), in which the series of dose-response data and the percentage of cytoviability were plotted together.

Table I. Antibacterial activity of Togeian islands soft corals

Soft corals	Mean Diameter of Inhibition Zone (mm) at 1000mg/mL	
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>
<i>Lobophytum sp</i>	-	-
<i>Sarcophyton sp</i>	12.75±0.08	12.25±0.50
<i>Sinularia sp 1</i>	12.36±0.46	13.32±0.16
<i>Sinularia sp 2</i>	15.80±0.30	15.73±0.41
Chloramphenicol	28.28±0.50	26.91±0.40

^a= Chloramphenicol was tested at 1mg/mL

Table II. Cytotoxic activity of Togeian islands soft corals

Soft corals	Inhibition Concentration, IC ₅₀ (µg/mL)					
	MCF-7		HCT-116		CDD	NDHF
	24h	48h	24h	48h	24h	24h
<i>Lobophytum sp</i>	91.415	92.116	162.978	162.978	ND	ND
<i>Sarcophyton sp</i>	168.824	260.557	264.783	264.783	ND	ND
<i>Sinularia sp 1</i>	43.315	24.02	1.505	<1.505	1.901	Non Detectable*
<i>Sinularia sp 2</i>	52.337	46.807	47.186	47.186	166.029	143.616
Doxorubicin	0.024	ND	ND	ND	ND	ND
Fluorouracil	ND	ND	8.915	ND	ND	ND

ND= Not determined; *Non Detectable= sample used is range 1000-63.5µg/mL and percentage of died cells obtained is around 93-94%, IC₅₀ cannot be calculated

Annexin V-FITC apoptosis assay

Annexin V-FITC apoptosis assay of cancer cells (MCF-7 and HCT-116) were seeded as described above and then incubated with different treatments for 24h. Cells were harvested, washed twice with PBS and centrifuged. In brief, 1x10⁵ of cells were treated with annexin V-FITC and propidium iodide (PI) using the apoptosis detection kit (BD Biosciences, San Jose, CA) according to the manufacturer’s protocol. Annexin V-FITC and PI binding were analyzed by flow cytometry on FACScanto II (BD Biosciences, San Jose, CA) without gating restrictions using 10.000 cells. Data were collected using logarithmic amplification of both the FL1 (FITC-A) and the FL2 (PI-A) channels. Quadrant analysis of coordinate dot plots was performed with CellQuest software. Unstained cells were used to adjust the photomultiplier voltage and for compensation setting adjustment to eliminate spectral overlap between the FL1 and the FL2 signals.

Identification of terpenoids

The presence of terpenoids was identified by using p-anisaldehyde-sulphuric acid spraying reagent on thin layer chromatography (TLC).

RESULT AND DISCUSSION

The aims of this study is to screen for the most potent antibacterial and anticancer activity of four soft corals collected from Togeian Islands. The four soft corals was identified as *Lobophytum sp*, *Sarcophyton sp*, *Sinularia sp 1*, and *Sinularia sp 2* (Figure 1) Meanwhile, the result of antibacterial and anticancer activity (Table I and II).

Antibacterial test was performed by well agar diffusion method. *Staphylococcus aureus* and *Escherichia coli* was chosen as tested bacteria as the representative of gram positive and gram negative bacteria. In table I only three samples have inhibition to *Staphylococcus aureus* and *Escherichia coli*, means that these samples have broad spectrum of antibacterial activity. *Sinularia sp 2* are the most potent with the

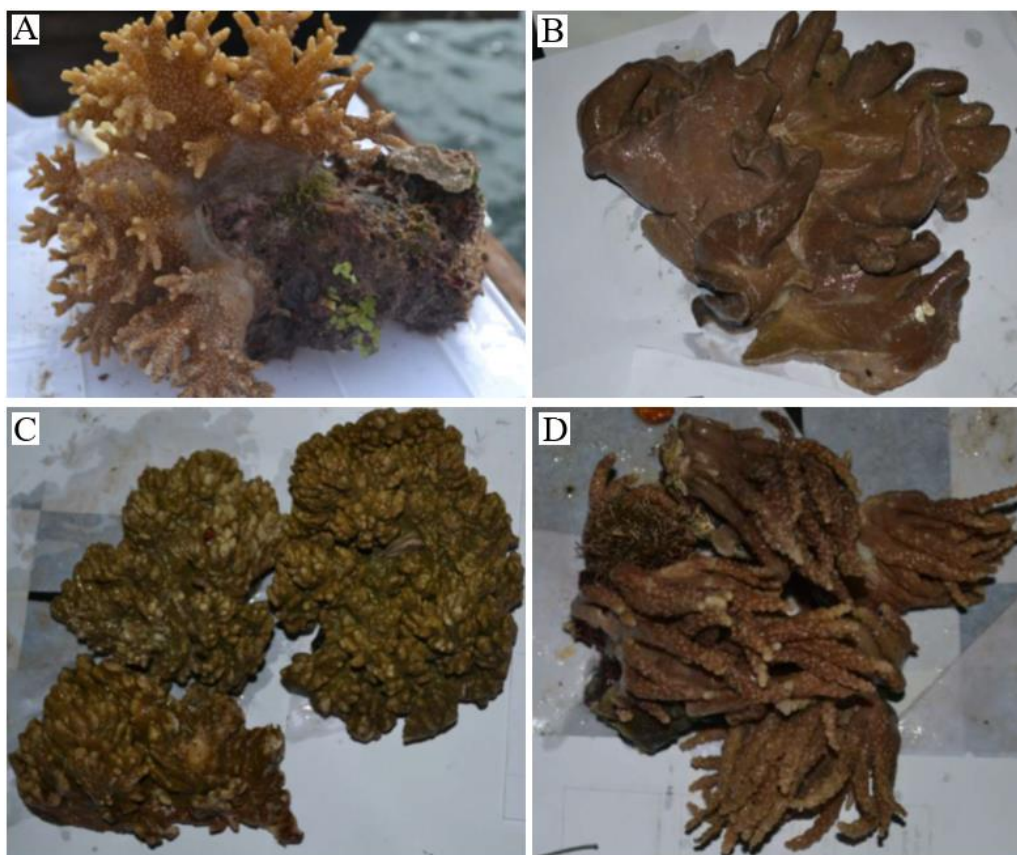


Figure 1. Soft corals collected from Togean Islands. A: *Lobophytum sp*, B: *Sarcophyton sp*, C: *Sinularia sp 1*, D: *Sinularia sp 2*

highest diameter of inhibition zone of 15.80 ± 0.30 mm and 15.73 ± 0.41 mm, followed by *Sarcophyton sp* and *Sinularia sp 1*. *Lobophytum sp* did not possess the inhibition on *S. aureus* and *E. coli*.

The cytotoxic activity on human colon carcinoma (HCT-116) of soft corals extracts, found that *Sinularia sp 1* methanolic extract have time dependent potent cytotoxicity at 24 and 48h with the IC_{50} of 1.505 and $<1.505 \mu\text{g/mL}$, respectively (Table II). Moreover, it is also showed cytotoxicity on MCF-7 with the IC_{50} of 24.02 $\mu\text{g/mL}$ after 48 h incubation. *Sinularia sp 2* extract can be categorized also to have potent cytotoxicity on HCT-116 and MCF-7 where the IC_{50} of 46.807 and 47.186 $\mu\text{g/mL}$, respectively. *Sinularia sp 2* extract showed high selectivity on cell growth inhibition where it is found to be not toxic on both CCD and NHDF normal cells.

Meanwhile, *Sinularia sp 1* found not selective as it has toxicity to both CDD and NHDF normal cells. Further identification of the possible apoptosis mechanism of the potential extracts of *Sinularia sp 1* and *Sinularia sp 2* was done by Annexin V-FITC assay. The result showed that methanolic extract of *Sinularia sp 1* and *Sinularia sp 2* have significant percentage of early and late apoptosis with the value of 51.90% and 42.90% on MCF-7 and 51.20% and 49.60% on HCT-116 cell lines, respectively (Figure 2)

Sinularia has been commonly known to contain class of terpenoid compounds that possessing broad antimicrobial and potent cytotoxicity activity. For example, a casbane diterpenes called 10-hydroxydepressin from *Sinularia depressa*, inhibit *S. aureus* and *E. coli* at 17 μM and possess cytotoxic on HepG2 and SW-1990 with the IC_{50} of 61 and 37 μM , respectively (Li *et al.*, 2010), sinulariolide and flexibilide

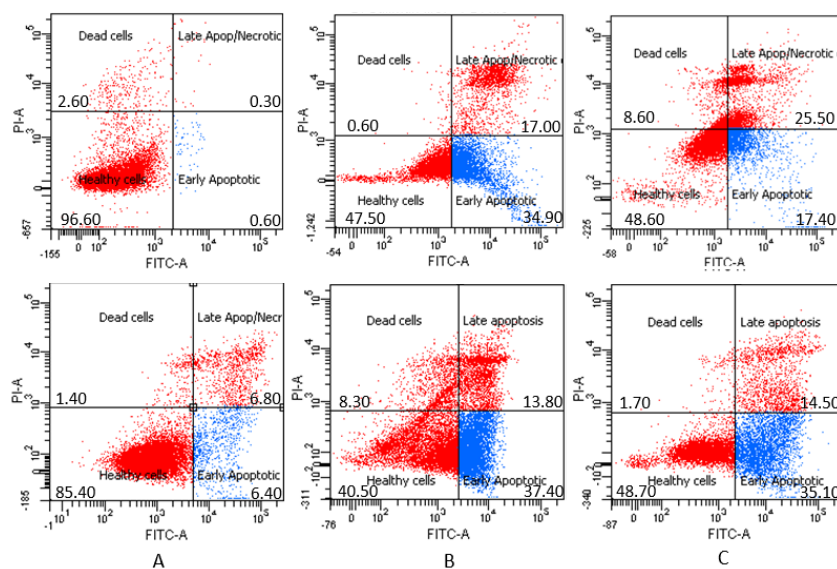


Figure 2. Effect of Untreated (A), *Simularia sp 1* (B) and *Simularia sp 2* (C) methanolic extract on MCF-7 (Above) and HCT-116 (Below) on annexin V-FITC-positive staining. The four quadrants identified as LL (healthy cells); LR (early apoptotic); UR (late apoptotic) and UL (necrotic).

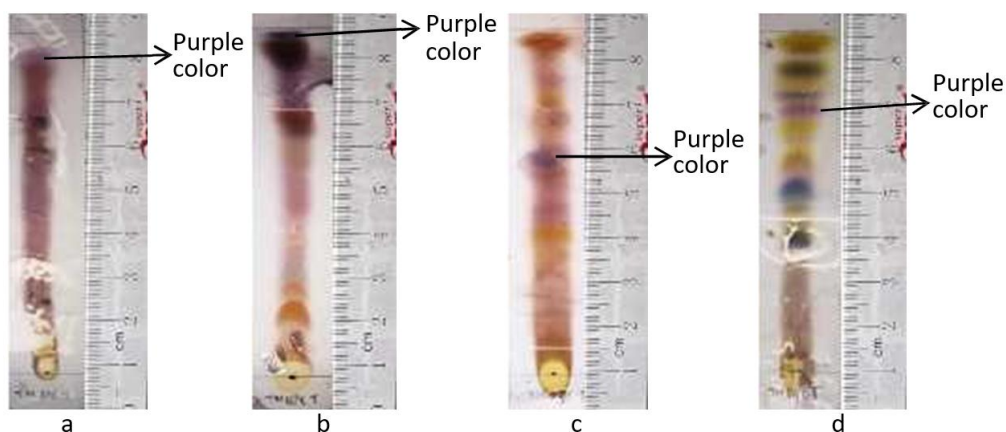


Figure 3. TLC Identification using p-anisaldehyde-sulphuric acid of the extract *Lobophytum sp* (a), *Sarcophyton sp* (b), *Simularia sp 1* (c), dan *Simularia sp 2* (d)

from *S. flexibilis*, exhibited growth inhibition of gram-positive bacteria. at concentrations of 10 and 5 ppm, respectively (Aceret *et al.*, 1998). A new cembrenolid diterpene isolated from the hybrid softcoral *Simularia maxima* and *S. polydactyla* showed strong cytotoxicity on the breast cancer SK-BR3 cell line and cervix cancer HeLa and HeLa-Apl cell lines with GI₅₀ values of 0.039, 0.48, and 0.56mM, respectively (Kamel *et al.*, 2007). Flexilarins D isolated from *S. flexibilis* exhibited potent cytotoxicity against

Hep G2 tumor cells with an ED₅₀ value of 0.07ug/mL (Lin *et al.*, 2009). Based on these studies, we further identify terpenoids content on the extract. Identification was done by thin layer chromatography using anisaldehyde-sulphuric spraying reagent (Figure 3). It is clearly shown that *Simularia sp 1* and *Simularia sp 2* extracts are possible to contains terpenoids as they shown one spot (purple color after spraying) that means positive for terpenoids.

CONCLUSION

Simularia sp 2 is the most potential extract that has broad spectrum of antibacterial activity and potent cytotoxic activity. Moreover, it selectively inhibited MCF-7 and HCT-116 and less toxic to normal cells. Meanwhile, *Simularia sp 1* showed potent cytotoxicity on all cell line studied and more toxic on normal cells compare to *Simularia sp 2*. Apoptosis mechanism were proposed as mechanism of *Simularia sp 1* and *Simularia sp 2* extracts. The presence of terpenoids suggested them as bioactive compounds of soft corals. It is needed for further isolation and purification of these terpenoid compounds

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