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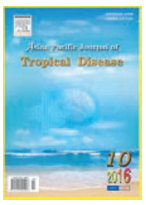

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Phenolic content, anti-oxidant, anti-plasmodium and cytotoxic properties of the sponge *Acanthella cavernosa*

Masteria Yunovilsa Putra[1*], Tutik Murniasih[1], Joko Tri Wibowo[1], Tri Aryono Hadi[1], Febriana Untari[1], Amalia Choirun Nisa[2], Respati Tri Swasono[2]

[1]*Research Center for Oceanography, Indonesian Institute of Sciences, Jl. Pasir Putih I, Ancol Timur, Jakarta 14430, Indonesia*

[2]*Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Gadjah Mada, Sekip Utara, Yogyakarta 55281, Indonesia*

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ABSTRACT

Objective: To investigate the total phenolic content, anti-oxidant capacity and cytotoxic activity present in the *n*-hexane, ethyl acetate, *n*-butanol and aqueous fractions of an extract collected at Selayar Island, Indonesia.

Methods: The antioxidant activity was performed by the 1,1-diphenyl-2-picrylhydrazyl radical scavenging method and β-carotene bleaching assay. All fractions from the crude extract of *Acanthella cavernosa* (*A. cavernosa)* were examined for their cytotoxicity using brine shrimp lethality bioassay and heme polymerization inhibitory activity assay for antimalarial activity.

Results: The highest phenolic content was found in the *n*-butanol fraction, followed by the ethyl acetate, aqueous and *n*-hexane fractions. The highest antioxidant activity, as determined by the β-carotene bleaching assay, was observed in the *n*-hexane fraction. On the other hand, the *n*-hexane fraction was most effective in suppressing 1,1-diphenyl-2-picrylhydrazyl radicals and neutralizing 50% of free radicals at the concentration of 171.86 μg/mL. Various fractions of the *A. cavernosa* extract showed the ability to inhibit heme polymerization indicating an anti-*Plasmodium* function. In this regard, the ethyl acetate fraction achieved an IC_{50} value of 3.3 μg/mL. The aqueous fraction showed moderate cytotoxic activity against the brine shrimp *Artemia* sp.

Conclusions: This study provided information on antioxidant, total phenolic content and antimalarial activities as well as the cytotoxicity of all fractions from the crude extract of *A. cavernosa*. The natural anti-*Plasmodium* compounds are of particular interest. Further studies are needed for a more extensive screening and characterization of the bioactive components in this sponge.

1. Introduction

Many investigations have shown that sponges of the genus *Acanthella* yield a large number of highly diversified natural products such as diterpenes and sesquiterpenes that contain various nitrogenous functional groups, including isonitrile-, isothiocyanate-, isocyanate- and formamide groups. Diterpenes and sesquiterpenes isolated from this genus have also been reported to exhibit cytotoxic, anthelmintic, anti-malarial, anti-microbial, anti-fungal and anti-fouling activities[1-3].

As a part of our research on bioactive secondary metabolites from Indonesian marine organisms, we studied the chemical composition and biological characteristics of the marine sponge *Acanthella cavernosa* (*A. cavernosa)* collected from Selayar Island. We examined various phytochemical properties, including the total phenolic content (TPC), anti-oxidant capacity, anti-plasmodium activity as well as cytotoxicity of different fractions (*n*-hexane, ethyl acetate, *n*-butanol and water) prepared from a crude extract of *A. cavernosa*.

2. Materials and methods

2.1. Chemicals and reagents

Ethyl acetate, *n*-hexane, n-butanol, methanol, chloroform, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene were purchased from Sigma Chemical Company (St. Louis, USA). Butylated hydroxytoluene (BHT), linoleic acid, gallic acid, ferrous chloride, sodium carbonate, Tween-40, dimethyl sulfoxide (DMSO) and

*Corresponding author: Masteria Yunovilsa Putra, Research Center for Oceanography, Indonesian Institute of Sciences, Jl. Pasir Putih I, Ancol Timur, Jakarta 14430, Indonesia.
Tel: +62 2164713850
E-mail: mast001@lipi.go.id

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ethylene diamine tetraacetic acid were purchased from Merck (Darmstadt, Germany). All chemicals used were of analytical grade.

2.2. Sponges

Sponges, identified as *A. cavernosa*, were collected by scuba diving in Selayar Island, South Sulawesi, Indonesia, at a depth of 10 m in June 2015. A voucher specimen was deposited in the Research Center for Oceanography, Indonesian Institute of Sciences, under the registration number SLYR SP-5.

2.3. Extraction in organic solvents

The sponge *A. cavernosa* (500 g wet weight) was homogenized and extracted with MeOH: $CHCl_3$ (3:1) at room temperature to obtain a crude extract. The extract was partitioned against various organic solvents to give fractions of *n*-hexane (non-polar), ethyl acetate (semi polar), *n*-butanol (polar) and water. The fractions were coded *n*-hexane (1.47 g), ethyl acetate (0.48 g), *n*-butanol (1.53 g) and water (1.46 g), respectively. Each fraction was subjected to a preliminary phytochemical screening to estimate its total phenol content, anti-oxidant capacity, anti-plasmodium activity, as well as cytotoxicity.

2.4. Phytochemical screening

All fractions were subjected to a preliminary phytochemical screening for the presence of selected secondary metabolites, following a standardized conventional protocol described by Abioye *et al.*[4].

2.5. TPC assay

TPC was measured using the Folin-Ciocalteu assay with slight modifications as described by Carciochi *et al.*[5]. The various test samples, *viz.,* the calibration standards (10 μL of gallic acid), the fractions (1 mg/mL in DMSO) and quercetin (1 mg/mL in DMSO) as the positive control were added to Folin-Ciocalteu reagent (500 μL). After 5 min, 300 μL of Na_2CO_3 solution (115 g/L) was added to each reaction mixture and thoroughly mixed. The mixtures were incubated at room temperature for 2 h before measuring the absorbance at 765 nm using the Infinite® 200 PRO microplate reader (Tecan Austria GmbH, Grödig, Austria). TPCs in the various fractions of *A. cavernosa* extract were expressed in mg gallic acid equivalent (GAE)/g of extract.

2.6. DPPH radical scavenging activity assay

DPPH radical scavenging activity in fractions of the crude *A. cavernosa* extract was determined using the method described by Krishnanunni *et al.*[6] with slight modifications. The fractions of the sponge extract (500 μL) was mixed with 7 mL of methanol and then added to DPPH solution (1 mL) in 96-well plates. The mixture was kept at room and incubated in the dark for 30 min, and then the absorbance values were read at 517 nm using the Infinite® 200 PRO microplate reader (Tecan Austria GmbH). Methanol was used as the blank. DPPH solution without addition of the extract was served as the control, and BHT was used as the calibration standard. The percentage of DPPH scavenging effect was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)/percent inhibition} = (A_0 - A_1/A_0) \times 100\%$$

Where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard. The IC_{50} value of the sample, the concentration of the sample required for 50% inhibition of the DPPH free radical, was obtained from the dose-response curve plotted as a linear regression between percent inhibition and concentrations.

2.7. Beta-carotene bleaching (BCB) assay

The BCB assay was performed using the method of Olugbami *et al.*[7] with some modifications. A stock solution of β-carotene/linoleic acid was initially prepared by dissolving 5 mg of β-carotene in 50 mL of chloroform. And 0.2 mL of the carotene-chloroform solution was pipetted into boiling linoleic acid (20 mg) and Tween-40 (200 mg). Chloroform was evaporated at reduced pressure for 5 min, and distilled water (50 mL) was added to the residue slowly with vigorous agitation to form an emulsion. An aliquot of 250 μL β-carotene/linoleic acid emulsion was pipetted into the 96-well plates containing 0.2 mL of the test solutions. An equal amount of methanol was used in the control. The plates were incubated at 50 ℃, and the absorbance was then measured at 470 nm after 60 min using the Infinite® 200 PRO microplate reader (Tecan Austria GmbH). BHT was used as antioxidant reference compound. The anti-oxidative activity was expressed as percent inhibition with reference to the control after 60 min of incubation and calculated as follow:

$$\text{Degradation rate (DR) of β-carotene} = [\ln (a/b)/60]$$

$$\text{Antioxidant activity (\%)} = [(\text{DR control} - \text{DR sample})/\text{DR control}] \times 100\%$$

Where *a* is absorbance at time 0, and *b* is absorbance at 60 min.

2.8. Heme polymerization inhibitory activity assay

The heme polymerization inhibitory activity assay was conducted using the method developed by Basilico *et al.*[8]. A 100 mL solution of 1 mmol/L hematin in 0.2 mol/L NaOH was pipetted into a 96-well micro-culture plate, and 50 mL of the test sample of concentrations, ranging from 1.25 to 10.00 μg/mL were added to each well. A total of 50 mL of glacial acetate acid at pH 2.6 was added to the wells to initiate a heme polymerization reaction. The micro-culture plate was then incubated for 24 h at 37 ℃ to complete the polymerization process. Following this, the micro-culture plate was centrifuged, and the resulting deposits were washed 3 times with 200 mL of DMSO. NaOH (200 mL, 0.1 mol/L) was subsequently added to each well of the micro-culture plate. Absorbance values were read at 405 nm using the Infinite® 200 PRO micro-plate reader (Tecan Austria GmbH). Heme polymerization inhibitory activity was expressed as IC_{50}. Aquadest and chloroquine were used as negative and positive controls, respectively. The percent inhibition of heme polymerization was calculated by the following formula:

$$\%\text{Inhibition} = (\beta\text{-hematin}_0 - \beta\text{-hematin}_1)/\beta\text{-hematin}_0 \times 100$$

Where $\beta\text{-hematin}_0$ is the concentration of the negative control and