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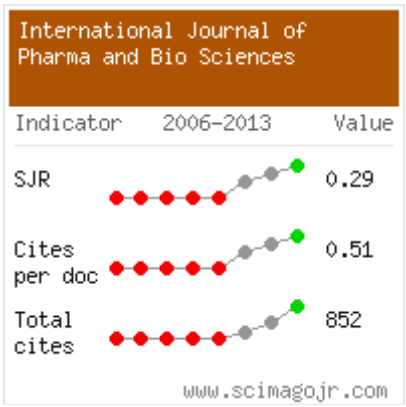

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Research Article **Microbiology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

TOXICITY, ANTICANCER AND ANTIOXIDANT ACTIVITY OF EXTRACTS FROM MARINE BACTERIA ASSOCIATED WITH SPONGE *Jaspis* sp.

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ABSTRACT

Sponges associated with microorganism can produce bioactive compounds such as antioxidant and anticancer. The aim of this study determines the toxicity, anticancer and antioxidant potency of marine bacteria which associated with sponges *Jaspis* sp. The bioactive compounds were extracted with ethyl acetate. The toxicity of extract was analyzed trough by brine shrimp lethality test (BSLT), while anticancer activity againts HeLa cell was analyzed by MTT assay and antioxidant capacity was analyzed by cupric ion reducing antioxidant capacity (CUPRAC) assay. The result showed that extract consisted of flavonoid, alkaloid, and triterpenoid which were potential as an antioxidant and anticancer. The extract had a toxic effect with the range LC_{50} value was 251.18 to 390.50 µg/mL. The extract could inhibited HeLa cell in the range IC_{50} value was 242.54 to 267.03 µg/mL. Antioxidant capacity of extract were ranged from 156.94 to 649.92 µmol trolox/g extract.

KEYWORDS: anticancer, antioxidant, marine bacteria, toxicity.

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INTRODUCTION

Cancer is the second leading cause of mortality in the world[1]. This disease, caused by uncontrolled cell growth, which interfere the function of another cell. The uncontrolled growth because of mutations in genes that regulate the cell cycle[2]. Apparently, most cancer cells are under oxidative stress from free radicals[3]. The free radicals disrupts the DNA bases of genetic information, and continue with the formation of cancer cells. Antioxidants play an important role in the human body in neutralizing free radicals that lead to degenerative diseases including cancer [4]. Biologically active natural product from sponges had potency as anticancer, antioxidant, and anti-inflammatory[5,6]. However, the development of bioactive natural product from sponge requires a large sponge biomass for extracting bioactive natural product in its application in the medical field[7]. Sponges have been studied that have a close association with various microorganism, in general microorganism associated can reach 50-60% of sponges volume[8]. Microorganism associated with sponges are reported involved in the production of bioactive natural product[9,10]. Production of bioactive natural product by microorganism is a strategy to produce a variety of biologically active natural product in large quantities through the production of microbial culturing. Bioactive natural product from marine bacteria associated with sponges had potency as antibiotics againts human microbial pathogen[11]. It also had herbicidal activity and growth promotor activity for plant[12]. The potency extract of marine bacteria associated with sponges as anticancer and antioxidant was studied in this study. Toxicity test was done as an initial test to determine the potency of biologically active natural product in pharmaceuticals development. Brine Shrimp Lethality Test (BSLT) is considered as a preliminary screening for the presence of antitumor or anticancer[13]. This study aimed to determine the toxicity, anticancer, and antioxidant potency of marine bacteria which associated with sponges *Jaspis* sp.

MATERIALS AND METHODS

(i) Culture of bacteria assosiated with sponge [14]

Bacterial isolates used in the study were SAB E-31, SAB E-41, SAB E-57 which were isolated from the sponge *Jaspis* sp. The bacterial isolates were cultured in medium to Sea Water Complete (SWC).

(ii) Extraction of bacteria assosiated with sponge[15,16]

The bacterial isolates were cultured in 500 mL Erlenmeyer flask with SWC broth medium. The cultures were incubated in shaker incubator 100 rpm at 30ºC for 72 hours. After incubation, bacterial cultures (500 mL each) were mixed with 500 mL of ethyl acetate. The mixture were kept 30 ºC for 12 hours stirred. The ethyl acetate layer was separated and the ethyl acetate was evaporated. The crude extract was stored below 5 ºC until further use.

(iii) *Chemical identification[17]*

The secondary metabolites in extract of marine bacteria associated with sponge were analyzed by qualitative method. The secondary metabolites that analyzed were alkaloid, flavonoid, saponin, tannin, terpenoid and steroid.

(iv) Toxicity test (Brine Shrimp Letality Test) [18]

Artemia Salina eggs (0.1 g) were inoculated with 100 ml of seawater and incubated in a Hatcher at 30 ºC with strong aeration, under continuous light regime. Approximately 24 hours after hatching, 20 individuals nape was collected with micropipette from the light side and placed in a small vial. Concentration of extracts were 0 µg/ml as control, 10 µg/ml, 100 µg/ml, 250 µg/ml, 500 µg/ml, and 750 µg/ml. Each sample concentration was tested in triplicate. The numbers of dead individuals were counted after 24 hours of exposure.

$$\% \text{ Mortality} = \frac{\Sigma \text{ Mortality larva nauplii} - \Sigma \text{ Mortality in control}}{\Sigma \text{ initial larva nauplii}} \times 100\%$$

The data was corrected using the following formula. LC_{50} value was calculated using Probit analysis[19].

(v) Anticancer activity by MTT assay[6]

Cancer cells used in the study of cervical cancer cells was human cervix HeLa cells ATCC CCL-2 from Primate Study Center Bogor Agriculture University in Indonesia. Hela cells 100 mL (5×10^3 cells/wells) were inoculated in Dubecco's Modified Eagle's Medium (DMEM) for 24 hours. A total of 100 mL with a concentration of active extracts 5 µg/mL, 25 µg/mL, 125 µg/mL, and 625 µg/mL was added to the inoculant then incubated in CO_2 incubator at 37°C for 24 hours. Furthermore, MTT (3 - [4,5-dimetiltiazol-2-yl] 2,5-difeniltetrazolium bromide) 100 µL was added in each well and incubated for 4 hours at 37ºC. The absorbance of formazan were measured by ELISA reader at 595 nm wavelength.The percentage inhibition of HeLa cells was calculated by the following formula:

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 100\%$$

The extract concentration which inhibit 50% HeLa cell (IC_{50}) was determined using the relationship curve between the concentration of extract (x) and the persentage inhibition (y).

(vi) Antioxidant activity by Cupric Ion Reducing Antioxidant Capacity (CUPRAC) assay[20]

An 1 mL $CuCl_2$ 1×10^{-2} M, 1 mL Neocuproine 7.5×10^{-3} M, 1 mL NH_4Ac 1 M, and 0.1 mL H_2O was added with 1 mL of the extract in ethanol and it was incubated for 30 min. The absorbance was measured at a 450 nm wavelength. Each sample were tested in triplicate.The antioxidant capacity of trolox with concentration of 10, 25, 50, 75, 100, 150, 200, and 250 µM was measured as standard. The antioxidant capacity of extract was expressed in µmol trolox/g of extract.

RESULTS

1. Chemical Identification

Chemical identification of crude extract of marine bacterial associated with sponge posses bioactive properties were flavonoid, alkaloid, and terpenoid (Table 1).

Table 1

Chemical identification of extracts from marine bacteria assosiated with sponge

Bioactive Compound	Extract of bacteria assosiated with sponge		
	SAB E-31	SAB E-41	SAB E-57
Flavonoid	+	+	+
Alkaloid	+	+	+
Saponin	-	-	-
Tanin	-	-	-
Terpenoid	+	+	+
Steroid	-	+	-

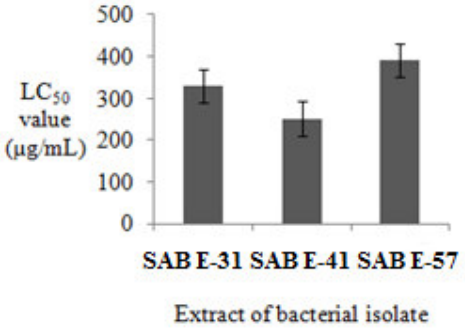
+ : present, □: absent

2. Toxicity

Brine Shirmp Letality Test (BSLT) was used to prescreening bioactivity of extract which had toxicity and it was determined in LC_{50} value. LC_{50} value is a concentration which may kill 50% of the test subject. Bioactivity test of extract marine bacteria SAB E-31, SAB E-41, and SAB E-57 showed a LC_{50} value were 328.04 µg/mL, 251.40 µg/mL, and 390.50 µg/mL. The lowest LC_{50} value was found in extract marine bacteria SAB E-41, while others had higher values (Graph 1).

Graph1

Toxicity of extracts from marine bacteria associated with sponge

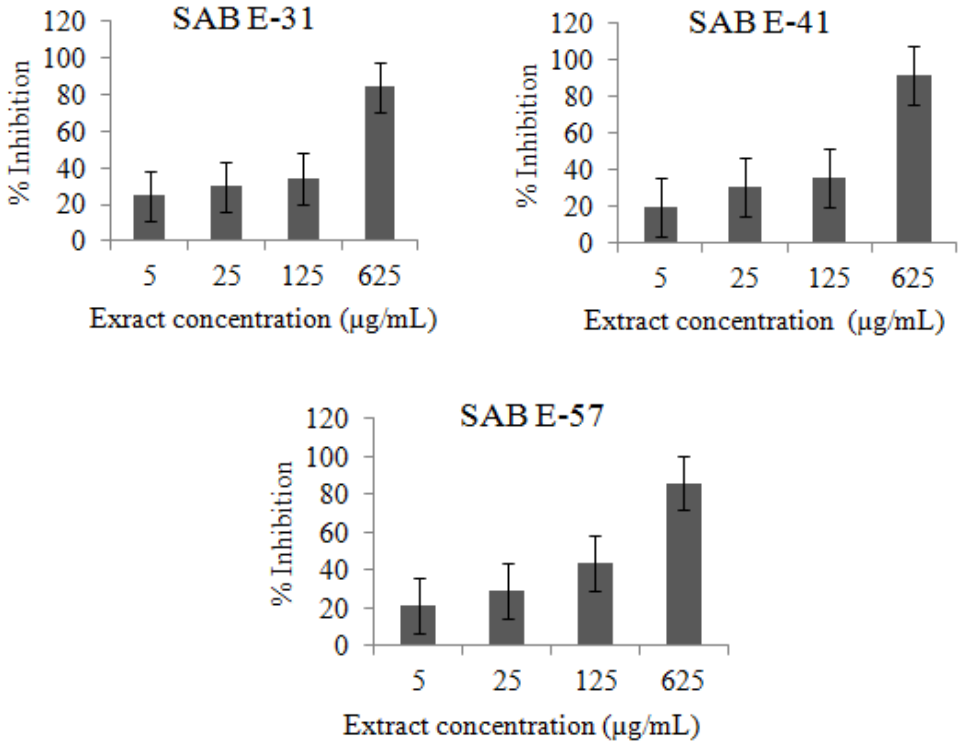


3. Anticancer activity

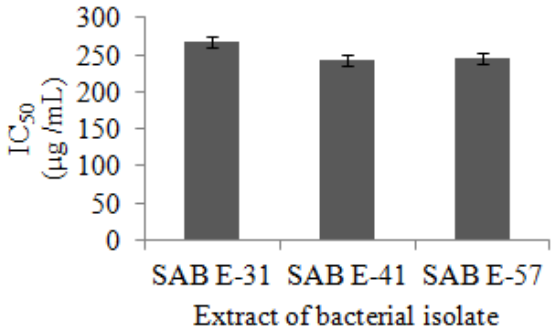
The extract against HeLa cells at a concentration of 5 µg/mL, 25 µg/mL ,125 µg/mL, and 625 µg/mL. The results that the all crude extract of SAB E-31, SAB E-41, and SAB E-57 against HeLa cells range from 20.2 % to 91.92% (Graph 2). Based on the percentage relationship of the curve HeLa cell inhibition and the concentration of the extract obtained IC_{50} values. IC_{50} values of extract marine bacteria associated with sponges SAB E-31, SAB E-41, and SAB E-57 were 267.03 µg/mL, 242.54 µg/mL, and 245.47 µg/mL (Graph 3).

Graph 2

Anticancer activity of extracts from marine bacteria associated with sponge



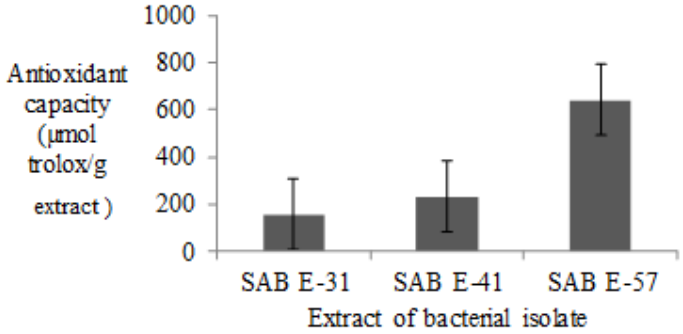
Graph3
IC50 value of extracts of bacteria associated with the sponge Jaspis sp. against HeLa cells



4. Antioxidant Capacity

The antioxidant capacity of extract marine bacteria associated with sponges SAB E-31, SAB E-41, and SAB E-57 were 156.94 µmol trolox/g extract, 233.86 µmol trolox/g extract, and 641.38 µmol trolox/g extract. The highest antioxidant activity of extracts of bacteria was SAB E-57 with antioxidant capacity was 641.38 µmol trolox/g extract (Graph 4).

Graph 4
Antioxidant capacity of extracts from marine bacteria associated with sponge



DISCUSSION

All of marine bacteria associated with sponge extraction in this studied consisted of flavonoid, alkaloid, and triterpenoid. Only, extract of marine bacteria SAB E-41 consisted of steroid (Table 1). Flavonoid, alkaloid, and terpenoid were reported having potency as anticancer and antioxidant[21, 22,23]. For example, biflavone isolated from *Selaginella willdenowii*, a flavonoid were significantly cytotoxic against a panel of human cancer cell lines[21]. Ecteinascidin-742 isolated from bacterial endosymbiont *Endoecteinascidia frumentesis* associated with a sponge *Ecteinnascadia tirbinata*, an alkaloid has potential as anticancer compounds against leukemia cells, cervix cancer cells and breast cancer cells[22]. Sponge *Jaspis stellifera* produced isomalabaricane triterpenes which are toxic against P388 leukemia cells[23]. Brine Shirmp Letality Test (BSLT) was used to prescreening bioactivity extract which had toxicity and it was determined in LC_{50} value. LC_{50} value is a concentration which may kill 50% of the test subject. Bioactivity test of extract marine bacteria SAB E-31, SAB E-41, and SAB E-57 showed an LC_{50} value was 328.04 µg/ml, 251.40 µg/ml, and 390.50 µg/ml (Graph 1). All of extract had a toxic effect on brine shirmps because they had LC_{50} value below 1000 µg/mL[18]. Toxic effect on brine shirmps by extract indicated the potency extract as

anticancer[13]. Extract of marine bacteria associated with sponges had cytotoxic activity against cancer cervix HeLa cells. Crude extracts of *α–Proteobacterium* from *sponges Suberites domuncula* at a concentration of 100 µg /mL inhibited ± 30% HeLa cells[6]. It was approached with the results that the all crude extract of SAB E-31, SAB E-41, and SAB E-57 against HeLa cells range from 33.97% to 43.57% at a concentration of 125 µg/ mL (Graph 2). Based on the percentage relationship of the curve HeLa cell inhibition and the concentration of the extract obtained IC_{50} values. According to the National Cancer Institute (NCI) IC_{50} value is the concentration of extract required to inhibit cancer cell growth until 50%[24]. IC_{50} values of extract marine bacteria associated with sponges SAB E-31, SAB E-41, and SAB E-57 were 267.03 µg/mL, 242.54 µg/mL, and 245.47 µg/mL (Graph 3). If they were compared with IC_{50} values extract of *Ficus pseudopalma* was 300 µg/mL and it was potential as chemopreventive agent against cancer[25]. Moreover, IC_{50} values of the crude extracts were determined under 300 µg/mL and may be used potential as chemopreventive agent against servix cancer. Antioxidant capacity using standard compounds trolox then it was expressed in TEAC (Trolox Equivalent Antioxidant Capacity)[20]. Antioxidant capacity trolox expressed in µmol/g of extract. The results CUPRAC assay of antioxidant capacity showed three extracts from bacteria associated with the sponge posses antioxidant activity. The antioxidant capacity of extract marine bacteria associated with sponges SAB E-31, SAB E-41, and SAB E-57 were 156.94 µmol trolox/g extract, 233.86 µmol trolox/g extract, and 641.38 µmol trolox/g extract. The high antioxidant activity of extracts of bacteria was SAB E-57 with antioxidant capacity was 641.38 µmol trolox/g extract (Graph 4). The result was approached with the antioxidant activity of *Apium nodiflorum* extract (110 µmol trolox/g extract), and *Cichorium intybus* extract (210 µmol trolox/g extract) which had potency as antioxidant[26]. The antioxidant activity value of SAB E-57 was approached with the antioxidant activity of turmeric extract (700 µmol trolox/g extract) from rhizomes of the plant Curcuma lounge which had potency as an antioxidant[27].

CONCLUSION

The crude extract of marine bacterial isolates associated with sponge *Jaspis* sp. posses bioactive properties were flavonoid, alkaloid, and triterpenoid. The exctract of marine bacteria displayed potential anticancer and antioxidant activities. The extract of marine bacterial isolates exhibits antioxidant activity and may be used as a chemopreventive agent againts cervix cancer.

ACKNOWLEDGMENT

Part of this work was supported by Directorate General of Higher Education of Indonesia through “Penelitian Strategis Institut Pertanian Bogor 2013” to ATW. We greatefully acknowledge for the support given to carry out this research.

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