Screening of Antioxidant and Anti-Acne Activities in 16 Sea Cucumber in Indonesia

A. Ardiansyah*, A. Nugroho, A. Rasyid, M.Y. Putra
Research Center for Biotechnology, Indonesian Institute of Sciences, Bogor, West Java, Indonesia.
*Corresponding Author: ardiardiansyah24@gmail.com

Abstract. Sea cucumbers are marine organism that have interesting biological activities and generally used for food, cosmetics, and medicine. The use of sea cucumbers in cosmetics due to sea cucumbers have good antioxidant and antibacterial activity. There have not been many studies on sea cucumbers in Indonesia that show sea cucumbers as antibacterial (especially bacteria that cause acne. This study was conducted on sixteen species of sea cucumber from genus Actinopyga, Bohadscia, Holothuria, Pseudocolochirus, and Stichopus to select sea cucumber species that have the best activity in counterring free radicals (antioxidant) and inhibits acne bacterial growth (anti-acne activities) on Propionibacterium acnes, Staphylococcus epidermidis, and Staphylococcus aureus. Antioxidant test is carried out using DPPH (2,2-diphenyl-1-picrylhydrazil) method while antibacterial test uses Plate Bioassay method with resazurin indicator. Test results on the sixteen samples of methanol extract of sea cucumber species showed that H. leucospilota was the species with the highest antioxidant activity with an IC50 value of 9.66 ± 0.15 mg.mL-1 and with an inhibition of 53.09 ± 1.20 % at a concentration of 10 mg.mL-1. Five species of sea cucumbers that have antibacterial activity in the three bacteria tested were Holothuria impatiens, Holothuria scabra, Pseudocolochirus sp., Stichopus vastus, and Holothuria atra.

1. Introduction
Indonesia is a country with a high diversity of marine species [1], one of which is the sea cucumber species, a marine organism that has benefit as source of new functional materials with biological activity that can be used in foods and drugs. With 1,716 species that have been found, this species is the largest biodiversity in Asia Pacific. In history, Indonesia is recorded as an old sea cucumber exporting country with 350 species owned and 26 species that have economic value [2]. Especially in China, sea cucumbers are included in marine organisms that are important in economic aspects in various Asian countries [3].

The high value of sea cucumbers in the economic aspect is closely related to the active properties they have in curing several diseases such as hypertension, eczema, arthritis, and wounds [4, 5]. These benefit properties come from the defence mechanism of sea cucumbers which are considered as natural marine products that have ecological functions as antifouling, anti-predation, and protection against UV rays [6]. Meanwhile, other activities such as free radical scavengers [3] and antibacterial [7] were also found in research results related to the benefits of sea cucumbers.

Free radicals are active molecules with unpaired valence electrons [8] which play an important role for the body, such as in the process of energy creation, cell growth, and immunity. However, these activities can cause various cell and tissue disorders that result in damage or modification of the DNA structure [9], that can lead to cell death, cell abnormalities, and cancer [10]. Antioxidants themselves have a positive effect on human health because they can protect the body from free radical damage [3]. On the other hand, antioxidant compounds have also been used in food product storage activities and are also used to reduce the process of decay and rancid odor in feed products. According to [11], fish feed exposed to air is easily oxidized. This causes chemical changes and causes nutrient degradation, and also produces a rancid odor so that the change in odor reduces the attractiveness of the feed. One type of synthetic antioxidant compound used, such as BHT, has negative side effects, such as toxic potential and carcinogens on reproductive effects in humans and other organisms [12]. Therefore, the search for new sources of natural antioxidants must continue.

Antibacterials are substances that can inhibit and even kill bacteria by disrupting metabolism. This is very useful for overcoming diseases caused by bacteria (pathogenic bacteria) including acne-
causing bacteria caused by *Propionibacterium acnes*, *S. epidermidis*, and *S. aureus* bacteria. Acne vulgaris is a disorder in the pilosebaceous unit characterized by blackheads, papules, cysts, nodules, and scarring [13] as well as being a very common condition with a lifetime prevalence of around 85% and mostly occurring during adolescence [14]. The occurrence of this disease causes psychological disorders such as poor self-image, depression, and anxiety, which leads to a negative impact on quality of life [15]. One of them is the *S. aureus* bacteria, a type of bacteria that is classified as highly pathogenic and can cause disease in animals to humans [16] with the second largest rate after *Clostridium difficile* in the list of infectious disease treatments in the United States [17]. On the other hand, the bacterial resistance mechanism is also an important concern because it can occur naturally so that new antibacterial candidates are needed [18].

The diversity of marine resources is a great source of chemical compounds that have beneficial bioactivity for life because they can be a guide in finding new sources of medicine [19]. As much as 60% of the 877 drugs from 1988 to 2008 derived from natural ingredients have been commercialized [18]. Several studies related to the benefits of sea cucumbers on antioxidants have been conducted on *H. leucospilota* species [20], *H. scabra* [9], and *Stichopus chloronotus* [6], as well as benefits on acne-causing antibacterials in *S. aureus* [21] and *S. epidermidis* [22]. These things make research on this topic have a great opportunity to get develop. Sixteen species of sea cucumber belonging to five genus (Actinopyga, Bohadscia, Holothuria, Pseudocolochirus, and Stichopus) were used in this study for potential selection in counteracting free radicals (antioxidants) and inhibiting the growth of acne-causing bacteria (antibacterial).

2. Methodology

2.1 Sample preparation

Sea cucumber samples were obtained from the Lampung (LA) and Makassar (MA) waters. It was cleaned of dirt and sand using sea water and removed the stomach contents after identify also freeze before taken to the laboratory. There are sixteen species of sea cucumber which can be classified into five genus: Actinopyga (*Actinopyga lecanora* [LA], *Actinopyga miliaris* [LA]), Bohadscia (*Bohadscia similis* [MA], *Bohadscia sp.* [LA]), Holothuria (*Holothuria impatiens* [LA], *Holothuria leucospilota* [MA], *Holothuria scabra* [MA], *Holothuria atris* [LA], *Holothuria sp.* [MA]), Pseudocolochirus (*Pseudocolochirus sp.* [MA]), and Stichopus (*Stichopus hermani* [LA], *Stichopus horrens* [MA], *Stichopus noctivagus* [MA], *Stichopus ocellatus* [LA], *Stichopus vastus* [LA], *Stichopus sp.* [LA]). This sea cucumber sample then cut into small pieces (about 0.5 cm) for further processing.

2.2 Extraction by maceration

Sea cucumber samples that already prepared than conduct extraction process using the maceration method. The solvent used is methanol, which is done in several repetitions to obtain a clear filtrate. After the extract is obtained, it is then concentrated using a rotary evaporator at a temperature below 40°C.

2.3 Antioxidant Test with the DPPH Method

Sea cucumber extracts with different concentration variations in the range 0.1 mg.mL-1 to 50 mg.mL-1 were prepared for analysis. A total of 160 µL of extract solution at each concentration was added to 40 µL of 1 mM DPPH solution (Sigma, D9132) in one 96 well plates. Samples were incubated for 30 minutes at room temperature. The absorbance was measured at a wavelength of 515 nm using the Tecan® Microplate Reader. BHT (Butylated hydrotoluene) (Sigma, W218405-1KG-K) and Quercetin (Sigma, Q4951-10G) were used as standard standards under the same conditions. The concentrations used were 0.001 mg.mL-1 to 0.05 mg.mL-1 for BHT and 0.00025 mg.mL-1 to 0.01 mg.mL-1 for Quercetin. The measurement was repeated three times and the mean ± standard error was taken and the inhibitory activity was calculated. Free radical inhibitory activity is calculated using the formula:

\[
\text{% Inhibition} = (1 - \frac{A_{\text{sample}}}{A_{\text{control}}}) \times 100
\]
2.4 Anti-bacterial Test Using Microtiter Plate Bioassay

The sea cucumber extract was tested with the same concentration, 20 mg.mL-1. The test was carried out using a 96-well microplate using three wells as a triplicate. With the composition in each well; 25 µl of Nutrient broth media, 25 µl of tested bacterial suspension and 25 µl of tested extract were then incubated for 18-24 hours. Using the bacteria test *P. acnes, S. aureus, and S. epidermidis* with OD at 600 nm of 0.5. Indicators include positive control (erythromycin antibiotic), negative control (bacteria), media control (nutrient broth), and solvent control (DMSO 10%). The addition of 15 µL resazurin with a concentration of 0.1 mg.mL-1 used as an indicator. Observation of the color change was carried out after 15 minutes after resazurin was added and compared with the color that appeared on the control indicator. A change in color from blue to pink indicates a negative antibacterial result (bacteria remain alive), while no change in color or a blue color remains a positive result for antibacterial (dead bacteria).

3. Results and Discussion

3.1. Antioxidant Test Results

Sea cucumbers are natural products that have valuable ingredients, including their active pharmaceutical substances [23]. Knowing the difference in antioxidant potential in various types of sea cucumbers is very important in order to determine the source of natural products that have the best potential for utilization. In this study, the antioxidant activity test was carried out to select the sea cucumber species that had the best antioxidant activity using the DPPH (2,2-diphenyl-1-picrylhydrazil) method, a substance used as free radicals that produces a violet solution in methanol with the intensity of its color will reduced when reacting with antioxidant compounds to turn yellow or clear. The color change was used to evaluate the antioxidant activity of the extracts tested [24, 25]. The use of DPPH in this study has the advantage of being minimal cost, easy to do, and can be used to analyse large numbers of samples [26].

| Table 1. Antioxidant test results using the DPPH method |
|--------------------------------------------------------|
| **No.** | **Species** | **Concentration (mg.mL-1)** | **Inhibition (%)** | **Regression Value** | **IC_{50} (mg.mL-1)** |
|---------|-------------|-----------------------------|-------------------|---------------------|---------------------|
| 1       | *Actinopyga lecanora* | 50  | 16.38 ± 0.69 | 211.13 ± 30.81 |
|         |             | 20  | 9.74 ± 1.22  |                   |
|         |             | 10  | 9.74 ± 0.36  |                   |
|         |             | 5   | 7.55 ± 0.60  |                   |
|         |             | 2   | 5.84 ± 0.62  |                   |
| 2       | *Actinopyga miliaris* | 50  | 26.36 ± 1.04 | 101.75 ± 2.16 |
|         |             | 20  | 13.10 ± 0.19 |                   |
|         |             | 10  | 8.54 ± 0.71  |                   |
|         |             | 5   | 5.73 ± 0.02  |                   |
|         |             | 2   | 4.55 ± 1.65  |                   |
| 3       | *Bohadscia similis* | 50  | 22.66 ± 2.23 | 138.83 ± 24.91 |
|         |             | 20  | 16.11 ± 1.09 |                   |
|         |             | 10  | 11.88 ± 0.81 |                   |
|         |             | 5   | 9.36 ± 1.82  |                   |
|         |             | 2   | 7.95 ± 0.78  |                   |
| 4       | *Bohadscia* sp. | 50  | 24.94 ± 1.03 | 102.25 ± 3.08 |
|         |             | 20  | 8.93 ± 0.87  |                   |
|         |             | 10  | 4.96 ± 0.43  |                   |
|         |             | 5   | 2.88 ± 0.52  |                   |
|         |             | 2   | 1.68 ± 0.07  |                   |
| 5       | *Holothuria impatiens* | 20  | 46.13 ± 0.45 | 22.02 ± 1.04 |
|         |             | 10  | 23.78 ± 5.54 |                   |
|         |             | 5   | 16.80 ± 3.80 |                   |
|         |             | 2   | 11.08 ± 3.67 |                   |
|     | 1     | 5     | 10    |
|-----|-------|-------|-------|
| 6   | 4.33 ± 0.85 | 24.65 ± 0.86 | 14.26 ± 3.00 |
|     | 11.17 ± 0.83 | 6.81 ± 4.37 | 11.17 ± 0.83 |
|     | 9.66 ± 0.15 | 23.75 ± 2.26 | 23.75 ± 2.26 |
| 7   | 24.27 ± 0.85 | 17.41 ± 1.07 | 9.22 ± 0.63 |
|     | 9.17 ± 1.27 | 6.96 ± 1.01 | 9.17 ± 1.27 |
| 8   | 11.17 ± 0.65 | 15.96 ± 0.33 | 3.12 ± 0.09 |
|     | 6.81 ± 0.55 | 0.66 ± 0.55 | 0.66 ± 0.55 |
| 9   | 20.73 ± 0.30 | 16.89 ± 1.08 | 9.49 ± 0.42 |
|     | 4.08 ± 0.38 | 2.33 ± 0.71 | 2.33 ± 0.71 |
| 10  | 31.73 ± 0.44 | 15.90 ± 0.30 | 9.07 ± 0.16 |
|     | 7.00 ± 0.06 | 5.84 ± 0.52 | 7.00 ± 0.06 |
| 11  | 35.64 ± 0.85 | 17.31 ± 0.32 | 13.93 ± 0.47 |
|     | 5.63 ± 0.86 | 3.27 ± 1.15 | 5.63 ± 0.86 |
| 12  | 31.51 ± 0.18 | 15.90 ± 0.32 | 7.22 ± 0.90 |
|     | 5.76 ± 0.81 | 3.00 ± 0.34 | 5.76 ± 0.81 |
| 13  | 53.51 ± 1.40 | 15.93 ± 0.12 | 8.80 ± 0.94 |
|     | 7.09 ± 0.20 | 4.70 ± 0.07 | 7.09 ± 0.20 |
| 14  | 45.82 ± 0.61 | 23.86 ± 0.27 | 13.25 ± 0.29 |
|     | 8.02 ± 1.20 | 4.74 ± 0.08 | 8.02 ± 1.20 |
| 15  | 21.83 ± 0.86 | 12.35 ± 0.47 | 15.08 ± 0.62 |

- **Holothuria leucospilota**: \( Y = 4.6978X + 4.6139 \)
- **Holothuria scabra**: \( Y = 1.252X + 6.6537 \)
- **Holothuria sp.**: \( Y = 3.1205X + 0.25 \)
- **Pseudocolochirus sp.**: \( Y = 3.9468X + 0.0999 \)
- **Stichopus hermanii**: \( Y = 0.5311X + 5.3047 \)
- **Stichopus horrens**: \( Y = 0.6729X + 2.4757 \)
- **Stichopus noctivagus**: \( Y = 0.589X + 2.0369 \)
- **Stichopus ocellatus**: \( Y = 1.0298X + 0.0881 \)
- **Stichopus vastus**: \( Y = 0.8482X + 4.3809 \)
- **Stichopus sp.**: \( Y = 1.3924X + 1.1151 \)
The test results on the sixteen samples of sea cucumber extract of methanol show that *H. leucospilota* from Lampung waters was the species with the best IC₅₀ value with 9.66 ± 0.15 mg.mL⁻¹. Meanwhile, BHT and Quercetin as control indicators had a better value than the test samples with 0.03 ± 0.00 mg mL⁻¹ for BHT and 0.01 ± 0.00 mg mL⁻¹ for Quercetin. It can also be seen in the inhibition diagram based on the test concentration of 10 mg mL⁻¹, it shows the highest inhibition with 53.09 ± 1.20 % by *H. leucospilota* from Lampung waters and followed by the species *Pseudocolochirus sp.* with 40.73 ± 0.30% and species of *Holothuria sp.* with 31.17 ± 0.65% from Makassar waters.

![Figure 1. Inhibitory percentage at a concentration of 10 mg.mL⁻¹.](image)

This free radicals activity has also been conducted by [27] for the *H. leucospilota* species in water extract and organic solvents and obtained better values with 3.91 ± 0.12 mg.mL⁻¹ for water extract and 5.44 ± 0.15 mg.mL⁻¹ for organic solvents, while the results of the study [20] using dichloromethane solvent produced an IC₅₀ value of 1 mg.mL⁻¹. In another similar study conducted by [9] using ascorbic acid as a comparative indicator found that the results of the crude extract activity of *H. scabra* were lower than the control indicator, but the purified compounds had better values than crude extract and control indicators. This is likely to occur in each sample tested, given the extract being tested is also in a crude form. Meanwhile, the types of compounds that have a major relationship to the contribution of antioxidant activity contained in sea cucumbers are generally phenolic compounds as stated by [4]. It stated that the *H. atra* species contains several components of phenolic compounds such as chlorogenic acid, pyrogallol, coumaric acid, and catechins.
3.2 Microtiter Plate Bioassay Antibacterial Test Results

This study also conducted the potential for antibacterial activity, especially acne-causing bacteria (P. acnes, S. aureus, and S. epidermidis) using the resazurine indicator on a microtiter plate bioassay. Resazurin indicator is used to differentiate between a positive test result for antibacterial or a negative indication. Repetition is done three times for a more valid interpretation. This method has been used by [28] with similarities in work principles but different in the sample and composition comparison used.

Both microtiter plate and disc diffusion methods have often been used as non-quantitative indicators to measure the antibacterial activity contained in natural products [29]. However, for now, there are no definite interpretation criteria on the disc method because it will only revolve around the inhibition zone value [30]. This method (microtiter plate) has advantages such as being standardized, accurate, cheap and easy to implement [31], coupled with technical problems such as inaccurate diffusion power of a test sample in the diffusion method due to differences in polarity between the test compounds contained in agar used in the disc method, so that it has an influence on the diffusion process which will affect the test results [32]. Therefore, this microtiter plate method was chosen to select potential antibacterial activity in sea cucumber extracts which still contain various or varied compounds.

Table 2. Microtiter plate bioassay antibacterial test results.

| No. | Name of Sea Cucumber Species | P. acnes | S. epidermidis | S. aureus |
|-----|-----------------------------|----------|----------------|----------|
| 1   | Actinopyga lecanora         | +        | +              | -        |
| 2   | Actinopyga miliaris         | -        | -              | -        |
| 3   | Bohadsia similis           | +        | -              | +        |
| 4   | Bohadsia sp.               | -        | -              | -        |
| 5   | Holothuria impatiens        | +        | +              | +        |
| 6   | Holothuria Leucospilota     | -        | -              | -        |
| 7   | Holothuria scabra           | +        | +              | +        |
| 8   | Holothuria sp.              | -        | +              | -        |
| 9   | Pseudocolochirus sp.        | +        | +              | +        |
| 10  | Stichopus hermanii          | +        | +              | -        |
| 11  | Stichopus horrens           | +        | +              | -        |
| 12  | Stichopus noctivagus        | +        | -              | -        |
| 13  | Stichopus ocellatus         | -        | -              | -        |
| 14  | Stichopus vastus            | +        | +              | +        |
| 15  | Stichopus sp.               | -        | -              | -        |
| 16  | Holothuria atra             | +        | +              | +        |
| 17  | Eritromisin (Indicator)     | +        | +              | +        |
| 18  | Bacteria (Indicator)        | -        | -              | -        |
| 19  | Media (Indicator)           | -        | -              | -        |
| 20  | DMSO 10% (Indicator)        | -        | -              | -        |

Note: (+): indicated to have antibacterial activity
      (-): Indicated to have no antibacterial activity
The presence of antibacterial activity possessed by sea cucumbers is closely related to the compounds contained therein, such as the discovery of various types of saponin compounds [33, 34] and the content of terpenic compounds in sea cucumbers [7, 35]. This supports the magnitude of the correlation between the potential for antibacterial activity with the sea cucumber extract tested, for example the genus Holothuria has antibacterial activity [36] especially the *H. atra* test species have activity on *S. aureus* [5].

The use of the resazurin indicator makes it easier to observe the results of the antibacterial activity test by the mechanism of changing its color, turning pink or remaining blue. This is because resazurin is a blue redox dye which turns pink when the enzyme oxidoreductase is reduced to resofurin by the surviving bacterial cells [37]. So that with a sufficiently contrasting change, the use of this indicator can make it easier to observe and determine test results.

We did not study further why the result of antioxidant and antibacterial activity differed for the water areas tested. However, in general it may be caused by a number of factors such as physiological characteristics, environmental conditions, habitat and life cycle [38].

### 4. Conclusion

The test results of the sixteen samples of the methanol extract of sea cucumber species found *H. leucospilota* from Lampung waters as the species with the highest antioxidant activity with an IC\textsubscript{50} value of 9.66 ± 0.15 mg.mL\textsuperscript{-1} and an inhibitory of 53.09 ± 1.20 % at a concentration of 10 mg.mL\textsuperscript{-1}. However, it is still below the value of BHT and Quercetin as the control used. Variable results were not only found in the antioxidant activity test results, but also on the antibacterial activity. There were five species of sea cucumbers that had antibacterial activity on the three tested pathogenic bacteria that cause acne which are *H. impatiens* from Lampung waters, *H. scabra* from Makassar waters, *Pseudocolochirus sp.* from Makassar waters, *S. vastus* from Lampung waters, and *H. atra* from Lampung waters.

**Acknowledgement**

The authors would like to their grateful acknowledgement to COREMAP-CTI, which has funded this research activity to completion through the RPKP program 2019.
References

[1] Kasanah N, Triyanto, Seto DS, Amalia W and Isnansetyo A 2015 Antibacterial compounds from red seaweeds (rhodophyta) Indonesian J. of Chemistry 15(2) pp 201-209

[2] Pangestuti R and Arifin Z 2018 Medicinal and health benefit effects of functional sea cucumbers J. of Traditional and Complementary Medicine 8(3) pp 341-351

[3] Pangestuti R, Murniasih T, Putra MY, Rasyid A, Wibowo JT, Ardiansyah A and Untari F 2016 Free radical scavenging activity of selected sea cucumber species from Mataram- Lombok, Indonesia J. Teknologi 78(4-2) pp 179–185

[4] Esmat AY, Said MM, Soliman AA, Masry KSH and Badiea EA 2012 The bioactive compound, antioxidant potential and hepatoprotective activity of sea cucumber (Holothuria atra) against thioacetamide intoxication in rats Nutrition 29(1) pp 258-267

[5] Dhinakaran DI and Lipton AP 2014 Bioactive compounds from Holothuria atra of indian ocean, Springer Plus 3(1) pp 673

[6] Murniasih T, Putra MY and Pangestuti R 2015 Antioxidant capacities of Holothuria sea cucumbers. Annales Bogorienses 19(2) pp 21-26

[7] Li K, Li X, Gloer JB and Wang B 2012 New nitrogen-containing bromophenols from the marine red alga Rhodomela confervoides and their radical scavenging activity Food Chemistry 135(3) pp 868-872

[8] Mahmood NF and Shipman AR 2017:The Age-old problem of acne International J. of Women's Dermatology 3(2) pp 71–76

[9] Tan AU, Schlosser BJ and Paller AS 2018 A review of diagnosis and treatment of acne in adult female patients. International J. of Women's Dermatology 4(2) pp 56–71

[10] Kobayashi S D, Malachowa N, and Deleo FR 2015 Pathogenesis of Staphylococcus aureus abscesses The American J. of Pathology 185(6) pp 1518-1527

[11] Hu Y, Chen J, Hu G, Yu J, Zhu X, Lin Y, Chen S and Yuan J 2015, Statistical research on the bioactivity of new marine natural products discovered during the 28 years from 1985 to 2012 Marine Drugs 13(1) pp 202-221

[12] Soltani M and Baharara J 2014 Antioxidant and antiprolifervative capacity of dichloromethane extract of Holoturia leucospilota sea cucumber International J. of Cellular & Molecular Biology 13 pp 1-9

[13] Adibpour N, Nasr F, Nematpour F, Shakouri A and Ameri A 2014.Antibacterial and antifungal activity of Holothuria leucospilota isolated from Persian Gulf and Oman Sea Jundishapur J. Microbiol 7(1) pp 8708
[22] Shakouri A, Shoushizadeh MR and Nematpour F 2017 Antimicrobial activity of sea cucumber (Stichopus variegatus) body wall extract in Chabahar bay, Oman sea. Jundishapur J. of Natural Pharmaceutical Products 12(1) pp 1-5

[23] Qi H, Ji X, Liu S, Feng D, Dong X, He B, Srinivas J and Yu C 2017 Antioxidant and anti-dyslipidemic effects of polysaccharic extract from sea cucumber processing liquor Electronic J. of Biotechnology 28 pp 1–6

[24] Blois MS 1958 Antioxidant determinations by the use of a stable free radical Nature 181(4617): pp 1199–1200

[25] Abdille MH, Singh RP, Jayaprakasha GK and Jena BS 2005 Antioxidant activity of the extracts from Dillenia indica fruits Food Chemistry 90(4) pp 891–896

[26] Sveinsdottir H, Hamaguchi PY, Bakken HE and Kristinsson HG 2014 Method for assessing the antioxidative activity of aquatic food compounds. In: Hordur G.K. (ed.). Antioxidant and functional component in aquatic foods. John Wiley & Sons Ltd. p 151-174

[27] Althunibat OY, Hashim RB, Taher M, Daud JM, Ikeda M and Zali BI 2009 In vitro antioxidant and antiproliferative activities of three malaysian sea cucumber species European J. of Scientific Research 37(3) pp 376-387

[28] Pesic A, H.I. Baumann, K. Kleinschmidt, P. Ensle, J. Wiese, R.D. Süssmuth, and J.F. Imhoff. 2013. Champacyclin, a new cyclic octapeptide from Streptomyces strain c42 isolated from the Baltic sea Mar. Drugs 11(12) pp 4834-4857

[29] Yemoa A, Gbenou J, Affolabi D, Moudachirou M, Bigot A, Anagonou S, Portaels F, Quetin-Leclercq J, and Martin A 2011 Buruli ulcer: a review of in vitro tests to screen natural products for activity against Mycobacterium ulcerans Planta Medica 77(06) pp 641–646

[30] Elshikh M, Ahmed S, Funston S, Dunlop P, McGaw M, Marchant R and Banat IM. 2016. Resazurin-based 96-well plate microdilution method for the determination of minimum inhibitory concentration of biosurfactants Biotechnology Letters 38(6) pp 1015–1019

[31] Jorgensen JH and Ferraro MJ 2009 Antimicrobial susceptibility testing: a review of general principles and contemporary practices Clin Infect Dis 49(11) pp 1749-1755

[32] Sanchez JB and Kouznetsov VV 2010 Antimycobacterial susceptibility testing methods for natural products research Brazil J. Microbiol 41(2) pp 270–277

[33] Bahrami Y, Zhang W and Franco CMM 2018 Distribution of saponins in the sea cucumber Holothuria lessoni; the body wall versus the viscera, and their biological activities Mar. Drugs 16(423) pp 1-30

[34] Zhao YC, Xue CH, Zhang TT, Wang YM 2018 Saponins from sea cucumber and their biological activities J. of Agricultural and Food Chemistry 66(28) pp 7222-7237

[35] Sukmiwati M, Ilza M, Putri AE and Siauruk SW 2019 Antibacterial activity of sea cucumber (Holothuria atra) against Pseudomonas aeruginosa. IOP Conference Series: Earth and Environmental Science 404(012047) pp 1-6

[36] Bordbar S, Anwar F and Saari N 2011 High-value components and bioactives from sea cucumbers for functional foods-A review Mar. Drugs 9(10) pp 1761-1805

[37] Sarker SD, Nahar L and Kumarasamy Y 2007 Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in vitro antibacterial screening of phytochemicals Methods J. 42(4):321-324

[38] Diniz GS, Barbarino E and Lourenço SO 2012 On the Chemical Profile of Marine Organisms from Coastal Subtropical Environments: Gross Composition and Nitrogen-to-Protein Conversion Factors IntechOpen London p 297-320