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Original Article

Macrophage Immunomodulatory Activity of Unsaturated Fatty Acid Isolated from the Crown-of-thorns Star Fish (*Acanthaster planci*)

M Janib Achmad[1], Alim Isnansetyo[2], Noer Kasanah[2],Ustadi[2]

ABSTRACT

Introduction: Immunomodulator are chemical compounds that can improve the body's defense mechanisms both specific and non-specific, and non-specific induction of both cellular and humoral defense mechanisms. **Objectives:** The objectives of this study were to investigate immunomodulator activity and to identify the chemical constituents of active fractions from star fish *Acanthaster planci,* based on bioassay guided isolation. **Materials and Methods:** *A. planci* was collected from Ternate Island, North Moluccas, extracted with distilled methanol, partitioned with gradient chloroform-hexane-water and fractionated in column chromatography using silica gel and gradient hexane-ethyl acetate. Profiling chemical constituent was done by thin layer chromatograpahy and GC-MS. The immunomodulator activity was measured based on percentage of phagocytic capacity (PC) and phagocytic index (PI). **Results:** The result showed that 3 partition fractions exhibited immunomodulator activity. Data analysis exhibited that the best fraction was hexane fraction, and the best dosesmost effective doses of PC and PI were at 0.5 mg/kg body weight (BW) and 0.7 mg/kg BW, respectively. Data analysis of the 3 hexane fractions exhibited that the best fraction was fraction 3 and the best doses of PC was at 0.5 mg/kg BW and that of PI was at 0.7 mg/kg BW. Metabolites analysis using GC-MS yielded a number of chemical constituents of fraction 2 dan fraction 3 that dominated by unsaturated fatty acid. The study concluded that star fish *A. planci* from Ternate Island has a potential source of immunomodulator.

Key words: *Acanthaster planci*, Immunomodulatory, Phagocytic capacity, Phagocytic index, Ternate island, North moluccas.

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INTRODUCTION

Environmental conditions with high pollution, erratic weather, unhealthy eating pattern, less exercise and high level of stress, could decrease body's immunity or fail the immune response. These agents cause pathological damages that would eventually kill hospes.[1] More specifically, the factors create an easy condition for infectious agents to contaminate the body at any time and cause tissue damages or diseases such as flu, diarrhea, cough, fever, or even more serious diseases like pneumonia and cancer. At this condition, high level of immunity would be very essential.[1]

Particularly, the immune system serves to protect the body from infections by microorganisms, help the healing process and to dispose or repair damaged cells when any infections occurred.[2,3] For normal individuals, most infections only stay for a limited period of time and cause a minor permanent damage, since that the immune system works against infectious agents by controlling or destroying them. Importantly, increasing in immunity could be achieved by improving the function of the immune system using material that stimulates the immune system which is identified as immunomodulator.[1]

Immunomodulator could naturally strengthen body's resistance against numerous viruses and bacteria infections, or assist the treatment of diseases associated with immune system impairment. Immunomodulator works by stimulating the main factors of immune system, among others, via phagocytosis, complement system, secretion of antibody ribs, release of interferon α and γ, T and B lymphocytes, system of specific and cytokines antibody and synthesis of lung's surfactant.[3] Specifically, immunomodulator has three main functions, that are, as an immunostimulator to improve the functioning and the activity of the immune system, as an immunoregulator to

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regulate the immune system, and as an immunosupresor to impede or impair the activity of the immune system.[4,5,6]

Several natural materials, recognized as biological response modifiers (BRM), could stimulate the immune system as having important chemical compounds such as fatty acid, polysaccharides, terpenoid, alkaloid and flavonoid.[4,5,6] These compounds help to regulate and improve the immune functions on infections, respiratory, and to help the healing process of chemotherapy and anti-inflammation in allergies.

High biodiversities in Indonesian waters is very potential for the invention of new active materials, seeing that sea organisms have unique chemical compounds and bioactivities that are valuable for the field of pharmacological and health.[7-10] One of the sea organisms that could be investigated as immunomodulator is *Acanthaster planci*. *A. planci* is a starfish from class *Stelleroidea*, family *Acanthateridea* which is covered by poisoning thorn. The starfish is the main predator of coral which lives in a lagoon or in 3-10 meters depth in coral reefs area. As the main feed of *A. planci* is coral, it is estimated that the existence of the starfish would balance the development of coral reefs. Unfortunately, large quantity of *A. planci* would also responsible for the coral reefs devastation.[11]

Previous studies reported that *A. planci* has important chemical compounds as primarily and secondary metabolites such as: uracil deoxyribosidase, docosahexenoic acid, metil arachidonic acid α-linolenic acid, venom, terpenoid, alkaloid and flavonoid.[11-14] The compounds have biological activities consist of antifungal, antibacterial, cytotoxicit, anticoagulant and immunomodulator which are valuable for pharmacological industry.[7,15,16]

The current research is expected to provide information about the prospective benefits of star fish *A. planci* as a sources of immunomodulator substance. Briefly, the aims of current study were to (1) investigate the immunomodulator activity from *A. planci.*

(2) purify and identify the unsaturated fatty acid compounds which are responsible for immunomodulator activites.

MATERIALS AND METHODS

The specimen was identified at Animal Taxonomy Laboratory, Faculty of Biology and immunomodulating activity test was conducted at LPPT, University of Gadjah Mada Yogyakarta. Materials for this research were water, methanol, n-hexane, ethyl acetate, chloroform, 30 mice balbc weigh 19-21 g. *RPMI*-1640 (Sigma), HEPES, FBS (Gibco) 10% natrium carbonate, 10% fungizon 1 µl/ml (Gibco), penicillin streptosimin (Gibco) 2% ammonium carbonate 0,17M, (Con-A) (Sigma. C-5275), MTT (Sigma. C-5655), latex 3,0 µm (Sigma. LB-3*0*) metanol absoluteand giemsa 15% PBS pH 7.

Instrumentation used in this study included rotary evaporator(180^{0}C. 270 RPM. Heidolph. German), TLC (genestic type 300), chromatography column (Pyrex 100 ml), freezerCNR B5400H (National Indonesia), cintrifuge 1500 RPM (Primdad. German), centrifuge 9000 (Broun. German), separator funnel (Pyrex. German), microscope 100x (Olympus. Japan), video camera (STO-CM 816C. US) and GC-MS (GCMS-QP2010S SHIMADZU).

Procedure

Isolation and identification

Sampling.*A. planci* samples were collected from Ternate Island at 0.80^0 N - 0.90^0 N and 127.30^{0}E-127.40^{0}E, at 5-10 meters depth by scuba diving,in October 2013. The collected samples were cleaned by rinsing with seawater and distilled water and transported in cool box to Fisheries and Marine Science Laboratory of Khairun University Ternate and kept frozen at -20^{o}C. Subsequently, the samples were transported to Hydrobiology Laboratory of Fisheries Department of Gadjah Mada University Yogyakarta. Identification of the species was based on the study of.[14] Sample of *A. planci* is shown in Figure 1

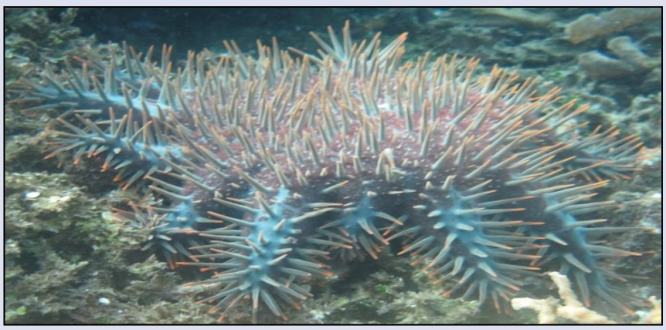

Figure 1: The Crown-of-Thorns starfish, *Acanthaster planci*
Phylum: Echinodermata; Class: Asteroidea; Order: Valvatida; Family: Acanthasteridae; Genus : *Acanthaster*; Species: *A. planci*(Linnaeus, 1759)

Extraction and partition

planci (700) was cut into small pieces and extracted by maceration method in methanol 90% (24 h) at room temperature. Afterwards, the extracts were evaporated in rotary evaporator 35^{o}C. These extracts were freeze dried and the yield was then calculated. Methanol extract was then partitioned with 187.5 ml hexane and 375 ml methanol to obtain hexane fraction. Subsequently, the 375 ml methanol was also partitioned with 187.5 ml chloroform and 187.5 ml water to obtain chloroform fraction and water fraction. At the end, the 3 fractions (hexane, chloroform and water) were evaporated and freeze dried to calculate the rendements.

Fractionationas having the highest activity, hexane fraction was chromatographed on glass column (50 cm) packed with silica gel 60 (0.2-05 mm). Elution was carried out using gradient 75% hexane to 25% ethyl acetate. Fractions were collected and checked by thin layer chromatography (TLC) by mobile phase hexane and ethyl acetate (7:3 v/v). The fractions with the same TLC spot were then combined which resulted in 3 fractions. The result of evaporation and drying of the 3 fractions yielded that 2.1 of fraction 1 had 0.7% yield, 2 of fraction 2 had 0.67% yield and 1.7 of fraction 3 had 0.57% rendement. Subsequently, fraction 1, 2 and 3 were subjected for further bioassay.

Gas Chromatography-Mass Spectrometry (GC-MS). Fraction with the highest activity was chosen as active fraction. The active fraction was then analyzed by GC-MS-QP2010S Shimadzu that equipped with RTX-5 MS column with 30 m length and 0.22 mm of internal diameter. The carrier gas used in this instrument was helium. The conditions of GC-MS instruments were 320^{0}C temperature injector, 13.7 kPa pressure, 40 ml/min total flow, 0.50 ml/min column flow, 25.90 cm/sec linear speed, 3 ml/min purge flow, 73.0 split ratio, programmed column temperature from 70^{0}C (hold for 5 min) until 300^{0}C (hold for 52 min), with the rate of temperature increase reached 10^{0}C/min. Schematic diagram of isolation and identification of chemical constituent from *A. planci* is shown in Figure 2.

Immunomodulator activity test

Blb-C mice (male 20 g) were used for *in vivo* testing. The fractions were injected i.p with 1 ml of fraction at doses of 0.1, 0.3, 0.5, and 0.7 mg/kg BW. The control mice were injected with 1 ml of PBS. On the fourth day after injection, the mice were killed by pervisceral dislocation, and peritoneal macrophage and isolated by standard procedure.[17] Ten ml cold RPMI was injected in the cavity in the peritoneum. Peritoneum liquid was taken and suspensioned, then centrifuged at 1200 rpm, 40^{o}C for 10 min.

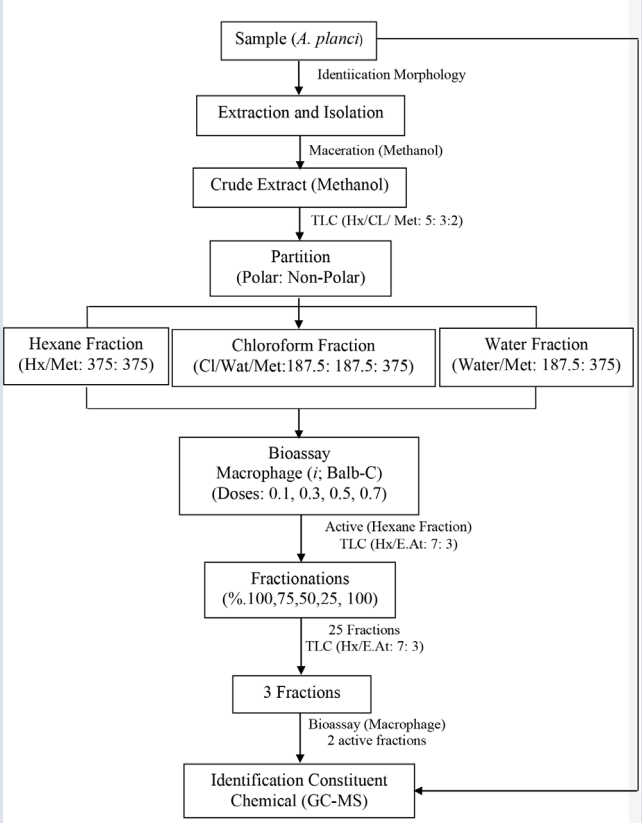

Figure 2: Schematic diagram of isolation and identification of chemical constituents from *A. planci*

Supernatant was then discharged and pellet was taken and added with 3 ml of complete medium.

Number of cells was enumerated by haemocytometer while cells viability was determined by trypan blue staining. The 50 µl and RPMI 950 µl cell medium diluted for 20 times were placed into 24 well micro plates with coverslipes and the cells were incubated for 24 h. Subsequently, the cells were cleaned with RPMI and added with 20 µl/well latex suspension and incubated for 60 min in a 5% CO_2 incubator at 37°C. Afterwards, the cellswere cleaned with PBS for 3 times to eliminate the unphagocytic latex. The cells were then dried at room temperature and fixed with methanol for 30 sec, then, methanol was eliminated and the coverslipes were idled until dry. Twenty percent (1 ml) of geimsa was then added to each dry well for 20 min, rinsed with aquades and dried at room temperature. The cells were observed with a microscope (400x), and 100 macrophage cells were calculated to obtain the phagocytic capacity(PC) and phagocytic index(PI) accordance with the formula of.[18]

(i) Phagocytic Capacity (PC)

$$PC = \frac{\text{Number of Macrophages Phagocytozing}}{\text{Total of Macrophages Counted}} \times 100$$

(ii) Phagocytic Indeks (PI)

$$PI = \frac{\text{Number of latex inside macrophages}}{\text{Number of macrophages phagocytozing}}$$

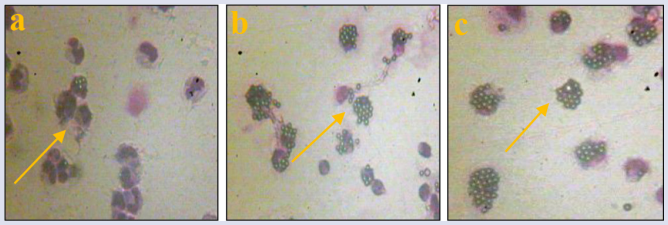

Figure 3: Macrophages that phagocytozing latex of (a) water fraction, (b) chloroform fraction and (c) hexane fraction.

RESULTS AND DISCUSSIONS

Immunomodulator Activity

Crude extract and partition fraction

The result of evaporation and drying of the 3 fractions yielded that 2.1 of fraction 1 had 0.7% yield, 2 of fraction 2 had 0.67% yield and 1.7 of fraction 3 had 0.57% rendement. Subsequently, fraction 1, 2 and 3 were subjected for further bioassay. Result showed that 65 g of methanol extract had 21.7% rendement.

The impact of immunomodulatory activity of a natural product on body's immune system could be analyzed by observing macrophages activity. The activity of macrophages is the number of macrophage cells that actively phagocytozing in 100 cells.[19] Increasing activity of macrophages indicates an improvement in immune system to protect the body infected by any pathogens.[17]

Immunomodulatory activity was measured based on the abilityof macrophages to ingest latex particles in. Macrophages that phagocytozing latex is shown in Figure 3.

The test results of macrophages activities on 3 fraction partitions showed that in water fraction the average value of PC at 5 doses was ranged from 69.00 to 99.50, where the highest was at doses of 0.5 mg/kg BW and the lowest was on the negative control (1ml PBS). Meanwhile, the average value of PI at 5 doses was ranged from 1.66 to 2.83, where the highest was at doses of 0.7 mg/kg BW and the lowest was on the negative control (1 ml PBS). The result of the analysis of variance shows that PC at doses of 0.5 mg/kg BW was significantly different to the negative control, nevertheless, not significantly different to the positive control. Meanwhile, PI was not significantly different to the negative control.

The chloroform fraction exhibited an average value of PC at 5 doses that was ranged from 69.00 to 105.00, where the highest was at doses 0.3 mg/kg BW and the lowest was on the negative control (1ml PBS). Moreover, the average value of PI at 5 doses was ranged from 1.66 to 2.98, where the highest was at doses of 0.7 mg/kg BW and the lowest was on the negative control (1ml PBS). The result of analysis of variance showed that PC at doses of 0.5 mg/kg BW was significantly different to the negative control, but not significantly different to the positive control. Meanwhile, PI was not significantly different to negative control.

The hexane fraction showed that the average value of PC at 5 doses was ranged from 69.00 to 119.00, where the highest was at doses of 0.7 mg/kg BW and the lowest was on the negative control (1 ml PBS). Besides, the average value of PI at 5 doses was ranged 1.66 to 3.17, where the highest was at doses of 0.7 mg/kg BW and the lowest was on the negative control (1 ml PBS). The result of the analysis of variance showed that PC and PI at doses of 0.7 mg/kg BW were significantly different to negative control, but not significantly different to positive control. The data analysis of 3 partition fractions exhibited that the best fraction was hexane fraction, and the best doses of PC was at 0.5 mg/kg BW and IP was at 0.7 mg/kg

Table 1: Phagocytic capacity (PC) and phagocytic index (PI) of fraction water, chloroform and hexane

Doses (mg/kg BW)	(%)Phagocytic Capacity (PC)				Phagocytic Index (PI)			
	Water Fraction	Cloroform Fraction	Hexane Fraction	Average	Water Fraction	Cloroform Fraction	Hexane Fraction	Average
Control (+)	106,00±6,00[a]	106,00±6,00[a]	106,00±6,00[a]	106,00±0,00[a]	2,69±0,38[a]	2,69±0,38[a]	2,69±0,38[a]	2,69±0,00[a]
Control (-)	69,00±8,49[b]	69,00±8,49[b]	69,00±8,49[b]	69,00±6,57[c]	1,66±0,15[a]	1,66±0,15[a]	1,66±0,15[b]	1,74±0,15[b]
0.1	66,50±4,95[b]	72,00±12,73[b]	94,00±19,80[ab]	77,50±16,88[bc]	2,03±0.49[a]	2,70±0,42[a]	2,81±0,94[a]	2,51±0,59[ab]
0.3	77,00±15,56[b]	105,00±4,24[a]	99,50±2,12[a]	93,83±15,13[ab]	2,21±0,11[a]	2,67±0,30[a]	2,81±0,10[a]	2,56±0,32[a]
0.5	99,50±9.19[a]	83,00±8,49[ab]	115,50±6,36[a]	99,33±15,83[a]	2,37±0,23[a]	2,76±0,56[a]	2,75±0,78[a]	2,63±0,48[a]
0.7	74,50±6,36[b]	87,00±16,97[ab]	119,00±9,00[a]	93,50±22,51[a]	2,83±1,16[a]	2,98±0,78[a]	3,17±0,21[a]	3,00±0,65[a]
Average	77,30±14,28[a]	83,20±15,79[ab]	106,60±32,83[b]		2,25±0,57[a]	2,58±0,56[a]	2,53±0,69[a]	

Note: Differ significantly at P<0,05 indicated by different notation

Control (+) = Echinacea (0.4 mg/kg BW)

Control (-) = PBS 1 ml

Table 2: Phagocytic capacity (PC) and phagocytic index (PI) from 3 hexane fractions.

Doses (mg/kg BW)	(%)Phagocytic Capacity (PC)				Phagocytic Index (PI)			
	Fraction 1	Fraction 2	Fraction 3	Average	Fraction 1	Fraction 2	Fraction 3	Average
Control(+)	106,00±6,00[a]	106,00±6,00[a]	106,00±6,00[a]	106,00±0,00[a]	2,69±0,38[a]	2,69±0,38	2,69±0,38[a]	2,69±0,00[a]
Control (-)	69,00±8,49[b]	69,00±8,49[a]	69,00±8,49[b]	69,00±6,57[c]	1,66±0,15[a]	1,66±0,15[a]	1,66±0,15[b]	1,74±0,15[b]
0.1	66,50±4,95[b]	72,00±12,73[b]	94,00±19,80[ab]	77,50±16,88[bc]	2,03±0.49[a]	2,70±0,42[a]	2,81±0,94[a]	2,51±0,59[ab]
0.3	77,00±15,56[b]	105,00±4,2[4a]	99,50±2,12[a]	93,83±15,13[ab]	2,21±0,11[a]	2,67±0,30[a]	2,81±0,10[a]	2,56±0,32[a]
0.5	99,50±9.19[a]	83,00±8,49[ab]	115,50±6,36[a]	99,33±15,83[a]	2,37±0,23[a]	2,76±0,56[a]	2,75±0,78[a]	2,63±0,48[a]
0.7	74,50±6,36[b]	87,00±16,97[ab]	119,00±9,00[a]	93,50±22,51[a]	2,83±1,16[a]	2,98±0,78[a]	3,17±0,21[a]	3,00±0,65[a]
Average	77,30±14,28[a]	83,20±15,79[ab]	106,60±32,83[b]		2,25±0,57[a]	2,58±0,56[a]	2,53±0,69[a]	

Note: Differ significantly at P<0,05 indicated by different notation

Control (+) = Echinacea (0.4 mg/kg BW)

Control (-) = PBS 1 ml

BW. The values of PC and PI from 3 partition fractions are presented in Table 1.

Fractionation

Fractionation of hexane fraction resulted in 3 fractions.

Fraction 1 showed that the average value of PC at 5 doses was ranged from 69.00 to 99.50, where the highest was at doses of 0.5 mg/kg BW and the lowest was on the negative control (1ml PBS). In addition, the average value of PI at 5 doses was ranged from 1.66 to 2.37, where the highest was at doses of 0.7 mg/kg BW and the lowest was on negative control (1ml PBS). The result of analysis of variance showed that PC at doses of 0.5 mg/kg BW was significantly different to the negative control, meanwhile, PI was not significantly different to negative control.

Fraction 2 showed that the average value of PC at 5 doses was ranged from 69.00 to 105.00, where the highest was at doses 0.5 mg/kg BW and the lowest was on the negative control (0.4 mg/kg BW). Besides, the average value of PI at 5 doses was ranged from 1.66 to 2.37, where the highest was at doses of 0.7 mg /kg BW and the lowest was on negative control (1 ml PBS). The result of analysis of variance showed that PC at doses of 0.5 mg/kg BW was significantly different to the negative control, meanwhile, PI was not significantly different to negative control.

Fraction 3 showed that the average value of PC at 5 doses was ranged from 69.00 to 115.50, where the highest was at doses 0.7 mg/kg BW and the lowest was on the negative control (1ml PBS). Further, the average value of PI at 5 doses was ranged from 1.66 to 2.98, where the highest was at doses of 0.7 mg/kg BW and the lowest was on negative control (1ml PBS). The result of analysis of variance showed that PC and PI at doses of 0.7 mg/kg BW were significantly different to the negative control. Data analysis of the 3 hexane fractions exhibited that the best fraction was fraction 3 and the best doses of PC was at 0.5 mg/kg BB and that of PI was at 0.7 mg/kg BW. The results of PC and PI from 3 fractions are presented in Table 2.

Identification of Chemical Compounds from Active Fraction

The bioactive compounds presented in fractions 2 and fraction 3 were analyzed by GC-MS. The dominant peaks at different retention time were analyzed and identified. Fraction 2 was contained fatty acid and identified as 8-11-14 eicosatrienoic acid and 9-octadecenoic acid. Fraction 3 was contained hexadecatrienoic acid and octadecatrionoic acid. Chemical constituents identified from fractions 2 and fraction 3 of *A. planci* are shown in Figure 4-13.

According[20] eicosatrienoic acid is a polyunsaturatedomega-3 fatty acid, with linear formula $C_{20}H_{34}O_2$. ETrA found in fish fatty and algae.[21] Moreover, beneficial effects of ETrA are to prevent inflammation and autoimmunity process. Octadecenoic acid is a monounsaturated C-18 fatty acid with linear formula $C_{18}H_{34}O_2$.[20] Moslty, the fatty acid is found in marine organisms such as fish, algae, and some echinodermata.[21] Octadecenoic acid has beneficial effects in immunological process, help to decrease inflammatory, help to prevent arteriosclerosis and high blood pressure, and as an antitumor.

Hexadecatrienoic acid is a polyunsaturatedomega-3 fatty acid, with molecular formula $C_{16}H_{26}O_2$.[20] The fatty acid is also found in fish fatty and algae. Beneficial effects of hexadecatrienoic are to prevent inflammation and autoimmunity process.[23,24] Octadecatrionoic acid is a conjugated polyunsaturated fatty acid, with molecular formula $C_{18}H_{34}O_2$.[20] The fatty acid is mostly found in fish oil and algae.[21,23] Octadecatrionoic acid has positive effects on treatment of diseases such as inflammation (particularly rheumatoid arthritis and asthma), hypertension, arthritis, atherosclerosis, depression, adult-onset diabetes mellitus, myocardial infarction, thrombosis and some cancers.

Chromatogram fraction 2

The study of [22] reported that omega-3 (ω-3) fatty acids docosahexaenoic acid and eicosapentaenoic acid, along with γ-linolenic acid and antioxidants, modulated systemic inflammatory response and improved oxygenation and outcomes in patients with acute lung injury. Previous studies reported that some fatty acids such as metil arachidonat acid, ricosapentaenoic acid and docosahexaneoic acidhave an immunomodulator activity.[22,23,24] Reported that *A. planci* had low lipid content and

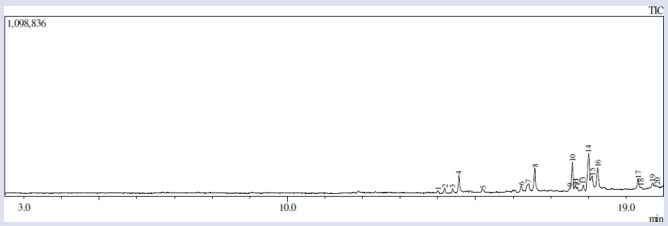

Figure 4: Chromatogram active fraction 1 of *A. planci*.

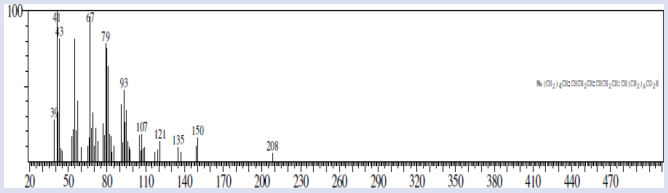

Figure 5: Peak MS no:15 retention time 18.099 min.
8-11-14 Eicosatrienoic acid

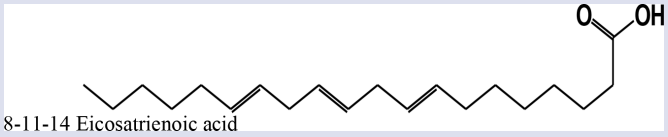

Figure 6: Prediction of chemical constituents structure peak no: 15 (MW.306).

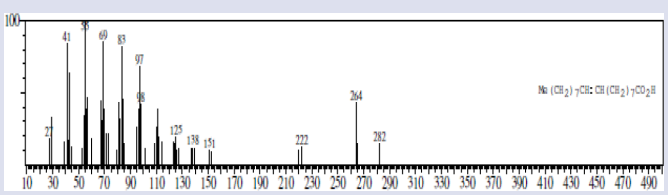

Figure 7: Peak MS no: 35 retention time 18.242 min.

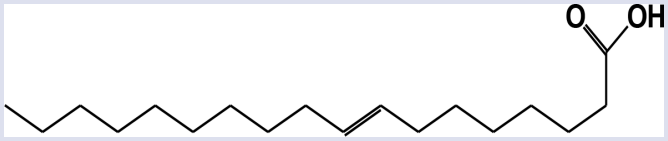

Figure 8: Prediction of chemical constituents structure peak no:35 (MW.282).

Chromatogram fraction 3

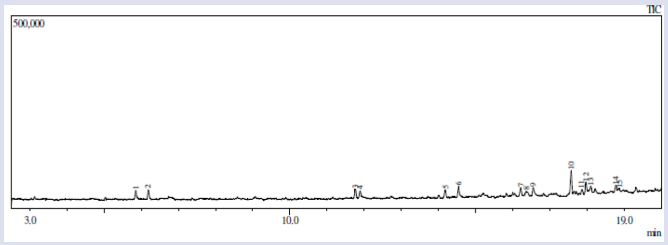

Figure 9: Chromatogram active fraction 2 of *A. planci*.

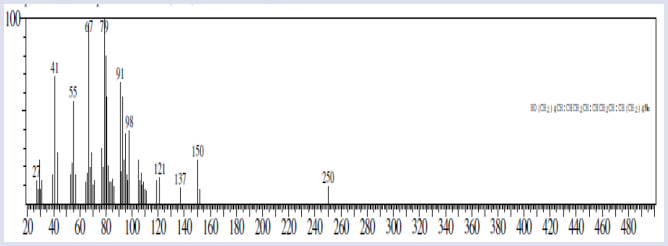

Figure 10: Peak MS no:10 retention time 17.557min.
Hexadecatrienoic acid

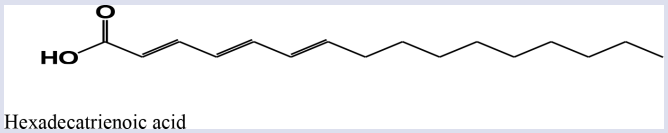

Figure 11: Prediction of chemical constituents structure peak no:9 (MW.250).

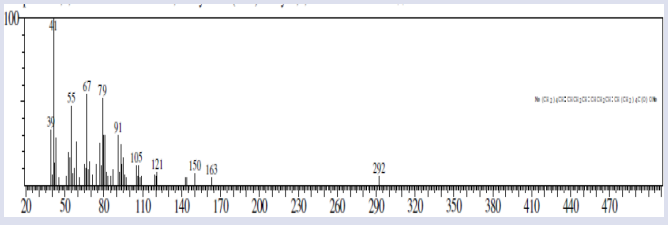

Figure 12: Peak MS no:15 retention time 17.573min.
Octadecatrionoic acid

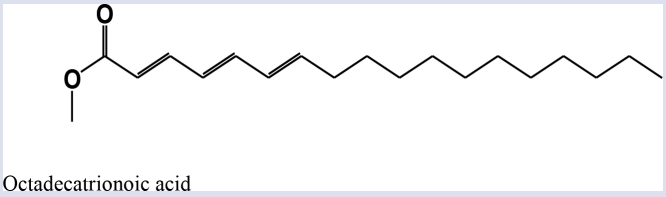

Figure 13: Prediction of chemical structure constituen peak no:15 (MW.292).

a good profile of fatty acid composition, which was manifested by the results that unsaturated fatty acids reached at 59.84 to 68.36% of total fatty acid and polyunsaturated fatty acids accounted for half of the unsaturated fatty acids. Polyunsaturated fatty acids contained timnodonic acid (EPA) (C20:5 ω-6, 1.31 to 2.73%) docosahexaenoic acid (DHA) (C22:6 ω-3,0.89-1.71%) which had important physiological functions to humans and animals. According to the study of,[7] those fatty acid compounds were derived from algae and coral that consumed by *A. planci*.

Nonetheless, there is no study reported the immunomodulator activity of unsaturated fatty acids from *A. planci* such as 8-11-14 eicosatrienoic acid, 9-octadecenoic acid, hexadecatrienoic acid and octadecatrionoic acid.

CONCLUSION

The study concluded that star fish *A. planci* from Ternate Island is a potential source of immunomodulator. The data analysis of 3 partition fractions exhibited that the best fraction was hexane fraction, and the best doses of KF was at 0.5 mg/kg BW and IF was at 0.7 mg/kg BW. Data analysis of the 3 hexane fractions exhibited that the best fraction was fraction 3 and the best doses of PC was at 0.5 mg/kg BB and that of PI was at 0.7 mg/kg BW. In summary, the result that analyzed and identified by GC-MS of fractions 2 and 3 contained:8-11-14 eicosatrienoic acid, 9-octadecenoic acid, docosahexaneoic acid and pentadecanoic acid.

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CONFLICT OF INTEREST

This research is a part of my unpublished Ph.D. dissertation research. Although Ph.D. study was funded by my institution (University of Khairun), the process and result of the research did not affected by the institution.

ABBREVIATIONS

A. planci: *Acanthaster planci*; **BW:** Body Weight; **BRM:** Biological Response Modifiers; **DHA:** Docosahexaenoic Acid; **E. Acetate:** Ethyle Acetate; **FBS:** Fetol Bovine Serum; **Fr:** Fraction; **GC-MS:** Gas Chromatography-Mass Spectrometry; **IP:** Index phagocytic; **MTT:** Dimethylthiazol; **MW:** Molecule weight; **PC:** Phagocytic capacity; **PBS:** Phosphate Buffered Saline; **RPMI:** Roswell Park Memorial Institute; **TLC:** Thin Layer Chromatography

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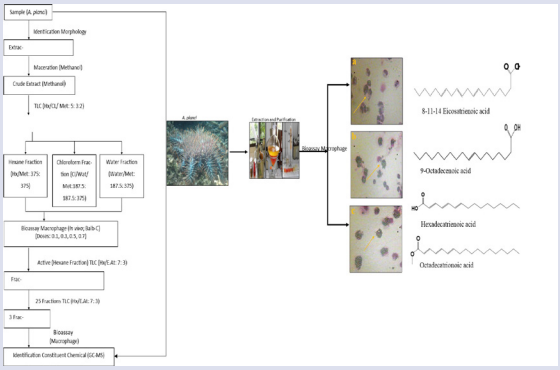
GRAPHICAL ABSTRACT



SUMMARY

This research focuses on marine natural product which *A. planci* is the main object. The organism is echinoderm of asteroidea class, acanthasteridae family and acanthaster genus. The study aims to determine the immunomodulatory activity of three fractions (water fraction, chloroform fraction and hexane fraction) from extract of *A. planci,* by calculating CP and PI. The test results showed that the hexane fraction had CP and PI values higher than the water fraction and chloroform fraction. The hexane fraction was further purified by hexane and ethyl acetate, with a percentage ratio of 100%, 75%, 50%, 25%, the purification results obtained by three fractions. The three fractions (fraction 1, fraction 2 and fraction 3) were then tested for immunomodulatory activity. The result of the test showed that fraction 2 and fraction 3 have CP and IP values better than fraction 1. These two fractions are then identified in the fatty acid compound by GC-MS analysis. The identification obtained four fatty acid compounds, and these compounds are suspected to have immunomodulatory activity.

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