P-ISSN : 2527-4627
Warmadewa Medical Journal
Available online http://ejournal.warmadewa.ac.id/index.php/warmadewa_medical_journal

WMJ (Warmadewa Medical Journal), Vol. 7 No. 1 Mei 2022, Hal. 23-32

In Vitro Study: Antimalarial Activity of Rivet Sea Cucumber Extract (Holothuria atra) With Ethyl Acetate Solvent Against *Plasmodium falciparum*

Zuhro Putri Febrianti[1], Prawesty Diah Utami[2], Verna Biutifasari[3]

[1]*Faculty of Medicine Hang Tuah University, Surabaya, Indonesia*
[2]*Parasitology Departement, Faculty of Medicine Hang Tuah University, Surabaya, Indonesia*
[3]*Pathology Clinic Departement, Faculty of Medicine Hang Tuah University, Surabaya, Indonesia*
Email[1]: prawesty.diah@hangtuah.ac.id

Abstract

Indonesia is experiencing a decreased cure rate of malaria caused by the species Plasmodium falciparum. Alternative medicine, in this case, uses marine biota, namely lollyfish (Holothuria atra), which has a lot of active compound content; The use of marine biota is stimulated by reports of resistance to artemisinin-based combination therapy (ACT). The purpose of this study was to find out the antimalarial activity of Holothuria atra extract with ethyl acetate solvent at doses of 0.01; 0.1; 1; 10; and 100 Ug/ml against Plasmodium falciparum in vitro. Extract lollyfish using the maceration method. The lollyfish powder will be dissolved with ethyl acetate solvent for 24 hours. Antimalarial activity testing is measured through 4 parameters, namely the measurement of parasitemia rates, % parasite growth, % inhibitory rate, and IC50. Antimalarial activity with ethyl acetate solvent belongs to a very active category with an IC50 value of 1.52 µg/mL. These results can kill the P. falciparum parasite with a percentage that reaches 0%. A decrease in the percentage of growth occurs when the dose given is too low so that the parasite can grow and survive. The result of higher concentrations of extracts will have a higher percentage of resistance to the growth of parasites. The potential for antimalarial activity in lollyfish extract is influenced by the presence of active content such as alkaloids, flavonoids, catechins, and pyrogallol owned by marine biota animals. This study suggests that lollyfish (H. atra) can be developed as an alternative treatment for malaria.

Keywords: Holothuria atra, antimalarial, Plasmodium falciparum, in vitro

INTRODUCTION

Malaria is an infectious disease of the *Plasmodium* parasite, transmitted through the bite of the *Anopheles sp* that has various symptoms such as cold chills and persistent fever because the area is too dense with poor sanitation that can facilitate the transmission of the disease. People who migrate to endemic areas can facilitate the spread of malaria [1–3]. The three states attributed for 99.5 percent of all malaria infections in Southeast Asia, with India providing the most incidents (87.9%), Indonesia (10.4%), and Myanmar (1.2%). There are five species of malaria in humans (*P. falciparum*, *P.vivax P.ovale*, *P.malariae*, and *P.knowlesi*) [4], but *Plasmodium falciparum* infection can result in severe circumstances and fatality [5–7].

ACT resistance has been identified in numerous countries, including Cambodia, Myanmar, Thailand, Vietnam, and the Democratic Republic of Laos. The role of parasite molecular markers in the occurrence of resistance due to the occurrence of mutations in parasite receptors associated with delayed clearance of parasites. Failure of ACT therapy to ensure treatment is productive or not, WHO has advised conducting regular monitoring at least once every 24 months in a row to detect changes that occur and efficacy in therapy [8]. Due to artemisinin resistance in *P.falciparum* malaria has spread globally around the world, therefore it takes alternative antimalarial treatments, one of which comes from marine biota [9,10].

Indonesia's vast territorial waters make it a source of marine life that has a

therapeutic effect on various diseases. So coastal areas with the potential for marine life can be used as an alternative medicine for a new regimen for malaria [11–13]. Sea cucumbers (*Holothuria atra*) are healthy food ingredients that contain various physiologically active substances, including vitamins A, C, B1, B2, B3, iron, magnesium, calcium, zinc, chondroitin sulfate, and saponin glycosides. Sea cucumber extract also produces effects to inhibit tumor cell growth and as an antiprotozoal activity [14,15]. *Holothuria atra,* also known as sea cucumber or *lollyfish,* is a species of sea cucumber commonly found in eastern Indonesia's waters. This species is most commonly found in coral reefs and seagrass meadows [16,17]. Sea cucumber extract *(Holothuria atra)* with ethyl acetate solvent in phytochemical tests showed positive to contain a group of alkaloid compounds, steroids, and saponins. This research is important to explore a new antimalarial regiment.

METHODS

This research was in vitro study with a posttest-only control group design. The experimental group was divided into three groups, of which (1) The negative control group included ten culture media of *P. falciparum* without antimalarial drugs or *H. atra* extract; (2) The positive control group included ten culture media of *P. falciparum* with antimalarial drug administration (chloroquine) but no extract administration; and (3) A treatment group included ten culture media of *P. falciparum* with ethyl acetate extract *H. atra* administration, but no antimalarial administration [18].

The study was conducted in 4 stages: preparation, extraction, in vitro antimalarial activity examination (by measuring levels of parasitemia, inhibitory rate, and inhibitory concentration 50/IC50), and descriptive – statistical analysis.

Preparation:

The research sample was *P. falciparum* (3D7 type, chloroquine-sensitive strain) culture media obtained from the ITD/ Institute Tropical Disease, Medicine Faculty of Airlangga University.

Sea cucumber, *H. atra* was obtained from the waters of Madura Sabuntan Village Sapeken Subdistrict and extracted at Veterinary Faculty Airlangga University. *H. atra* taxonomic test was conducted in the Lab. MIPA ITS. The ethics committee of Hang Tuah University's medical faculty has approved that this research be carried out

Extraction Process

The sample of sea cucumber *(H. atra)* is extracted by the maceration technique by drying the previously small cut sample first. Samples that have been dried are smoothed to widen their surface so that samples and solvents can give maximum results. *H. atra* powder will be dissolved with ethyl acetate solvent for 24 hours. The results are collected and evaporated with a vacuum rotary evaporator and put into an airtight container and stored in the refrigerator until use[17,19,20].

Examination of Antimalarial Activity

The antimalarial properties were evaluated using the Trager and Jansen method with Giemsa staining. The extract concentration dose used in this study was 0.01; 0.1; 1; 10; and 100 µg/ml. The test material was put into 24 disposable microwell plates with 1% parasite breed suspension and 5% hematocrit. The culture is inserted into the candle jar and incubated for 48 hours at a temperature of 37°C. Culture results are made preparations of thin blood removal with 20% Giemsa staining. Removal of dried blood is carried out by the microscopic examination with a magnification of 100x. Observation of the percentage of parasitemia and percentage of inhibition is done after the microscopic examination[19–21].

1. The first parameter used to assess antimalarial activity is parasitemia level. The calculation formula for the level of parasitemia levels is:

DOI: https://doi.org/10.22225/wmj.7.1.4287.23-32

$$\frac{\sum \text{of infected erythrocytes} \times 100\%}{5000 \text{ erythrocytes}}$$

2. The second parameter used to assess in vitro antimalarial activity is an inhibitory percentage. The calculation formula of inhibition percentage is:

$$\% \text{ Inhibition} = 100\% - \frac{Xu}{Xk} \times 100\%$$

Xu represents the percentage of parasitemia tests and Xk is the percentage growth on negative controls.

3. The last parameter to measure antimalarial activity in vitro is to calculate IC 50 using probit statistical analysis.

Descriptive and Statistical Analysis

The scale used to measure the percentage of parasitemia, and the percentage of inhibition is the ratio data. The data is entered and calculated in the parameters of parasitemia and inhibitory rate levels using excel software. Statistical analysis to calculate IC50 is obtained using probit analysis.

RESULT

The results of antimalarial activity tests with different types of concentrations in negative control groups, positive control, and control with sea cucumber extract of *H. atra* in different concentration doses can be shown in table 1.1 for parasitemia levels, table 1-2 for a percentage of parasite growth, table 1-3 for a percentage of inhibition and table 1-4 for IC 50.

Table 1-1 Percentage of parasitemia levels before and after administration of H. atra extract with ethyl acetate solvent against P. falciparum

Concentration (µg/mL)	Group Research		
	G1	G2	G3
100	**6.73**	**-**	**0.29**
10	**6.73**	**2.92**	**2.82**
1	**6.73**	**2.09**	**4.16**
0.1	**6.73**	**2.79**	**5.27**
0.01	**6.73**	**3.67**	**6.27**

Group 1 was a negative control group that got the same results because negative control was not treated without the administration of antimalarial drugs *or H. Atra* extract. Group 2, namely positive control with the administration of antimalarial drugs (chloroquine), showed low levels of parasitemia at a dose of 10 µg / ml, which is 2.92%. Group 3 showed the lowest levels of parasitemia at a dose of 10 µg/ml at 2.82% so the levels of group 3 parasitemia were lower than group 2 at 10 µg/ml.

DOI: https://doi.org/10.22225/wmj.7.1.4287.23-32

Table 1-2 Percentage growth of P. falciparum parasite

Concentration (µg/mL)	Group Research		
	G1	G2	G3
100	5.73	-	0
10	5.73	0.43	1.79
1	5.73	1.06	3.12
0.1	5.73	1.76	4.23
0.01	5.73	2.64	5.23

Group 1 on negative control obtained a fixed result because no treatment was done. Group 2 on positive control did not measure the growth of parasites at doses of 100 µg/ mL because the administration of chloroquine dose of 10 µg / mL has been able to suppress parasites close to zero (0). Group 3 on the growth of *P. falciparum* with an extract dose of 100 µg/mL showed results (0) in the absence of parasite growth at that dose so a large concentration dose provides a low percentage of parasite growth even if parasites cannot grow at that dose.

Table 1-3 Inhibitory rate P. falciparum

Concentration (µg/mL)	Group Research		
	G1	G2	G3
100	0	-	100
10	0	92.46	68.6
1	0	81.4	45.17
0.1	0	69.13	25.70
0.01	0	53.69	8.16

Group 1 is 0 because no treatment is done. Group 2 on positive control showed that at doses of 10 µg/mL, the percentage of inhibition reached 92.46%, which was close to 100% so that at doses of 100 µg/ mL, no more measurements were taken. The percentage inhibition of group 3 is greatest in extract *H.atra* ethyl acetate solvent with a concentration of 100 µg/mL, which is 100 percent. According to all the data, the higher the concentration of *H. atra* extract, the greater the inhibitory activity.

Table 1-4 Inhibitory Concentration extract of H. atra against P. falciparum parasites

Active Compounds	Extract Dose (µg/mL)					IC50 (µg/mL)
	0,01	0,1	1	10	100	
***H atra* with ethyl acetate solvent**	**8.16**	**25.70**	**45.17**	**68.60**	**100**	**1.52**
Klorokuin	**53.68**	**69.12**	**81.40**	**92.46**	**-**	**0.04**

Probit analysis results on IC50 values used to inhibit the growth of *P. falciparum* with ethyl acetate solvent of 1.52 µg/ml. This means that *H.atra* extract has strong antimalarial potential (IC50 < 5 g/ml). Chloroquine has an IC50 value of 0.04 µg/ml meaning that chloroquine produces a very active antimalarial potential compared to ethyl acetate solvent *H.atra* extract.

DISCUSSION

Calculation of parasitemia levels was done to determine the difference in the number of *P. falciparum* parasites at each concentration tested. The results of processing data in table 1-1 showed that the higher the dose of *H atra* extract was inversely proportional to the level of parasitemia because the larger the dose of the extract had the greater ability to suppress parasite growth [17,22]. At a dose of 100 µg/mL extract, it showed the highest suppression of parasitemia levels (0.29 %), the same as the G2 group that received chloroquine (with a dose of 100 µg/mL, it was able to suppress the parasite level until it was undetectable or 0%) [17,23].

This research used the parasite's growth rate to calculate the percentage growth of *P. falciparum* after using *H. atra* extract with ethyl acetate solvent at various doses. The larger the dose of concentration tested, the smaller the growth of the *parasite P. falciparum.* A decrease in the growth percentage occurs when the dose given is high so that the parasite can't survive and grow. The results of the resistance percentage measurement showed that the highest inhibition effect on sea cucumber extract with ethyl acetate solvent was obtained at a concentrated dose of 100 µg/mL with inhibition reaching 100%. Chloroquine inhibition ability on positive control indicates that a dose of 10 µg/mL can inhibit the development of parasites with achievements close to 100%. This result suggests that chloroquine inhibition ability was greater when administered at doses of 10 µg/mL compared to the group that got extract. The increase in the dose of the concentration of extracts tested, the greater the inhibitory activity. It is difficult to compare the results of this study with other similar studies, as no studies have been found exploring the antimalarial effects of *H.atra* [17,20].

Observations of the growth of parasites on negative controls showed the highest percentage. This is thought to occur due to the natural response of the *P. falciparum* parasite to defend itself in environmental conditions that are not suitable for its survival, so the parasite seeks to speed up the process of its growth [23,24]. The negative control group contained only media containing erythrocytes and *P.falciparum* strains of 3D7 without the administration of drugs or extract ingredients that could inhibit its growth. Because of this, the growth in the control group showed the highest value among the other two groups [17,20].

Positive controls in this study used chloroquine. Chloroquine is a group of 4-aminoquinoline compounds used as antimalarial drugs. Chloroquine can inhibit the growth of parasites through its schizonticide mechanism and blood gametocytocidal in all types of human plasmodium [20,25]. The mechanism of action of chloroquine is the acidic nature of chloroquine in the food vacuole that can increase the pH of organelles so that the metabolism of parasites is disrupted and toxic substances contained in

DOI: https://doi.org/10.22225/wmj.7.1.4287.23-32

chloroquine will poison the vacuoles thereby inhibiting food intake and parasites starve to death [26,27].

Interventions conducted in this study used an extract of sea cucumber *H. atra* with ethyl acetate solvent. Biodiversity in marine waters is much higher than on land. Many potential marine species are used as medicine and are very beneficial for humans. Sea cucumber or also called *Echinoderms* is a marine animal with invertebrate phylum that has many pharmacological benefits [28,29]. Compounds from sea cucumbers have pharmacological activities including antimalarial, antitumor, anticoagulant, antithrombotic, antioxidant, antiinfective, antiviral, and antifungal [30–32].

The body wall of sea cucumber contains polysaccharides consisting of fucan sulfate and *Fucosylated Chondroitin Sulfates* (FuCS), which have pharmacological activity as anticoagulants and antithrombotics [31,33]. Phytochemical test of antifungal activity of *H. atra* extract with positive ethyl acetate solvent contains triterpene glycoside compounds that can be used as antifungals [34–36]. Phenolic compounds and alkaloids in the internal organs of *H. atra* can also act as antioxidants. Saponin compounds act as antibacterials with their mechanism of work that kills bacteria by removing proteins and enzymes from inside the cell [37–39]. Sulfate saponin compounds in sea cucumbers can also act as antitumors by inhibiting the proliferation of colorectal cancer cells [40,41].

Identification of active compounds in *H. atra,* which are flavonoid, phenolic, and alkaloid compounds has been done in phytochemical tests and is suspected to have antimalarial activity [23,42,43]. Alkaloids are active nitrogenous compounds derived from animals and plants. Alkaloids have been successfully used for the treatment of malaria. Alkaloids can inhibit the formation of proteins, DNA, and RNA *P. falciparum* by inhibiting the enzyme *Plasmodium falciparum Dihydrofolate Dehydrogenase* (PfDHFDH) which synthesizes RNA and DNA in parasites [44–46]. Hetero flavone compounds of the flavonoid group have the highest and most active antimalarial activity due to the lowest IC50 values. One group of flavonoids, chalcone, can lower hemolysis levels from erythrocytes infected with *P. falciparum*. Catechins and pyrogallol are two phenolic compounds that have antimalarial properties. In the trophozoite and schizont phases, catechins can *prevent P. falciparum* from growing, so that merozoites cannot come out to infect erythrocytes [43,47]. Pyrogallol resulting from the hydrolysis of tannins from gallic acid can inhibit enzymes in *P. falciparum* parasites, the enzyme *Dihydrofolate Reductase* (DHFR) that synthesizes folate so that the growth of parasites will be disrupted [48,49]. This is with various active compounds contained in *H.atra* has the potential as an antimalarial that can suppress the development of *P.falciparum* in line with the results of this in vitro study.

The chance of *H. atra* extracts with ethyl acetate solvent as an alternative malaria drug can be proven by having very active antimalarial activity with its IC50 value. Ethyl acetate solvent *H. atra* with a concentrated dose of 100 µg/mL can kill the parasite *P. falciparum* with a growth percentage of 0%. The semipolar solvent ethyl acetate has been shown to have antimalarial activity [17,33,50] and further research is needed to find out and explore compounds in *H. atra* extract that have antimalarial activity.

CONCLUSION

In vitro studies show that the extract of sea cucumber (*Holothuria atra*) with ethyl acetate solvent at doses of 0.01; 0.1; 1; 10 and 100 µg/ml has antimalarial against *P. falciparum*. This is evidenced by a decrease in the percentage growth of parasites in the administration of ethyl acetate extract *H. atra* compared to negative controls. The greater the dose of extract concentration, the higher the percentage resistance to the growth of parasites *H.atra* extracts with ethyl acetate solvent was shown to produce highly active antimalarial activity with an IC50 value of 1.52 µg/mL. Although the value is still lower than

IC50 chloroquine, this is likely due to the use of chloroquine-sensitive *P.falciparum* strains.

ACKNOWLEDGEMENT

The authors of the article express their gratitude to the Faculty of Medicine at Universitas Hang Tuah and the Laboratory Institute of Tropical Disease at Airlangga University for their contributions to the in vitro research.

REFERENCES

1. Sutarto dan Eka Cania. 2017. Faktor Lingkungan, Perilaku dan Penyakit Malaria. Agromed Unila. Vol 4, No 1, pp. 173-184.
2. Alim A, Adam A, Dimi B. 2020. Prevalensi Malaria Berdasarkan Karakteristik Sosio Demografi. Jurnal Ilmu Kesehatan. Vol 19, No 1, pp. 1-9 .doi:10.33221/jikes.v19i01.399
3. Mousa A, Al-Taiar A, Anstey NM, Badaut C, Barber BE, Bassat Q, et al. 2020.The impact of delayed treatment of uncomplicated P. falciparum malaria on progression to severe malaria: A systematic review and a pooled multicentre individual-patient meta-analysis. PLoS Med. Vol 17, No 10, pp. 1-28. doi:10.1371/journal.pmed.100339
4. World Health Organization. 2016-2030 Global Malaria Programme World Health Organization. WHO Geneva. 2016.
5. Langi ET, Bernadus JBB, Wahongan GJP. 2016. Perbandingan deteksi Plasmodium falciparum dengan metode pemeriksaan mikroskopik dan teknik real-time polymerase chain reaction. Jurnal e-Biomedik. Vol 4, No 1.doi: 10.35790/ebm.4.1.2016.11058.
6. Xia J, Wu D, Wu K, Zhu H, Sun L, Lin W, et al.2020. Epidemiology of plasmodium falciparum malaria and risk factors for severe disease in Hubei Province, China. Am J Trop Med Hyg.Vol 103, No 4, pp 1534-1539. doi: 10.4269/ajtmh.20-0299.
7. Utami PD, Hadi U, Dachlan YP, Suryokusumo G, Loeki Enggar Fitri R, Yudo V. 2021. Protection against brain histopathological damage in experimental cerebral malaria models after exposure to hyperbaric oxigent. Res J Pharm Technol. Vol 14, No7, pp. 3833-3838. doi:10.52711/0974360X.2021.00665.
8. World Health Organization. Artemisinin and artemisinin-based combination therapy resistance. WHO. 2017.
9. Nowin E, Warouw V, Rimper J, Paulus J, Pangkey H, Sumilat D. 2018. Penapisan (skrining) aktivitas antibakteri beberapa ekstrak spons dari Teluk Manado. Jurnal Pesisir dan Laut Tropis. Vol 6, No 1, pp. 52-60. doi: 10.35800/jplt.6.1.2018.20198.
10. Sumarna S, Azis M, Sangadji SS, Idrus IN. 2019. Bioaktivitas Antimalaria Ekstrak Metanol Spons Petrosia ficiformis Asal Tanjung Rum Kepulauan Tidore. Techno Jurnal Penelitan. Vol 8, No 2, pp. 328-333. doi: 10.33387/tk.v8i2.1352.
11. Muharuddin M. 2019. Peran Dan Fungsi Pemerintah Dalam Penanggulangan Kerusakan Lingkungan. JUSTISI. Vol 5, No 2, pp 97-112. doi: 10.33506/js.v5i2.544.
12. Widyartono M, Rahmadian R.2019. Potensi Otec Di Provinsi Papua Indonesia. Inajeee Indonesian Journal of Electrical Eletronics Engineering. Vol 2, No 1, pp 17-21.doi:10.26740/inajeee.v2n1.
13. Hidayati AR, Widyawaruyanti A,

Ilmi H, Tanjung M, Widiandani T, Siswandono, et al.2020.Antimalarial activity of flavonoid compound isolated from leaves of artocarpus altilis. Pharmacogn Journal.Vol 12, No 4, pp 835-842.doi: 10.5530/pj.2020.12.120.

14. Rasyid A. 2008. Biota Laut Sebagai Sumber Obat-obatan. Oseana. Vol 33, No 1.pp 11-18.
15. Mulawarmanti D. 2019. Biota Laut Sebagai Alternative Bahan Obat (Pemanfaatan Teripang Emas Sebagai Terapi Ajuvan Di Kedokteran Gigi). Prosiding Seminar. pp 1-10.
16. Elfidasari D, Noriko N, Wulandari N, Perdana AT, Biologi PS, Sains F, et al. 2012. Identifikasi Jenis Teripang Genus Holothuria Asal Perairan Sekitar Kepulauan Seribu Berdasarkan Perbedaan Morfologi. Jurnal Al-Azhar Indonesia Seri Sains dan Teknologi.Vol 1(3),pp. 140–146.doi: 10.36722/sst.v1i3.53.
17. Basir A. 2013. Aktivitas antimalaria ekstrak teripang keling (Holothuria atra) terhadap Plasmodium falciparum secara in vitro.pp 1- 67.
18. Utami PD, Yudho V. 2021. High Antiplasmodial Activity of Golden Gamat (S.hermanni) Extract Through In Vitro Study. Eur J Biol Biotechnol. Vol 2, No 5, pp 19-23. doi: 10.24018/ejbio.2021.2.5.260.
19. Trager W, Jensen JB. 1976. Human malaria parasites in continuous culture. Science Journal. Vol 193, pp 673-675.doi: 10.1126/science.781840.
20. Nur' Aisyah Kusumaningrum. 2016. Uji aktivitas Antimalaria Daun Helianthus annuus L. Dengan Ekstraksi Bertingkat Terhadap Plasmodium falciparum Secara In Vitro. ADLN-Perpustakaan Univ Airlangga. pp 1-79.
21. Indriani I, Aminah NS, Puspaningsih NNT. 2020. Antiplasmodial Activity of Stigmastane Steroids from Dryobalanops oblongifolia Stem Bark. Open Chem.Vol 18, No 1, pp 259-264. doi: 10.1515/chem-2020-0027
22. Özbilgin A, Çavuş İ, Haghi M, Özel Y. 2020. In vitro Cultivation of Plasmodium falciparum. Turkiye parazitolojii Derg. Vol 44, No 4, pp 159-165. doi:10.4274/tpd.galenos.2020.7148
23. Budiarti M, Maruzy A, RK NR, Brotojoyo E. 2020. Aktivitas Antimalaria Daun Gempol (Nauclea orientalis (L.) L) terhadap Plasmodium falciparum. Media Penelitan dan Pengembangan Kesehatan. Vol 30, No 2, pp 135-146. doi: 10.22435/mpk.v30i2.3044.
24. Achmad F, Ardhistia R, Wiwied Ekasari N.2014. Uji aktivitas antimalaria ekstrak air daun johar (Cassia siamea Lamk) terhadap Plasmodium berghei secara in vivo. Jurnal Farmasi dan Ilmu Kefarmasian Indonesia. Vol 1, No 1, pp 6-9.
25. Darlina D, E.S. HN, A. AR. 2014. Efektifitas Klorokuin Terhadap Pertumbuhan Plasmodium falciparum Radiasi Secara In Vitro. Jurnal Forum Nuklir. Vol 8, No 1, pp 21-25.
26. Skrzypek R, Callaghan R. 2017. The "pushmi-pullyu" of resistance to chloroquine in malaria. Essays in Biochemistry.Vol 61, pp 167-175. doi: 10.1042/EBC20160060
27. Arnida A, Nurlely N, Kartinah N, Sutomo S. 2020. Aktivitas Penghambatan Polimerisasi Hem Ekstrak Etanol Daun Cambai Utan (Piper porphyrophyllum). Majalah Farmasetika. Volume 4, pp 173-177. doi: 10.24198/mfarmasetika.v4i0.25877
28. Ngantung A, Bara R, Sumilat D. 2017. Uji Aktivitas Antibakteri dari Spons Dictyonella funicularis dan Phyllospongia lamellosa yang Diambil pada Perairan Bunaken. Jurnal

Pesisir dan Laut Tropis. Vol 4, No 2, pp 10-16. doi: 10.35800/ jplt.4.2.2016.13035

29. Ratu K, Simbala HEI, Rotinsulu H. 2019. Uji Aktivitas Antimikroba Ekstrak Dan Fraksi Spons Phyllospongia lamellosa Dari Perairan Tumbak, Minahasa Tenggara Terhadap Pertumbuhan Mikroba Escherichia coli, Staphylococcus aureus dan Candida albicans. Pharmacon.Vol 8, No 4, pp 815-824. doi:10.35799/pha.8.2019.2938.

30. Kaur K, Jain M, Kaur T, Jain R.2009. Antimalarials from nature.Bioorganic and Medicinal Chemistry. Vol.17, pp 3229-3256.doi: 10.1016/ j.bmc.2009.02.050

31. Khotimchenko Y. 2018. Pharmacological potential of sea cucumbers. International Journal of Molecular Sciences. Vol. 19, pp 1-42. doi: 10.3390/ijms19051342

32. Sarhadizadeh N, Afkhami M, Ehsanpour M. 2018. Evaluation bioactivity of a Sea cucumber, Stichopus hermanni from Persian Gulf. European Journal of Experimental Biology. Vol 4, No 1, pp 254-258.

33. Siringo-Ringo V, Kadiwijati LR, Yuniantika O, Murniasih T.2017. Formulasi Kapsul Ekstrak Kental Etanol 96% Teripang Keling (Holothuria atra) dan Uji Aktivitas Antibakteri Ekstrak. Indonesia Natural Research Pharmaceutical Journal.Vol 2, No 1, pp 1-9.

34. Septiadi T, Pringgenies D, Radjasa O karna. 2013. Uji Fitokimia dan Aktivitas Antijamur Ekstrak Teripang Keling (Holoturia atra) Dari Pantai Bandengan Jepara Terhadap Jamur Candida albicans. Diponegoro Journal Marine Research. Vol 2, No 2, pp 76-84. doi: 10.14710/ jmr.v2i2.2355.

35. Evi M, Alimuddin AH, Destiarti L. 2015. Pemanfaatan Ekstrak Landak Laut (Diadema setosum) Dari Pulau Lemukutan Sebagai Antijamur Candida albicans. Jkk. Vol 4, No 4, pp 61-65.

36. Parisihni K, Revianti S.2013. Antifungal effect of Sticophus hermanii and Holothuria atra extract and its cytotoxicity on gingiva-derived mesenchymal stem cell.Majalah Kedokteran Gigi. Vol 46, No 4, pp 218.

37. Dewi O, Mulyani Y, Rochima E. 2015. Aktivitas Antioksidan Dan Antibakteri Ekstrak Jeroan Teripang Holothuria atra Dari Perairan Pulau Biawak Kabupaten Indramayu. Jurnal Perikan Kelaut. Vol 6, No 2, pp 1-6.

38. Mahyuni S.2018.Aktivitas Antibakteri Ekstrak Daun Filicium decipiens (Wight & Arn.) Thwaites Terhadap Staphylococcus aureus, Escherichia coli dan Candida albicans. Fitofarmaka Jurnal Ilmiah Farmasi. Vol 8, No 2, pp 20-27. doi: 10.33751/jf.v8i2.1571.

39. Ungogo MA, Ebiloma GU, Ichoron N, Igoli JO, de Koning HP, Balogun EO. 2020. A Review of the Antimalarial, Antitrypanosomal, and Antileishmanial Activities of Natural Compounds Isolated From Nigerian Flora.Frontiers in Chemistry. Vol. 8, pp 1-28. doi: 10.3389/ fchem.2020.617448

40. Yu S, Ye X, Chen L, Xie X, Zhou Q, Lian XY, et al. 2015. Cytotoxic and anti-colorectal tumor effects of sulfated saponins from sea cucumber Holothuria moebii. Phytomedicine. Vol 13, No 50, pp 1112-1119. doi: 10.4103/pm.pm_571_16

41. Zhao YZ, Zhang YY, Han H, Fan RP, Hu Y, Zhong L, et al. 2018. Advances in the antitumor activities and mechanisms of action of steroidal saponins. Chin J Nat Med. Vol 16, No 10, pp 732-748. doi:10.1016/ S18755364(18)30113-4.

42. Widyawaruyanti A, Zaini NC. 2011. Mekanisme dan Aktivitas Antimalaria dari Senyawa Flavonoid yang Diisolasi dari Cempedak (Artocarpus Champeden). Jbp. Vol 13, No 2, pp 67-77.

43. Moelyadi F, Utami PD, Dikman IM.2020. Inhibitory effect of active substances of Lollyfish (Holothuria atra) against the development of plasmodium falciparum based on in silico study. Ilmu Kelautan Indonesian Journal Marine Science.Vol 25, No 4, pp 135-142. doi:10.14710/IK.IJMS.25.4.135-142

44. Vyas VK, Qureshi G, Ghate M, Patel H, Dalai S. 2016.Identification of novel PfDHODH inhibitors as antimalarial agents via pharmacophore-based virtual screening followed by molecular docking and in vivo antimalarial activity. SAR QSAR Environmental Research. Vol 27, No 6. pp 1-14. doi:10.1080/1062936X.2016.1189959

45. Lang L, Hu Q, Wang J, Liu Z, Huang J, Lu W, et al. 2018. Coptisine, a natural alkaloid from Coptidis Rhizoma, inhibits plasmodium falciparum dihydroorotate dehydrogenase. Chemical Biology and Drug Design. Vol 92, No 1, pp 5080-5087 .doi: 10.1111/cbdd.13197

46. Talapko J, Škrlec I, Alebić T, Jukić M, Včev A. 2019. Malaria: The past and the present. Microorganisms. Vol. 7, pp 1-17. doi:10.3390/microorganisms7060179

47. Abdulah R, Suradji EW, Subarnas A, Supratman U, Sugijanto M, Diantini A, et al.2017. Catechin isolated from Garcinia celebica leaves inhibit Plasmodium falciparum growth through the induction of oxidative stress. Pharmacogn Magazine. Vol 13, No 50, pp 301-305. doi: 10.4103/pm.pm_571_16

48. Arsianti A, Astuty H, Fadilah, Bahtiar A, Tanimoto H, Kakiuchi K. 2017. Design and screening of gallic acid derivatives as inhibitors of malarial dihydrofolate reductase by in silico docking. Asian Journal Pharmaceutical Clinical Research. Vol 10, No 2, pp 330-334. doi: 10.22159/ajpcr.2017.v10i2.15712

49. Pacheco MA, Schneider KA, Cheng Q, Munde EO, Ndege C, Onyango C, et al.2020. Changes in the frequencies of Plasmodium falciparum dhps and dhfr drug-resistant mutations in children from Western Kenya from 2005 to 2018: the rise of Pfdhps S436H. Malarial Journal. Vol 19, No 1, pp 1-9. doi:10.1186/s12936-020-03454-8.

50. El Houda Lezoul N, Belkadi M, Habibi F, Guillén F.2020. Extraction processes with several solvents on total bioactive compounds in different organs of three medicinal plants. Molecules. Vol 25, No 20, pp 1-15. doi: 10.3390/molecules25204672