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Hepatoprotective Activity of Ethanolic Extract of Fresh and Fermented Clam *Meretrix meretrix* from Kalimantan, Indonesia

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ABSTRACT

Molluscs, apart from being a food ingredient, animals belonging to this class, also can be used as a source of potentially bioactive compounds. Shellfish are generally often used to treat liver disease. Thus, clam *Meretrix meretrix* may become a hepatoprotector. This research was conducted to determine the phytochemical components and hepatoprotective activity by measuring the levels of SGPT and SGOT from experimental animals that had been intervened by using ethanol extract of fresh and fermented clam *Meretrix meretrix*. The results of phytochemical screening showed the ethanol extract of the fresh sample only contained alkaloid compounds, and the ethanol extract of the fermented sample contained alkaloids, steroids, and saponins. The measurement of the SGPT value after treated with the sample extracts at concentrations of 100 ppm, 150 ppm, and 200 ppm for three days varied of 19.49 - 22.97 IU. The same also found in SGOT measurements after-treatment of the sample extracts at concentrations of 50 ppm, 100 ppm, and 150 ppm for three days, varied of 28.08 - 34.60. Our study revealed that ethanol extract from fresh and fermented clams showed a potential hepatoprotective activity, as seen from the effect of reducing SGPT and SGOT values after treatment for three days.

Keywords: *Meretrix meretrix,* hepatoprotective activity, SGPT, SGOT

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INTRODUCTION

Indonesia's marine natural resources are the potential to be developed. Today, marine becomes the main target for researchers to discover new bioactive compounds. The exhibition for new active compounds yet is carried out on marine biota. Molluscs are the second largest animal group after the Arthropod phylum. About 80,000 species belonging to this group are widely distributed in various habitats and good ability to adapt to their environment. Apart from being a food ingredient, animals belonging to the molluscs class also can be used as a source of potentially bioactive compounds. Some of the known potentials of molluscs include anti-bacteria (Arumugasamy and Cyril, 2017; Duddu et al., 2017; Giftson et al., 2014; Raj et al., 2014), antioxidants (Arumugasamy and Cyril, 2017; Gayathri et al., 2017; Hasan et al., 2015), anti-cancer (Benkendorff et al., 2010) and hepatoprotective capacity (Ajithkumar et al., 2012).

Meretrix meretrix is one of the molluscs from the Bivalvia family, which has high economic value. *Meretrix meretrix* is known as milk shells, white clams, or called ale-ale in the local area (West Kalimantan, Indonesia). Famous location of gathering the *Meretrix meretrix* found in several areas, namely, Pandeglang, Banten, Jakarta Bay, Tuban, Gresik, the East Coast of Sumatra, South Sulawesi, and Kalimantan (Setyobudiandi et al., 2004). Based on empirical experience, apart from being a food ingredient, shellfish are generally often used to treat liver disease (hepatitis). Thus, *Meretrix meretrix* may have the same potential as a hepatoprotector. This research was conducted to determine the phytochemical components and hepatoprotector activity of ethanol extract of fresh and fermented *Meretrix meretrix*.

MATERIALS AND METHODS

Sample extraction

Fresh and fermented *M. meretrix* gathered from Ketapang, West Kalimantan, Indonesia was cleaned, then weighed 200 grams and mashed, then put into a maceration tube added with ethanol absolute Pro A 'Merck' with a ratio of 1: 1 and left to stand for 3x24 hours. The extract was then filtered to separate the filtrate from the residue and concentrated using a vacuum evaporator (Minsas et al., 2020; Warsidah et al., 2020). The extract was ready to be used for further analysis.

Phytochemical screening

The screening was conducted to identify several phytochemicals in ethanol extract from fresh and fermented *M. meretrix.* The phytochemicals were including alkaloid, flavonoid, triterpenoid, steroid, saponin, and phenols. The observation was according to method of (Masriani et al., 2020). The following steps exhibited an alkaloid presence. The extract that was concentrated using a vacuum evaporator was diluted with ethanol. Then each filtrate was put into test tubes. Furthermore, the test tube was given reagents of Dragendrof, Mayer, and Wagner. The presence of alkaloid compounds was indicated by the appearance of reddish-orange deposits (Dragendroff), white deposits (Meyer), and brown deposits (Wagner). The presence of flavonoids was identified by diluting extract into test tubes. Then, the test tube with the flavonoid code was given a 10% NaOH solution. The presence of flavonoid compounds was indicated by the appearance of a yellowish-brownish green color. Terpenoids and steroids were identified in the following ways. Each diluted extract was put into test

tubes and added a Liberman-Burchad reagent. The formation of a red-orange or purple color indicates the presence of triterpenoid compounds, and green or blue was indicated the presence of steroid compounds.

Some steps were carried out for saponin identification. Each diluted extract was put into test tubes. Then the test tubes were added with hot water, cooled, and shaken vigorously for 10 seconds. The presence of saponins was indicated by the presence of foam that does not disappear for not less than 30 seconds and at the time of adding 2 N HCl. The presence of phenols observed by the following mechanism. Each extract was put into test tubes and then added 10 drops of $FeCl_3$.

Hepatoprotective activity evaluation

Hepatoprotective activity was determined through experiments carried out on experimental animals. The experimental animals used were five Sprague Dawley rats in each group. The number of experimental animals used was calculated based on Federer's formula: (n-1) (t-1) ≥ 15. All the experimental animals were induced with CCl_4 to suffer liver damage marked by an increase in SGPT and SGOT values, to be further treated according to the group. During the intervention period, all experimental animals were given standard feed and drink, ad libitum. There were 5 groups of experimental animals, namely the negative control group (the group that was induced by CCl_4 to experience liver damage), positive control group (the CCl_4-induced group then intervened with a phytopharmaca, namely curcumin extract for 3 days), K1 group (the CCl_4-induced group and then intervened with the extract 100 ppm sample for 3 days), K2 group (the CCl_4-induced group and then intervened with 150 ppm sample extract for 3 days), K3 group (the CCl_4-induced group then intervened with 200 ppm sample extract for 3 days).

In our research, the evaluation of hepatoprotector activity was held by the determination of SGPT and SGOT values. Blood samples were taken from the experimental animals through a vein located in the earlobe and then centrifuged to obtain blood serum. SGPT and SGOT measurements were taken by taking 100 µL of blood serum, added 1000 µL of SGPT and SGOT kits, and then measuring the absorbance at a wavelength of 340 nm using UV-VIS spectrophotometry.

Data analysis

Data of SGPT and SGOT values were analyzed by performing *one-way* ANOVA statistical analysis.

RESULT AND DISCUSSION

Phytochemical screening

Phytochemicals was proved as a main compound highly contributed to the antioxidant activities (Dewi et al., 2020a; Fadly et al., 2020), antimicrobial (Dewi et al., 2020b). The capability of The results of the phytochemical analysis in Table 1 indicate that the ethanol extract of the fresh sample contained only alkaloid compounds and the ethanol extract of the fermented sample contained alkaloid, steroid, and saponin compounds. The difference in the compound content of these two samples was thought to be due to the influence of the activity of the microorganisms such as Lactic Acid Bacteria (LAB), where LAB can produce metabolic compounds during the fermentation process.

According to (Nurhamidah, 2019), fermented Meretrix sp. positively contains Lactic Acid Bacteria (LAB), thus strengthening the evidence that compounds that were not present in Meretrix sp. fresh can be detected as a result of the metabolism of Lactic Acid Bacteria (LAB). This was consistent with (Madigan et al., 2006) statement that fermentation is a process in which the decomposition of organic compounds occurs to produce energy and convert the substrate into new products by microbes.

Table 1. Phytochemicals of the ethanol extract of fresh and fermented *Meretrix meretrix*.

No	Parameters	Fresh sample	Fermented sample
1	Alkaloids : Dragendroff Mayer Wagner	 + - -	 + - -
2	Flavonoids	-	-
3	Triterpenoids	-	-
4	Steroids	-	+
5	Saponins	-	+
6	Phenolics	-	-

Note: (+) Present, (-) Absent.

SGPT and SGOT levels

In our study, identification of hepatoprotective activity was carried out by determining two parameters, SGPT and SGOT. These two parameters are an enzyme that can be found in the liver, red blood cells, heart cells, muscle tissue, and other organs, such as the pancreas and kidneys. SGPT and SGOT are indicators used to determine the level of liver damage. The increase in the levels of SGPT and SGOT enzymes in the blood of the test animals can be caused by the influence of carbon tetrachloride (CCl_4), which enters the body and is metabolized by the liver to form free radicals that can damage liver cells. The

measurement of SGPT and SGOT levels is based on the work of antioxidants. Antioxidant may blockage the auto-oxidation via a rapid H^+ donation to free radicals (Fadly et al., 2020). Oxidation prevention leads to the inhibit the disease due to the poisoning of food and cosmetics (Sofiana et al., 2020). (Kalija, 2019) states that the methanol extracts from Meretrix sp. fresh and fermented have antioxidant activity that allows it to be used as a hepatoprotector because it can stabilize free radicals from the influence of carbon tetrachloride (CCl_4), a substance may lower the liver health.

Table 2. SGPT and SGOT levels after the intervention of ethanol extract of fresh and fermented *Meretrix meretrix.*

Treatment	SGPT (I.U.)			SGOT (I.U.)		
	Fresh	Fermented	P-value	Fresh	Fermented	P-value
Before treatment	19.54±1.22	21.44±2.11	(P>0.05)	26.77±3.21	30.00±0.54	(P>0.05)
Negative control (CCl_4)	23.67±3.24	23.67±0.44		35.34±2.33	35.34±0.32	
Positive control (phytopharmaca: curcumin extract)	21.3±1.32	21.32±2.31		27.36±4.22	27.36±2.31	
K1 (100 ppm Extract)	22.87±0.44	22,97±0.95		34.44±1.33	34.16±1.33	
K2 (150 ppm Extract)	20.98±0.66	21.96±0.88		30.28±3.11	32.66±3.22	
K3 (200 ppm Extract)	19.49±0.45	22.07±0.55		28.08±0.22	34.60±1.22	

Based on the measurement results of the SGPT and SGOT values from this study, it can be seen that the effect of CCl_4 on increasing the SGPT value was at 23.67 IU and the SGOT value was at 35.34 IU. The measurement of the SGPT value after intervened by phytopharmaca and ethanolic extracts within three days can be seen in Table 2. The results of statistical analysis of the SGPT and SGOT values after treatment of phytopharmaca and sample extracts (100, 150, and 200 ppm) did not show a significant difference (P> 0.05) to the control. The same also occurred to the SGOT, and the result is displayed in Table 2. The results of the statistical analysis of SGOT values also did not show a significant difference (P> 0.05) to control. According to the result, it revealed the more dose of sample extract given might contribute to lower SGPT and SGOT levels.

The level of liver damage that occurs can be seen from the value of SGPT and SGOT from the measurement results. Normal SGPT values in adults range between 5-35 IU and 5-40 IU for SGOT (Huang et al., 2006; Sacher and McPerson, 2011; Safitri, 2013). If the SGPT and SGOT values increase by 5-10 times or more than normal levels, it can be concluded that if the patient has acute liver damage or hepatitis. The increase in SGPT and SGOT values is 2-3 times from normal levels, indicated the patient has fatty liver bile. The cause of the rise in SGPT and SGOT values can be caused by various chemical toxins, including the use of antibiotics, chemotherapy, carbon tetrachloride (CCl4), excessive alcohol consumption, and microbes (Jannu et al., 2012). In or observation, the presence of lower SGPT and SGOT values in the group treated with both phytopharmaca (curcumin extract) and sample extract showed a performance of the hepatoprotective activity. The decrease in the value of the two indicators of liver damage in the group given the extract samples tended to be the same as the group given the curcumin extract as phytopharmaca. The existence of treatment for three days was able to repair liver damage even though statistically, the SGPT and SGOT values were not significantly different toward the negative control. This was due to the short amount of treatment time. So, following or finding, the ethanol extract from fresh and fermented clamps *Meretrix meretrix* has potential as a hepatoprotective activity.

CONCLUSION

The phytochemical content of the ethanol extract of the fresh clamp is different from that of fermented clams. The ethanol extract of the fresh sample only contained alkaloid compounds, and the ethanol extract of the fermented sample contained alkaloid, steroid, and saponin compounds. The SGPT and SGOT values in the experimental animal group, given the ethanol extracts, tend to be lower than the negative control group. Our study revealed that ethanol extract from fresh and fermented clams showed a potential hepatoprotective activity, as seen from the effect of reducing SGPT and SGOT values after treatment for three days. We suggest doing a longer observation to exhibit the significant impact of hepatoprotective activity from the extract of *Meretrix meretrix*.

REFERENCES

1. Ajithkumar, P., Jeganathan, N.S., Balamurugan, K., Radha, K., 2012. Evaluation of hepato-protective activity of Villorita cyprinoidesextract (Black water clams) against paracetamol -induced hepatic injury in albino rats. IJPBS 2, 123–127.
2. Arumugasamy, K., Cyril, R., 2017. Cytotoxicity, Antibacterial and Antioxidant Activities of the Tissue Extracts of Marine Gastropod Hemifusus Pugilinus (Born, 1778). J. Chem. Phamaticuetical Reasearch 9, 267–274.
3. Benkendorff, K., McIver, C.M., Abbott, C.A., 2010. Bioactivity of the Murex Homeopathic Remedy and of Extracts from an Australian Muricid Mollusc against Human Cancer Cells. Evid. Based Complement. Alternat. Med. 2011, enep042. https://doi.org/10.1093/ecam/nep042

4. Dewi, Y S K, Karunia, C.J.K., Fadly, D., 2020a. Antioxidant and Antimicrobial Activities of Methanolic Extracts of Scorodocarpus borneensis Becc. Syst. Rev. Pharm. 11, 246–252.
5. Dewi, Y S K, Lestari, O.A., Fadly, D., 2020b. Identification Phytochemicals and Antioxidant Activities of Various Fractions of Methanol Extracts from Bark of Kulim Tree (Scorodocarpus borneensis Becc.). Syst. Rev. Pharm. 11, 217–221.
6. Duddu, S.K., Gurugubelli, T., Gandham, K.G., Dogiparti, A., 2017. Antibacterial Activity of the Crude Extract of a Gastropod Cellana radiata (Born, 1778) from the Visakhapatnam Coast, Andhra Pradesh, India. Int. J. Mar. Sci. 7.
7. Fadly, D., Kusharto, C.M., Kustiyah, L., Suptijah, P., Muttalib, Y.S., Bohari, 2020. In Vitro Study of Antioxidant Activity of Carboxymethyl Chitosan derived from Silkworm (Bombyx mori L.) Pupa against Human Plasma Lipid Peroxidation. Syst. Rev. Pharm. 11, 76–81.
8. Gayathri, M., Ramasamy, M., Santhiya, N., Dineshkumar, G., 2017. Journal of Marine Biosciences In vitro antioxidant properties from tissue extract of Gastropods around Lower and Grand Anicut Reservoir, Tamil Nadu. JMBS 5, 145–151.
9. Giftson, H., Patterson, J., Devadason, S., 2014. Antibacterial Activity of the Shell Extracts of Marine Mollusc Donax faba against Pathogens. Int. J. Microbiol. Res. 5, 140–143.
10. Hasan, T., Wahab, A.W., Djide, N., Zakir, M., 2015. Antioxidant Activity of Bioactive Protein of Kerang Kepah (Atactodea striata) From South Sulawesi. Am. J. Biomed. Life Sci. 3, 111. https://doi.org/10.11648/j.ajbls.20150306.12
11. Huang, X.-J., Choi, Y.-K., Im, H.-S., Yarimaga, O., Yoon, E., Kim, H.-S., 2006. Aspartate Aminotransferase (AST/GOT) and Alanine Aminotransferase (ALT/GPT) Detection Techniques. Sensors 6, 756–782.
12. Jannu, V., Baddam, P., Boorgula, A., Jambula, S., 2012. A Review on Hepatoprotective Plants. Int. J. Drug Dev. Res. 4, 1–8.
13. Kalija, T.A., 2019. Komponen Bioaktif dan Aktivitas Antioksidan Ekstrak Kasar Kerang Ale-Ale (Meretrix sp.) Segar dan Fermentasi. Universitas Tanjungpura, Pontianak.
14. Madigan, M.T., Martinko, J.M., Brock, T.D., 2006. Brock biology of microorganisms. Pearson Prentice Hall, Upper Saddle River, NJ.
15. Masriani, Fadly, D., Bohari, B., 2020. α-Glucosidase Inhibitory Activity of Ethanol Extract Obtained from Dillenia suffruticosa and Pycnarrhena cauliflora. J. Glob. Pharma Technol. 12, 881–887.
16. Minsas, S., Nurdiansyah, S.I., Prayitno, D.I., Sofiana, M.S.J., Kalija, T.A., Fadly, D., Warsidah, 2020. Screening of Bioactive Compounds and Antioxidant Activity of Ale-ale Shellfish (Meretrix meretrix) Crude Extracts from West Kalimantan, Indonesia. Syst. Rev. Pharm. 11, 222–227.
17. Nurhamidah, A., 2019. Aktivitas Antibakteri dan Antioksidan Isolat Bakteri Asam Laktat (BAL) yang Diisolasi dari Ale-ale dan Cincalok. Universitas Tanjungpura, Pontianak.
18. Raj, J.M., Chelladurai, G., Ramasamy, P., Deepa, P.K., Sasirekhamani, M., 2014. Antibacterial Attributes of Marine Gastropod Hemifusus pugilinus (Born, 1778) against Human Pathogenic Bacteria. Int. J. Mar. Sci. 4.
19. Sacher, McPerson, 2011. Tinjauan Klinis Hasil Pemeriksaan Laboratorium. Penerbit Buku Kedokteran EGC, Jakarta.
20. Safitri, J., 2013. Uji Aktivitas Hepatoprotektor Fraksi Metanol Daun Kesum (Polygonum minus Huds.) pada Tikus Putih Jantan Galur Wistar Yang Diinduksi Cisplatin. J. Mhs. Farm. Fak. Kedokt. UNTAN 1.
21. Setyobudiandi, I., Soekendarsih, E., Yonvitner, Y., Setiawati, R., 2004. Bio-Ecologi Kerang Lamis (Meretrix Meretrix) di Perairan Marunda. J. Ilmu-Ilmu Perair. Dan Perikan. Indones. 11, 61–66.
22. Sofiana, M.S.J., Aritonang, A.B., Safitri, I., Helena, S., Nurdiansyah, S.I., Risko, Fadly, D., Warsidah, 2020. Proximate, Phytochemicals, Total Phenolic Content and Antioxidant Activity of Ethanolic Extract of Eucheuma spinosum Seaweed. Syst. Rev. Pharm. 11, 228–232.
23. Warsidah, Fadly, D., Bohari, 2020. Antibacterial and Anti-inflammatory Activities of Ethanol Extract Obtained from The Hooks of Uncaria tomentosa (Wild. Ex Schult) DC Originated Kalimantan, Indonesia. Syst. Rev. Pharm. 11, 65–70.