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Hepatoprotective and toxicological evaluation of tropical *Sargassum polycystum* C. Agardh collected from Sumbawa coast Indonesia, against carbon tetrachloride-induced liver damage in rats

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Abstract: Pharmacological activities of seaweed, including its antioxidant effect, have been demonstrated and can protect macromolecules from xenobiotic-induced damage. Understanding the potency of seaweed as a hepatoprotection and its toxicity remains underexplored. The aims of this study were to investigate the antioxidant and hepatoprotective activity, as well as the toxicological potencies of *S. polycystum* ethyl acetate extract against carbon tetrachloride-induced liver damage in rats. Total phenolic content and total flavonoid contents were quantified using standard spectroscopy-based methods. The antioxidant activity was measured using 1,1-Diphenyl- 2-picryl Hydrazil scavenging radical, while the composition of compounds was identified by LCMS/MS. After seven days of post-administrated rats with *S. polycystum* ethyl acetate extract, the serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) levels were tested. Total phenolic content, total flavonoid content and IC_{50} of *S. polycystum* ethyl acetate extract were 1.28±0.04 of GAE/g, 13.32±0.48 QE/g and 744.726μg/mL, respectively. *S. polycystum* ethyl acetate extract 150mg/kg BW provides a hepatoprotective effect with a significant improvement in the levels of SGOT (134.845 U/l±9.645) and SGPT (60.238 U/l ± 9.645) ($p<0.05$). *S. polycystum* ethyl acetate extract potentially protected the damage induced by CCl_4 in the rat's liver at a certain concentration, while a higher extract concentration requires further examination.

Keywords: *Sargassum polycystum*, antioxidant, DPPH, hepatoprotective, toxicological evaluation.

INTRODUCTION

The liver is one of the most important organs for detoxification and biotransformation of xenobiotics that, in certain conditions of intensive therapies, can generate acute injury and potentially increase the risk of liver failure. Therefore, it is necessary to find a substance to protect the liver. Natural herbs have significantly managed various liver diseases and offer cost-effective alternative treatments (Zhang *et al*., 2013). However, many natural herbs have yet to be accepted for treating liver disease. The contributing factors are the lack of scientifically based pharmacological data such as toxicological evaluation, natural product standardization, active ingredient identification and clinical trials. A scientific methodological evaluation must be applied to prove the safety and efficacy of a natural product by conducting preclinical and clinical studies. It would help explore the real therapeutic value of these agents and standardize dosages based on evidence-based findings (Stickel and Schuppan 2007).

Sargassum polycystum belongs to the family *Phaeophyceae*, a type of brown marine algae widely distributed along the Sumbawa coast. It contains various bioactive substances: polyphenols, carotenoids, terpenoids, steroids, polysaccharides, alkaloids, glycosides and tannins (Sathya *et al*., 2013; Tarigan, 2020). Several studies reported that brown algae possessed many pharmacological activities, including anti-cholesterol, antibacterial, antitumor, antiviral and antioxidant (Husni *et al*., 2022; Polo and Chow, 2022; Saraswati *et al*., 2021). Its hepatoprotective effect also has been investigated, especially in some *Sargassum* species such as *Sargassum licifolium* and *Sargassum fluitans* (Hira *et al*. 2021; Quintal-Novelo *et al*. 2018). The scientific evidence regarding the toxic effects of brown algae has not been widely reported. A previous study stated that *Sargassum wightii* was considered safe and can be recommended for long-term medicinal use (Ramu *et al*., 2020).

The information about the activity of *Sargassum polycystum* against liver damage has yet to be discovered. A current study evaluated the hepatoprotective dan toxicological activity of *S. polycystum*. The results of this study may provide insight into its hepatoprotective effect.

MATERIALS AND METHODS

Carbon tetrachloride (CCL_4) (Merck, Germany), ethyl acetate (Merck, Germany) and methanol (Merck,

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Germany), 1,1-Diphenyl- 2-picryl Hydrazil (DPPH) (Sigma Aldrich (Tokyo, Japan)). Silymarin was suspended in 1% carboxymethyl cellulose. All chemicals were obtained as an analytical grade.

Sargassum polycystum was collected from the coastal area of Sumbawa, West Nusa Tenggara, Indonesia. Morphological identification was performed at Research Center for Oceanography, National Research and Innovation Agency (BRIN). The voucher specimen was stored at the Department of Pharmacy, Atma Jaya Catholic University of Indonesia. Soon after being collected from the field, *S.polycystum* was washed, dried in the shade for two days and using the oven at 45°C for 8 hours (Karthikeyan *et al*., 2010). The dried sample was then powdered before extraction.

Seaweeds extraction

The seaweeds were extracted using the modified method of Karthikeyan *et al*. (2010). The powder of *S. polycystum* (1,880g) was extracted using a soxhlet extractor in ethyl acetate (100mL) for 8 hours at 77°C, carried out for 8 to 10 cycles. The filtrate was concentrated using a rotary evaporator (65°C) to afford *S. poycystum* ethyl acetate extract (SPEE) of 12.201g with a yield extract of 0,649%.

Antioxidant activity assay

The antioxidant activity was performed following the method reported by Hasanah *et al*. (2017). The SPEE was dissolved in methanol as a stock solution. It was diluted to obtain concentrations ranging from 600 to 1500 µg/mL, mixed with DPPH solution (2mL). The mixtures were shaken and incubated for 30 min at 37°C. Absorbance was read at 516 nm using a spectrophotometer. The inhibition percentage data were plotted to determine the value of the inhibitory concentration (IC_{50}).

Analysis of total phenolic content (TPC)

The TPC of SPEE was calculated using the Folin-Ciocalteu method described by Singleton *et al*. (1999). Gallic acid was dissolved in methanol as a standard solution (1000µg/mL). It was serially diluted to 40, 30, 20, 10 and 5µg/mL. Plant extracts (500µL) were added with distilled water to 4 mL, then Folin Ciocalteu's reagent (25h µL) was mixed thoroughly. After incubating for 8 minutes, sodium carbonate 20% (750µL) was mixed and kept at room temperature for 2 hours. Absorbance was read using a spectrophotometer at 765nm. The TPC value was defined as mg/g of the extract of Gallic acid equivalent.

Analysis of total flavonoid content (TFC)

The TFC of SPEE was measured using aluminum chloride colorimetric assay explained by Chang, Yang, Wen and Chern (2020). The standard quercetin curve was obtained by diluting methanol into 50, 30, 20 and 10µg/mL concentrations. Plant extracts (250µL) were diluted serially, similar to the standard. SPEE was added with distilled water (2mL) and NaNO2 5% (150µL). After 5 minutes, $AlCl_3$ 10% (150µL) was added. After 6 minutes, NaOH 1 M (2ml) was mixed and diluted with up to 5ml of distilled water. The mixture was vigorously shaken and read the absorbance was using a spectrophotometer at 510nm. The TFC value was defined in mg/g of extracts of quercetin equivalent.

LC-MS/MS analysis

LC-MS/MS analysis was performed using Waters Acquity UPLC I-Class System equipped with a binary pump following the procedure by Asmi *et al*., (2021). The full scan mode was carried out from m/z 100-1200 with column temperature at 120°C. Chromatographic separation was determined using Acquity UPLC® BEH C8 (2.1×100 mm, 1.7µm particle size). The composition of the mobile phase was H_2O+0,1% Formic Acid (solvent A) and acetonitrile+0,1% Formic Acid (solvent B) with a flow rate of 0.3mL/min and column temperature at 40◦C. The gradient program was as follows: 0 min 95:5 (A:B, v/v), 8 min 60:40 (A:B, v/v), 11 min 0:100 (A:B, v/v), 13 min 0:100 (A:B, v/v), 16min 95:5 (A:B, v/v). The injection volume was one µL. The constant parameters were: the capillary voltage was 2.0 kV; cone voltage was 30 V; drying gas (N2) flow was 1000 L/h; and 500°C for drying gas.

Animals

Sprague-Dawley male rats, aged 2-3 months, weighing 150-250 g, were taken from the Faculty of Veterinary Medicine, IPB University, Indonesia. They were housed in boxes (four rats each) and given food and water. The rats were kept in a controlled environment under standard temperature (23±1°C) and humidity (60%) conditions with 12 h alternating light/dark cycles. The animal research study was approved by the Ethics Committee of the School of Medicine and Health Science of the Atma Jaya Catholic University of Indonesia. The number was 20/02/KEP-FKIKUAJ/2021.

Experimental induction of hepatotoxicity

The rats were acclimatized for one week before the experiment. The dosage regimen of SPEE was based on the study by Altinok-Yipel *et al*. (2019); Batubara *et al*. (2016); Chale-Dzul *et al*. (2020); Khouzani *et al*. (2019); Raghavendran *et al*., (2004). CCl_4 was used to induce hepatotoxicity. Twenty-four rats were randomized and divided into six groups (n=4). Group I/normal (received only 2mL of Na-CMC 1% p.o). Group II/negative control (administered 2mL of Na-CMC 1% p.o+2mL/kg BW of CCl_4 i.p). Group III/ positive control (100 mg/kg BW of silymarin p.o+2mL/kg BW of CCl_4 i.p). Groups IV (150 mg/kg BW of SPEE p.o+2mL/kg BW of CCl_4 i.p). Group V (300mg/kg BW of SPEE p.o+2mL/kg BW of CCl_4 i.p). Group VI (600mg/kg BW of SPEE p.o+2mL/kg BW of CCl_4 i.p). The pretreatment of Silymarin and SPEE was orally administered for seven days. CCl_4 was injected

intraperitoneally on day 8. After 48h, the rat blood samples were taken and separated into serum for biochemical analysis. The liver organs were observed as macro pathological and fixed with 10% formalin.

Macro pathological observation

Immediately after the necropsy, their livers were excised and washed in ice-cold saline. Macro pathological observation was done by checking the liver condition visually. The severity of abnormalities, fluid accumulation, adhesion and liver color was scored as -: not identified, +: mild, ++: moderate, +++: severe.

Determination of SGOT and SGPT levels

The SGOT and SGPT levels were calculated using the photometric method reported by Sinaga *et al*. (2021). Blood serum (100µL) was added with SGOT/SGPT reagent (1000µL) and allowed to stand for 5 minutes at 37°C. Absorbance was measured triplicate for every minute using a spectrophotometer UV-Vis at 365nm (Shimadzu A11635480009ML).

STATISTICAL ANALYSIS

SGOT-SGPT levels data were analyzed among the experimental group of animals and statistical significance ($p<0.05$) between controls and treated groups was evaluated using a one-way analysis of variance (ANOVA) following Tukey's post hoc test with SPSS version 25 statistical software. All experiments were performed in four replicates. All data are presented as mean ± standard deviation.

RESULTS

IC_{50}, TFC and TPC values of SPEE

The IC_{50}, TPC and TFC values of SPEE were 744.726± 27.393µg/mL, 1.28±0.04 GAE/g and 13.32±0.48 QE/g, respectively.

LC-MS/MS analysis

LC-MS/MS was selected for the characterization of SPEE compounds. Digiprolactone was observed at 5.17 min with m/z 197.1171 in the mass spectral analysis (fig.1).

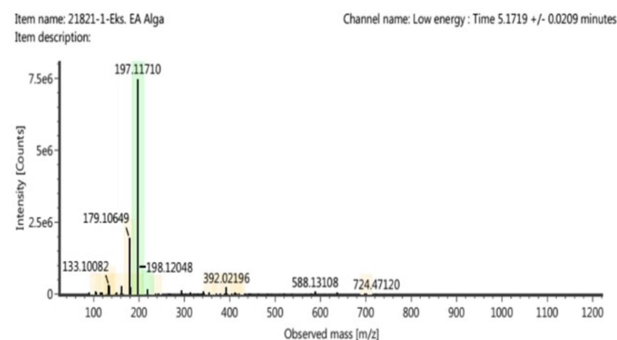

Fig. 1: MS/MS spectra of SPEE fraction

Macro pathological observation

The scores are concluded in table 1. There were no abnormalities in the liver organ of group I. In group II, the severity of liver adhesion and fluid accumulation in the abdomen cavity was severe. Silymarin as the positive control group was similar to the normal group. No abnormalities were observed in the SPEE 150mg/kg BW pretreatment group. However, significant liver damage was found at the higher SPEE dosage (300 and 600mg/kg BW), probably due to hepatotoxicity. Therefore, further investigation is needed.

SGOT and SGPT analysis

Administration of CCl_4 in rats caused liver damage, as evidenced by a significant increase in enzyme levels ($p<0.05$) compared to group I (normal). In addition, there was a significant reduction in SGOT and SGPT levels ($p<0.05$) in the silymarin and SPEE (150mg/kg BW) group (fig 2). SGOT values of silymarin and SPEE were 130.353 U/l±47.126; 134.845 U/l±9.645 and SGPT values were 62.350 U/l±10.45; 60.238 U/l±9.645, respectively. It has shown that they have similar potential as a hepatoprotective agent.

DISCUSSION

The hepatoprotective activity in plants relates to antioxidant compounds, namely flavonoids and polyphenols (Kelman *et al*. 2012). Flavonoids possess antioxidant activity, counteract free radicals and inhibit the induction of inflammatory mediators that affect hepatocyte cell damage (Kurniawan *et al*., 2015). Brown marine algae are rich in natural antioxidant compounds such as polyphenols essential for preventing lipid peroxidation and fucoxanthin as the primary carotenoid (Karthikeyan *et al*. 2010). Research by Jumaetri Sami *et al*. (2019) found that brown algae ethyl acetate extract had the most potent antioxidant activity than methanol and n-hexane extracts based on their IC_{50} values. Based on the IC_{50} value of SPEE in this study (744.726µg/mL±27.393) was similar to a study by (Darsih *et al*. 2021) who suggested the IC_{50} value of the brown algae *Sargassum duplicatum* and *Palmaria palmata* were 790.34µg/mL and 789.29µg/mL, respectively. Another study by Santoso *et al*. (2013) also found that the IC_{50} value of the ethyl acetate extract of *Padina australis*, another species of brown algae, was 1160.2µg/mL. Different results studies were shown by Arsianti *et al*. (2020) and Jumaetri Sami *et al*. (2019) that explained the IC_{50} value of *S. polycystum* extracted using the maceration method with ethyl acetate solvent was 411.80µg/mL and 298.32µg/mL respectively. This difference might be due to the difference in extraction methods. In the extracted context, the IC_{50} value is generally lower than pure isolates because many other compounds in plant extracts could react and affect the antioxidant activity. Compared to other studies, the difference in IC_{50} values might be caused by several factors, such as chemical compositions in each brown algae species, location and climate (Parthiban *et al*., 2014). Pigment levels also affect antioxidant activity,

chlorophyll and carotenoids, reducing free radical compounds from DPPH (Safafar *et al*., 2015).

Table 1: Effect of SPEE on macropathological changes on CCl_4-treated rats

Groups	Abnormalities	Fluids Accumulation	Liver adhesion	Liver color change
I (Normal)	-	-	-	-
II (Negative control)	+++	+++	+++	+++
III (Positive control)	-	-	-	-
IV (SPEE 150 mg/kg BW)	-	-	-	-
V (SPEE 300 mg/kg BW)	+	+	+	+
VI (SPEE 600 mg/kg BW)	++	++	++	++

-: not identified, +: mild, ++: moderate, +++: severe.

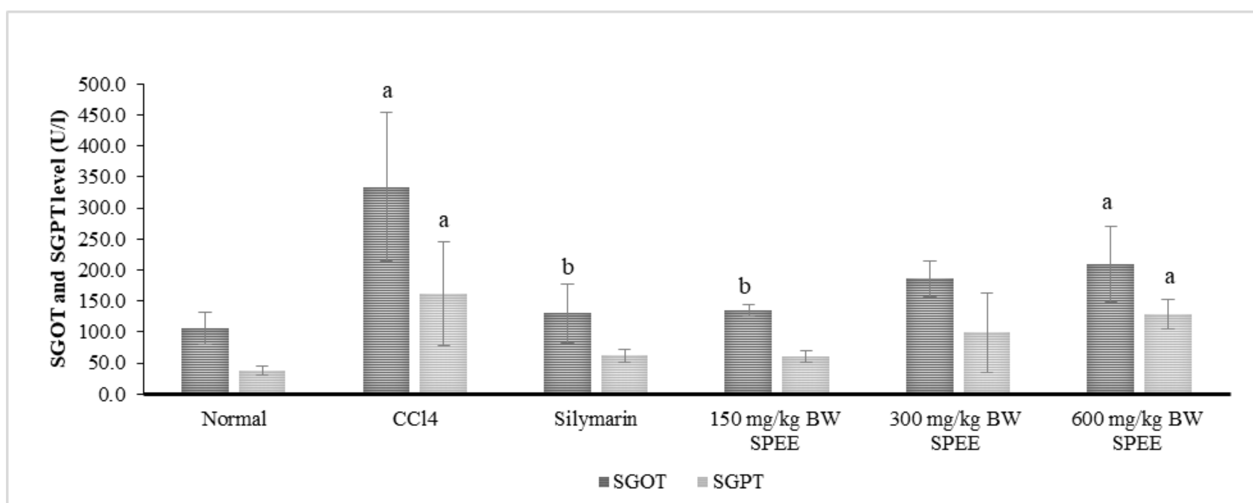

Fig. 2: SGOT and SGPT levels of SPEE on CCl_4-induced liver damage. Values are expressed in Mean ± SD (n=4). One-way ANOVA with a Tukey post hoc test. a=indicate significant differences ($p<0.05$) compared to the normal group; b=indicate significant differences ($p<0.05$) compared to the negative control group. CCl_4: Carbon tetrachloride (negative control); Silymarin (positive control); SPEE (*Sargassum polycystum* ethyl acetate extract).

The TPC value of SPEE in this study (1.28±0.04 GAE/g) was almost similar to Gazali *et al*. (2018), who found the most potent antioxidant effect was in ethyl acetate extract of *Sargassum sp. Agardh* from west of Aceh coastal (1.348±2.57 GAE/g). In addition, the total flavonoid content of SPEE (13.32±0.48 QE/g) was not too different from the study by Arsianti *et al*. (2020) and Neoh *et al*. (2021), which used the maceration method for extraction. They reported that ethyl acetate extract flavonoid content from *Sargassum polycystum* was 40.06µg/mL and 32.53 mg/g. Their study suggests that the antioxidant properties of brown algae ethyl acetate extract may be affected by its phenolic and flavonoid contents.

The LCMS/MS identified digiprolactone by ESI/MS fragmentation with m/z 197.1171 at 5.17 minutes (fig 1). It corresponds to the molecular formula of $C_{11}H_{16}O_3$. Digiprolactone, also known as loliolide, a monoterpene lactone, was found in several brown algae species, including *Sargassum sp* (Percot *et al*., 2009; Yang *et al*., 2011). Based on loliolide antioxidant assays using DPPH and H_2O_2 radical and intracellular ROS scavenging assays showed moderate activity. Loliolide can protect cells from damage or apoptosis (Yang *et al*. 2011). Based on literature studies, loliolide is the responsible compound related to the SPEE hepatoprotective and antioxidant activity.

Cytochrome P450 enzymes biotransform CCl_4 to form free radicals trichloromethyl (CCl_3-) and peroxy trichloromethyl (CCl_3OO-). Free radicals bind into cellular lipids and proteins or organelles covalently, initiating lipid peroxidation, changing enzyme activity and inducing hepatotoxicity. While the liver cell plasma membrane was damaged, the cytoplasmic enzymes (SGOT-SGPT) were released into the circulation. This elevation of serum enzyme level is also shown in the CCl_4 group in this study.

Based on macro pathological data, the silymarin (100 mg/kg BW) group provided potent protection liver against CCl_4 (table 1). SGOT and SGPT values of silymarin were 130.353U/l±47.126 and 62.350U/l±10.45, respectively. It showed a significant decrease compared to the negative control (fig. 2). Silymarin has been known as a potent hepatoprotective agent (Chandrashekhar, Muchandi, Sudi and Ganapty 2010). Silymarin can counteract free radicals and increase glutathione concentrations to improve liver function (Karimi *et al*., 2011).

Treatment with SPEE (150mg/kg BW) protected the liver from damage. It was supported by macro pathological data showing no abnormalities, fluid accumulation, liver adhesion, or liver color change (table 1). In addition, the SGOT (134.845U/l±9.645) and SGPT (60.238U/l±9.645) values were closely similar to the silymarin group (fig 2). Thus, we can say that this dose is as potent as silymarin. Altinok-Yipel *et al*. (2019), Khouzani *et al*. (2019) and Raghavendran *et al*. (2004) also found similar results. They explained that the optimum dosage of *Sargassum sp* as a hepatoprotective agent was 200mg/kg BW. This study suggests that SPEE contains several bioactive compounds that can protect the liver from damage via a free radical scavenging property (Begum *et al*. 2021). According to literature studies, the bioactive compounds in sargassum extract are polyphenols, steroids, alkaloids, phenols, triterpenoids, polysaccharides, phlorotannins and fucoxanthin as carotenoid compounds.

The SGOT and SGPT values of SPEE (300 and 600 mg/kg BW) have not shown any significant hepatoprotective effect (fig 2) and it was not significantly different from the negative control. Fluid accumulation, adhesion and inhomogeneous liver color on macro pathological were observed. SPEE at a concentration of 150mg/kg BW showed the most potent hepatoprotective effect than the higher doses, indicating the toxicity symptom. Wariz (2016) reported that administering *Sargassum sp.* extract (500, 1000, 1500, and 2000 mg/kg BW) showed mortality and toxicity symptoms at the highest dose. So, it was categorized as mild toxicity. In addition, using different solvents can also affect the toxicity symptoms level. However, further investigation is needed relating to the toxicity test of SPEE.

CONCLUSION

We conclude that *S. polycystum* could become a promising hepatoprotective agent. The optimum dose of SPEE 150 mg/kg BW can significantly decrease SGOT and SGPT levels in rats against CCl4. Its hepatoprotective effect might be partly due to its antioxidant properties. However, more research is needed.

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REFERENCES

Altinok-Yipel F, IO Tekeli, SY Ozsoy, M Guvenc, S Sayin and M Yipel (2019). Investigation of hepatoprotective effect of some algae species on carbon tetrachloride-induced liver injury in rats. *Arch Physiol Biochem*., **126**(5): 463-467.

Arsianti A, A Bahtiar, VK Wangsaputra, NN Azizah, W Fachri, LD Nadapdap, AM Fajrin, H Tanimoto and K Kakiuchi (2020). Phytochemical composition and evaluation of marine algal *Sargassum polycystum* for antioxidant activity and *in vitro* cytotoxicity on hela cells. *Pharmacogn J.,* **12**(1): 88-94.

Asmi N, A Ahmad, H Natsir, MN Massi and H Karim (2021). Identification and bioinformatics study of antibacterial peptides from symbiotic bacteria associated with macroalgae *Sargassum sp*. *Egypt J Chem.,* **64**(9): 4883-4888.

Batubara L, TN Kristina and B Rachmawati (2016). Effectiveness of brown algae extract to reduce serum malondialdehyde and protein carbonyl levels in streptozotocin-induced sprague dawley rats. *Sains Med: J Kedokt Kesehatan* **7**(2): 43-48.

Begum R, S Howlader, ANM Mamun-Or-Rashid, SM Rafiquzzaman, GM Ashraf, GM Albadrani, AA Sayed, I Peluso, MM Abdel-Daim and MS Uddin (2021). Antioxidant and signal-modulating effects of brown seaweed-derived compounds against oxidative stress-associated pathology. *Oxid Med Cell Longev,* **2021**(special issue): 1-22

Chale-Dzul J, R Pérez-Cabeza de Vaca, C Quintal-Novelo, L Olivera-Castillo and R Moo-Puc (2020). Hepatoprotective effect of a fucoidan extract from *Sargassum fluitans* Borgesen against CCl4-induced toxicity in rats. *Int J Biol Macromol.,* **145**(2020): 500-509.

Chandrashekhar VM, AA Muchandi, S v Sudi and S Ganapty (2010). Hepatoprotective activity of *Stereospermum suaveolens* against CCl4-induced liver damage in albino rats. *Pharm Biol*., **48**(5): 524-528.

Chang CC, MH Yang, HM Wen and JC Chern (2020). Estimation of total flavonoid content in propolis by two complementary colometric methods. *J Food Drug Anal.,* **10**(3): 178-182

Darsih C, AW Indrianingsih, CD Poeloengasih, DJ Prasetyo, and N Indirayati (2021) In vitro antioxidant activity of macroalgae *Sargassum duplicatum* and *Palmaria palmata* extracts collected from Sepanjang Beach, Gunungkidul, Yogyakarta. IOP Conf Ser: *Mater Sci Eng.,* **1011**(1)012052: 1-6

Gazali M, Nurjanah N and Zamani NP (2018). Eksplorasi senyawa bioaktif alga cokelat *Sargassum sp*. Agardh sebagai Antioksidan dari Pesisir Barat Aceh. *J Pengolah Has Perikan Indones.,* **21**(1): 167-178.

Hasanah M, B Maharani and E Munarsih (2017). Daya Antioksidan ekstrak dan fraksi daun kopi robusta (*Coffea Robusta*) terhadap pereaksi DPPH (2,2-difenil-1-pikrilhidrazil). *Indones J Pharm Sci Technol,* **4**(2): 42-49.

Hira K, H Farhat, N Sohail, M Ansari, J Ara and S Ehteshamul-Haque (2021). Hepatoprotective activity against acetaminophen-induced liver dysfunction and GC-MS profiling of a brown algae *Sargassum ilicifolium. Clin Phytoscience* **7**(1): 1-11.

Husni A, N Izmi, FZ Ayunani, A Kartini, N Husnayain and A Isnansetyo (2022). Characteristics and Antioxidant Activity of Fucoidan from *Sargassum hystrix*: Effect of extraction method. *Int J Food Sci.,* **2022**: 1-9

Jumaetri Sami F, N Hariani Soekamto, J Latip, J Kimia Universitas Hasanuddin Makassar and S Tinggi Ilmu Farmasi Makassar (2019). Uji aktivitas antioksidan beberapa ekstrak alga coklat *Sargassum polycystum* dan *Turbinaria deccurens* asal pulau dutungan sulawesi selatan terhadap radikal dpph. *J Kim Riset,* **4**(1): 1-6.

Karimi G, M Vahabzadeh, P Lari, M Rashedinia and M Moshiri (2011). "Silymarin", a promising pharmacological agent for treatment of diseases. *Iran J Basic Med Sci.,* **14**(4): 308-317.

Karthikeyan R, ST Somasundaram, T Manivasagam, T Balasubramanian and P Anantharaman (2010). Hepatoprotective activity of brown alga *Padina boergesenii* against CCl_4 induced oxidative damage in Wistar rats. *Asian Pac J Trop Biomed.,* **3**(9): 696-701.

Kelman D, E Kromkowski Posner, KJ Mcdermid, NK Tabandera, PR Wright and AD Wright (2012). Antioxidant activity of hawaiian marine algae. *Mar Drugs.,* **10**(2): 403-416.

Khouzani MRM, MR Shushizadeh, H Kalantari and ER Ghotrami (2019). Hepatoprotective effect of aqueous extract of persian gulf brown algae *Sargassum swartzii* against acetaminophen-induced hepatotoxicity in mice. *Jundishapur J Nat Pharm Prod.,* **14**(1):1-7

Kurniawan J, ; Pandu, I Bangsawan and Andriani (2015). Uji efek hepatoprotektor ekstrak etanol daun lidah buaya (*Aloe vera* l.) terhadap kadar malondialdehid plasma tikus jantan galur wistar yang diinduksi parasetamol. *J Mahasiswa PSPD FK Univ Tanjungpura*, **3**(1): 1-17

Neoh YY, P Matanjun and JS Lee (2021). Effects of various drying processes on malaysian brown seaweed, *Sargassum polycystum* pertaining to antioxidants content and activity. *2021. Trans Sci Technol.,* **8**(1): 25-37.

Parthiban C, Saranya C, Somasundaram ST and Anantharaman P (2014). Antioxidant activities of some selected seaweeds from tuticorin coast, tamilnadu, india. *Int. J. Phytopharm. Res.*, **5**(1): 36-41.

Percot A, A Yalçin, V Aysel, H Erduğan, B Dural and KC Güven (2009). Loliolide in marine algae. *Nat Prod Res.,* **23**(5): 460-465.

Polo LK and F Chow (2022). Variation of antioxidant capacity and antiviral activity of the brown seaweed *Sargassum filipendula* (Fucales, Ochrophyta) under UV radiation treatments. *Appl Phycol.*, **3**(1): 260-273.

Quintal-Novelo C, J Rangel-Méndez, Á Ortiz-Tello, M Graniel-Sabido, RPC de Vaca and R Moo-Puc (2018). A *Sargassum fluitans* borgesen ethanol extract exhibits a hepatoprotective effect *in vivo* in acute and chronic liver damage models. *Biomed Res Int.,* **2018**: 1-9

Raghavendran HRB, A Sathivel and T Devaki (2004). Hepatoprotective nature of seaweed alcoholic extract on acetaminophen induced hepatic oxidative stress. *J Health Sci,* **50**(1): 42-46.

Ramu S, A Murali, G Narasimhaiah and A Jayaraman (2020). Toxicological evaluation of *Sargassum Wightii* greville derived fucoidan in wistar rats: Haematological, biochemical and histopathological evidences. *Toxicol Rep.,* **7**(2020): 874-882.

Safafar H, J van Wagenen, P Møller and C Jacobsen (2015). Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. *Mar Drugs,* **13**(12): 7339-7356.

Santoso J, F Podungge and H Sumaryanto (2013). Chemical composition and antioxidant activity of tropical brown algae *Padina australis* from pramuka island, district of seribu island, indonesia. *J Ilmu Teknol Kelaut Tropis,* **5**(2): 287-297.

Saraswati, PE Giriwono, D Iskandriati and N Andarwulan (2021). Screening of *in-vitro* anti-inflammatory and antioxidant activity of *Sargassum ilicifolium* crude lipid extracts from different coastal areas in indonesia. *Mar Drugs,* **19**(252): 1-13.

Sathya R, N Kanaga, P Sankar and S Jeeva (2013). Antioxidant properties of phlorotannins from brown seaweed *Cystoseira trinodis* (Forsskål) C. Agardh. *Arab J Chem,* **10**(2): 1-7.

Sinaga E, A Fitrayadi, A Asrori, SE Rahayu, S Suprihatin and VD Prasasty (2021). Hepatoprotective effect of *Pandanus odoratissimus* seed extracts on paracetamol-induced rats. *Pharm Biol*, **59**(1): 31-39.

Singleton VL, R Orthofer and RM Lamuela-Raventós (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods Enzymol,* **299**(1999): 152-178.

Stickel F and D Schuppan (2007). Herbal medicine in the treatment of liver diseases. *Dig Liver Dis,* **39**(4): 293-304.

Tarigan N (2020). Eksplorasi keanekaragaman makroalga di perairan londalima kabupaten sumba timur. *Biosfer : J Biol Pendidikan Biol.,* **5**(1): 37-43.

Wariz R, Asfa NW, and Fauzi, A (2016). The toxicity of brown algae (*Sargassum sp*) extract to mice (Mus muscullus). *J of Dentomaxillofac Sci*, **1**(2): 109-115.

Yang X, M-C Kang, K-W Lee, S-M Kang, W-W Lee and Y-J Jeon (2011). Antioxidant activity and cell protective effect of loliolide isolated from *Sargassum ringgoldianum* subsp. coreanum. *Algae,* **26**(2): 201-208.

Zhang A, H Sun and X Wang (2013). Recent advances in natural products from plants for treatment of liver diseases. *Eur J Med Chem,* **63**(2013): 570-577.