Identification of Bioactive Compounds in Macr oalgae that have the Potential to Improve Male Infertility

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Abstract- Amongst marine sources, macroalgae are valuable resources because they have a structural diversity of bioactive compounds that important in developing pharmaceutical products. The use of marine macroalgae bioactive compounds as potential biological and industrial products is very important to obtain various health benefits, including improving human infertility, which has become a global problem. The objective of this study is to identify the content of antioxidant compounds in macroalgae in the coastal waters of the Minahasa peninsular that has the potential to improve male infertility outcomes. Amphiroa anceps (Lamarck) Decaisne and Gracilari sp obtained in this study were prepared and extracted. Phytochemical screening of the extracts was perfomed and the DPPH (1,1-diphenyl-2-picrylhydrazil) technique was used to determine the antioxidant activity of the macroalgae. The results indicated that the two macroalgae exhibit potent antioxidant activity. Amphiroa anceps (Lamarck) Decaisne and Gracilaria sp had IC50 values of 51.83 ppm and 51.00 ppm, respectively. The phytochemical analysis of these two macroalgae showed the presence of secondary metabolites, which are alkaloids, tannins, flavonoids, saponins, triterpenoids, and phenolics. In conclusion, the findings of this study suggest both macroalgae, Amphiroa anceps (Lamarck) Decaisne and Gracilaria sp, exhibit relatively high antioxidant activity and contain some secondary metabolites that may affect improving male infertility.

Index Terms- Amphiroa anceps, antioxidant, bioactive compounds, Gracilaria sp, infertility

I. INTRODUCTION

The marine environment is the most explored habitat because of its diversity. Marine organisms are potential sources of many bioactive compounds that are important in the development of pharmaceutical products. To dates, many valuable compounds isolated from marine organisms show beneficial biological activities such as antioxidant, antibacterial, antitumor, antiviral, anticoagulant, anti-inflammatory, antihypertensive, and antidiabetic. Amongst marine sources, macroalgae are valuable resources because they have a structural diversity of bioactive compounds (Jiameng, 2022).

Infertility is a global problem. A comprehensive study of 195 countries between 1990 and 2017 shows an increase in infertility cases worldwide (Sun, 2019). Other research suggests that around 100 - 140 million people (50 -70 million couples) worldwide have this problem (Szamatowicz, 2020). Another problem that is no less important is that the current diagnosis and treatment of infertility is expensive. The underlying cause of male infertility is oxidative stress related to environmental and lifestyle factors, including radiation, smoking, and systemic diseases such as diabetes and cancer (Agarwal et al., 2018). In addition, excessive levels of reactive oxygen species (ROS) can significantly damage sperm antioxidant systems and sub-fertile and infertile men have high levels of ROS in their reproductive tract that can damage reproductive cells (Martin-Hidalgo et al., 2019).

On the other hand, one of the current areas of focus is finding natural health products and natural product derivatives from marine organisms because of their safety, high nutritional content, and unique bioactive compounds that show beneficial health effects. Researchers from Flinders University's Center for Marine Bioproduct Development discussed the potential of bioactive compounds from marine organisms in overcoming human reproductive problems such as infertility (Hoang, 2022). Only a few marine organisms have been tested in clinical trials for their potential as treatments for human reproductive health. In addition, most of the potential bioactive compounds are still unknown, and the process of producing bioactive extracts and purified compounds is currently limited to a laboratory scale or is not sustainable in some species (Hoang, 2022).

A number of studies show that macroalgae contain biologically active metabolites, including polysaccharides, oligosaccharides, polyunsaturated fatty acids, sterols, polyphenolic proteins, carotenoids, ulvan, fucoidan, alkaloids, steroids, saponins, flavonoids, phenols, tannins, vitamins, and mineral. Several studies show macroalgae have promising potential in antitumor, antimicrobial, anti-inflammatory, immunomodulatory, antiangiogenic, antidiabetic, neuroprotective and infertility treatment (Barzkar, 2019; Jiameng, 2022).

II. MATERIAL AND METHODS

Source and preparation of samples

Macroalgae sampling was carried out at two locations in Minahasa waters:

1. N 01º40'41.8"; E 125º04'30.1"

2. N 01º44'35.6"; E 124º58'38.2"

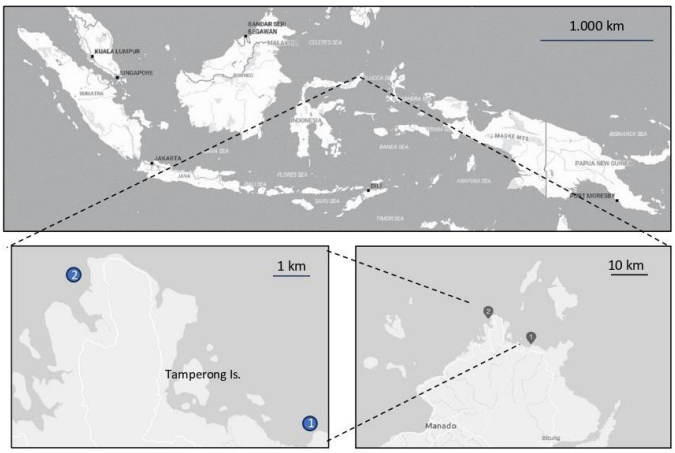

Figure 1. Study Site

Macroalgae were collected from the coastal water of the Minahasa Peninsula of Indonesia (Figure 1). Then we cleaned them under running fresh water to remove dirt, sand, silt, adhering organisms, and the surface salt about 3-4 times. After draining, we chopped the clean macroalgae into 5 cm pieces. In addition to increasing the contact area during the maceration process, this procedure makes storage and sample handling more practicable. The samples were dried in an oven at 400oC and then ground using a blender.

Preparation of extracts

The method of extraction is single extraction, using ethyl acetate solvent. The extraction procedure refers to the Santoso method et al., (2004) with modifications. The dry sample was weighed as much as 2 g, and 20 ml of ethyl acetate solvent was added and sonicated for 5 minutes. Then 25 ml of solvent was added, and macerated for 24 hours at room temperature with a magnetic stirrer and the final stage is second sonication for 5 minutes. Furthermore, Centrifugation was carried out for 10 minutes at 6000 rpm and 4°C, filtered using paper Whatman filter number 42. The filtrate product is evaporated with a rotary vacuum evaporator at 40°C until extract is turned into paste. The crude extract underwent phytochemical analysis and antioxidant activity test using the 1,1-diphenyl-2-picrylhydrazyl (DPPH).

III. RESULTS AND DISCUSSION

Algal sampling in the coastal waters of the Minahasa Peninsula was carried out using the exploration survey technique. Identification of macroalgae was using identification guides from the Field guide and atlas of the seaweed resources. The two species of macroalgae obtained were *Amphiroa anceps* (Lamarck) Decaisne and *Gracilaria sp.* Both macroalgae are a group of red algae (Rhodophyta) consisting of about 7,000 species of predominantly marine algae with taxonomic revisions ongoing. Red algae are a source of antioxidants and have a long history of use as food and pharmaceutical substances.

Amphiroa anceps (Lamarck) Decaisne (Fig 2): Phylum Rhodophyta – Class Florideophyceae – Order Corallinales – Family Corallinaceae – Subfamily Amphiroideae – Tribe Amphiroeae.

Determination of the antioxidant activity of the macroalgae extracts

In preparing a 0.1 mM DPPH solution, 0.39432 grams of DPPH powder (BM 394.32) were dissolved in 10 ml of methanol p.a. Using a micropipette, 100 ml of DPPH (0.1M) solution was obtained and transferred gradually to a 100 ml volumetric flask to produce a 0.1 mM DPPH solution. To determine the maximum DPPH wavelength, 2 mL of the 0.1 mM DPPH solution was placed into a test tube, and 2 ml of methanol p.a. was added, vortexed until the solution was homogenous, and then poured into a cuvette. The wavelength was measured between 400 and 800 nm with a UV-Vis spectrophotometer, with a maximum wavelength of 517 nm. In preparing the blank solution, 2 mL of a 0.15 mM DPPH solution was added along with 2 mL of methanol in a test tube. The solution was vortexed to ensure homogeneity before being incubated in the dark for 30 minutes. The wavelength of 517 nm was then used to measure the absorption. To prepare 1000 ppm of the main extract solution, 50 mg of the sample was diluted with methanol p.a. and poured into a 50 ml volumetric flask. The volume was then adjusted with methanol p.a. until the indicator was reached. Each concentrated test solution was diluted to a volume of 2 mL and added to 2 mL (0.15 mM) of DPPH in a test tube. Then, the solution was vortexed to ensure homogeneity before being incubated for 30 minutes in a dark room. The wavelength of 517 nm was then used to measure absorption on a UV-Vis spectrophotometer. The radical prophylactic activity was expressed as an inhibition percentage, which was calculated with the following formula:

$$\% \text{ DPPH inhibition} = \frac{\text{Control absorption} - \text{test solution absorption}}{\text{Control absorption}} \times 100\%$$

The x- and y-axes of the linear regression equation were used to plot the sample concentration and inhibition percentage separately. This equation was used to determine the value of IC 50 from each sample, with a y-axis value of 50 and an x-axis value that was obtained as the IC50 value.

Phytochemical screening of the macroalgae extracts

The method of Harborne is used for the phytochemical screening of the macroalgae extracts for the presence of alkaloids, phenol hydroquinone, flavonoids, tannins, saponins, steroids, and triterpenoids.

Figure 2. *Amphiroa anceps* (Lamarck) Decaisne

Thallus of *Amphiroa anceps* (Lamarck) Decaisne heavily calcified, in dense clumps, articulated, rigid, red-purple to grayish pink, 2–10(–15) cm high. Branching in one plane, dichotomous with very narrow angles in the upper part. Lower segments terete, compressed to flat above, nearly all of the same length, 6–10(–15) mm long, (0.5–)1–3(–4) mm broad. Joints conspicuous, non-calcified. In the longitudinal section, the segment consists of numerous curved tiers of medullary cells, 2–5 layers of longer cells (40–70 µm long) alternating with one layer of short cells, 10–25 µm long. Cortex composed of compact filaments of 3–8 isodiametric or slightly elongated cells, 7–12 µm diam., with terminal epithelial cells. Conceptacles are prominent, to 500 µm diameter, with a central pore, mainly on the surface of one side of the segments. Conceptacles with a central tuft of elongate cells and tetrasporangia. Tetrasporangia elongate-ovoid, 20–50 µm diam., zonately divided. Attachment by crustose holdfast, 2–10 mm across. Grow on solid substrates in the intertidal and upper subtidal zones. They live in subtidal areas of tropical and subtropical waters.

Figure 3. *Gracilaria sp*

Gracilaria sp (Fig 3) has general morphology: a cylindrical thallus, smooth surface, irregular branching, forms clumps, and the base of the thallus branching is narrow. Gracilaria sp grows both in brackish and coastal waters. Several species of Gracilaria sp grow in tidal area, characterized by muddy land, eutrophic waters, high temperatures, and sedimentation areas.

Table 1. Phytochemical analyses on *Amphiroa anceps* (Lamarck) Decaisne and *Gracilaria sp*

Compound	*Amphiroa anceps* (Lamarck) Decaisne	*Gracilaria sp*
Alkaloid (Dragendorf, Wagner, Meyer)	+++	+++
Flavonoid	+	+
Tannin	+	+
Saponin	+	+
Steroid	-	-
Triterpenoid	-	+
Phenol	+	+

The phytochemical analysis of these two macroalgae in Table 1 showed the presence of secondary metabolites; *Gracilaria sp* possesses alkaloids, tannins, flavonoids, saponins, triterpenoids, and phenolics, while *Amphiroa anceps* (Lamarck) Decaisne had the same properties except for triterpenoid. Both contained no steroids.

Table 2. Antioxidant activity test result of *Amphiroa anceps* (Lamarck) Decaisne and *Gracilaria sp*

	Amphiroa anceps (Lamarck) Decaisne			*Gracilaria sp*		
Concentration (ppm)	Mean absorption	% Mean inhibition	Mean IC_{50}	Mean absorption	% Mean inhibition	Mean IC_{50}
20	0.503	38.93		0.509	38.20	
40	0.456	44.68		0.456	44.64	
60	0.374	54.55	51.835487	0.376	54.39	51.008893
80	0.317	61.51		0.334	59.49	
100	0.276	66.49		0.296	64.06	
DPPH control	0.824			0.824		

As shown in Table 2, the result of this study indicated that the two macroalgae have high enough antioxidant activity, *Amphiroa anceps* (Lamarck) Decaisne and *Gracilaria sp* had IC50 values of 51.83 ppm and 51.00 ppm, respectively.

The underlying cause of infertility is oxidative stress related to environmental and lifestyle factors, including radiation, smoking, and systemic diseases such as diabetes and cancer (Agarwal et al., 2018). In addition, excessive levels of reactive oxygen species (ROS) can significantly damage sperm antioxidant systems, and sub-fertile and infertile men have high levels of ROS in their reproductive tract that can damage reproductive cells (Martin-Hidalgo et al., 2019). Antioxidants can be a promising solution in fighting oxidative stress because they have an essensial role in protecting the reproductive system at the cellular response level in fighting oxidative stress. Thus, interventions to prevent and improve male infertility can utilize natural antioxidants, such as the ones from Macroalgae.

IV. CONCLUSION

The findings of this study suggest that both red macroalgae, *Amphiroa anceps* (Lamarck) Decaisne and *Gracilaria sp,* have potent antioxidant activity and contain alkaloids, tannins, flavonoids, saponins, triterpenoids, and phenolics, for that reason, they may have the potential to improve male infertility.

ACKNOWLEDGMENT

We thank Sam Ratulangi University which has provided grants for the implementation of this research.

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