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# *Screening of Bioactive Compounds of the Brown Algae Turbinaria ornata* (Turner) J. Agardh, 1848 From the Coastal Waters of Aboru Village, Central Maluku Indonesia

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ABSTRACT

***Introduction:*** *One of the most important communities in the seawater ecosystems is the brown algae where Turbinaria ornate is a brown alga species which is rich in nutrients and bioactive compounds. The bioactive components of T. ornata are beneficial for people living along the coast, particularly in Maluku region, an archipelagic region, as well as for the medical and pharmaceutical sectors.*

***Objective:*** *This study aims to do screening of bioactive compounds of the Brown Algae Turbinaria ornata (Turner) J. Agardh.*

***Methods:*** *The research sample was the brown algae T.ornata obtained from the coastal waters of Aboru Village. Screening of bioactive compounds was carried out qualitatively using different reagents and indicated by the appropriate color change to ensure the presence of bioactive compounds which include phenolics, tannins, flavonoids, saponins, triterpenoids, steroids, alkaloids and anthocyanins.*

***Results:*** *This study's findings demonstrated that while triterpenoids, quinones, and anthocyanins were absent, tannins, saponins, alkaloids, steroids, phenolics, and flavonoids were present in high concentrations.*

***Conclusion:*** *In conclusion, T. ornata includes bioactive compounds like tannins, saponins, alkaloids, steorids, phenolics, and flavonoids, according to this study's findings*

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## INTRODUCTION

Indonesia is the nation with the greatest diversity with regard to marine biodiversity. On almost all Indonesian beaches, macroalgae is one of the marine biological resources that can be found. Seaweed is another name for macroalgae (1). *Turbinaria ornata* (Turner) J. Agardh, 1848, a type of large marine brown algae belonging to the Phaeophyceae family (2). This species is extensively present in Indonesia's coastal waters (3). The community, particularly traditional fishermen and those who live in the coastal area, use Turbinaria sp. despite the fact that it is thought to be a beach pollutant. They use it as animal feed, fertiliser liquid, and food ingredients (1). The use of *Turbinaria* sp. increased quickly over time. Fucoids and numerous other primary and secondary metabolites present in *T. ornata*, which have a high concentration, are to blame for this (2).

The chemical compounds of *T. ornata* such as carbohydrates, proteins, ash, lipids (4), amino acids (5), as well as macro and microminerals comprising Phosphor (P), Calcium (Ca), Magnesium (Mg), Potassium (K), Iron (Fe), Manganeses (Mn), and Zinc (Zn) (5,6). The *T. ornata* also encompass secondary metabolites including Saponin, Flavonoids, Phenolics, Alkaloids, Glycosides, Tannins and Steroids (7,8).

The bioactive components of *T. ornata* are a significant source and valuable medicinal components, including anti-diabetic, cardiovascular disease prevention, antibiotic, antioxidant, anticoagulant, anti-inflammatory, and anti-ulcer products, as well as suspending agents in radiological preparations (2,4,9,10,11). According to Unnikrishnan (2) *T. ornata* extract which was analyzed by GC-MS showed the presence of compounds such as hentriacontane, z, z-6, 28-heptatriactontadien-2-one, 8-heptadecene, and 1-heptacosanol, which can controlling hyperglycemia. Deepak (7) also reported about the presence of tetra decanoic acid in *T. ornata* that have antioxidant and anticancer activity.

Aboru Village is located in the littoral region of the Central Maluku Regency's Haruku Island Sub district. The marine biological resources in this region, which include *T. ornata* and other macroalgae, are extremely varied. *T. ornata* is utilised by the locals of Aboru Village in the shape of dried or fresh processed vegetables. Despite the fact that people have consumed it, the bioactive content of *T. ornata* from the coastal waters of Aboru has never been extensively researched and reported.

The only information regarding *T. ornata* from the coastal water of Aboru is the morphological characteristics that has been reported by Riry (12), while the other characteristics including phytochemical compounds is never been reported. Hence, the results of this research should shed light on the significance and usefulness information about the bioactive compounds of *T. ornata* that can be consider as a new source of bioactive compounds from marine ecosystem.

## METHOD

### Sample collection

*T. ornata* samples (**Figure 1**) were gathered from the littoral waters of Aboru Village in the Central Maluku Regency at latitude and longitude coordinates 128030'31.7" EL and 3036'45,4" SL. Using the tracking survey technique, sampling was done at two stations during the lowest tide, collecting samples of each type of algae that could be found there. The sample is then temporarily stored (± 8 hours) in a seawater-filled container before being used for further laboratory examination. Identification of *Turbinaria* was carried out at the Marine Science Laboratory, Faculty of Fisheries and Marine Sciences, Pattimura University.

### Sample extraction

Samples were extracted in accordance with Lailiyah (13) and Cazali (14). As much as 400 mL of an 85% methanol solvent were used to macerate up to 100 grams of *T. ornata* flour for 24 hours at a temperature of 27°C. The solution is then purified, the residue is re-macerated for another 24 hours, and the procedure is repeated for the residue. The methanol filtrate was then condensed using a rotary evaporator at 40$^{o}$C until a concentrated extract was obtained.

### Qualitative screening of bioactive compound

The brown algae *T. ornata* samples were screened qualitatively to identify the presence of bioactive compounds. These compounds include phenolics, tannins, flavonoids, saponins, triterpenoids, steroids, alkaloids, and anthocyanins. Up to 50 milligrams of *T. ornata* extract was mixed with 10 mL of aquadest for the tannin analysis, and the mixture was then heated until boiling. A few droplets of $FeCl_3$ were then added. Greenish hues suggest the presence of a tannin compound (15,16).

By mixing 2 ml of the extract with 10 mL of aquadest and giving it a gentle shake for 30 seconds, the saponin compound was evaluated. Stabil foam shows the presence of saponin compounds (15,17). The *T. ornata* extracts were dissolved in ten droplets of 2N sulfuric acid, and the presence of alkaloids was then determined using Meyer reagents. The development of a brown colour that indicated the presence of alkaloids provided a positive outcome (18).

The extract was analysed for steroid and triterpenoid content by combining 2 ml of it with a few droplets of anhydrate acetate and Liberman Buchard reagents. Greenish hues are indicative of steroid compounds (19), whereas reddish-brown hues are indicative of terpenoids (16).

For the Phenolics compound, as much as 1 mL of extract was added by two drops of $FeCl_3$ 5%. Positive reaction present by the formation of green color (15). Flavonoid identification was conducted by adding 2 mL of the sample with 2 mL of hot water, boiled for 5 minutes, and then filtered. 5 mL of filtrate was added with 0.05 mg of Mg powder and 1 mL of concentrated HCl, then shaken vigorously. Positive results are marked in red, yellow or orange (1). By adding a little 1N Natrium Hydroxide (NaOH) to the extract, the presence of quinones was confirmed by the appearance of a colour shift. A yellow

colour that forms indicates a favourable response.

Two procedures were used to examine anthocyanins. The first technique is heated for two minutes at 100ºC with the addition of 2 M of Hydrogen Chloride. A consistent crimson hue denotes successful outcomes. The second step involves adding 2 M of NaOH. A crimson colour that changes to blue in the presence of the reaction indicates a favourable response (20). As a qualitative study, the findings are explained in terms of how the final figure appears.

## RESULTAND DISCUSSION

To determine whether phytochemical compounds were present or absent in the brown algae *T. ornata*, a qualitative screening was conducted. Triterpenoids, phenolics, tannins, saponins, alkaloids, steroids, flavonoids, quinones, and anthocyanins were tested. The tests for triterpenoids, quinones, and anthocyanins yielded negative findings, while the tests for tannins, saponins, alkaloids, steroids, phenolics, and flavonoids yielded positive results (**Table 1**).

**Figure 1.** *Turbinaria ornata* at the rock substrate in natural habitat the coastal water of Aboru

To determine whether phytochemical compounds were present or absent in the brown algae *T. ornata*, a qualitative screening was conducted. Triterpenoids, phenolics, tannins, saponins, alkaloids, steroids, flavonoids, quinones, and anthocyanins were tested. The tests for triterpenoids, quinones, and anthocyanins yielded negative findings, while the tests for tannins, saponins, alkaloids, steroids, phenolics, and flavonoids yielded positive results (**Table 1**).

**Table 1.** Result of Qualitative Screening of *T. ornata* from Coastal Waters of Aboru

| Type of Test | Reagent | Results | | Information |
|---|---|---|---|---|
| | | Positive (+) | Negative (-) | |
| Tannins | Ferri Chloride (Iron III) | √ | | Greenish yellow color |
| Saponin | Hydrogen Chloride | √ | | Presence of stable foam ± 15 minutes |
| Alkaloids | Wagner reagents | √ | | Brownish red color |
| Steroids | Liberman-Buchard | √ | | Green color |
| Triterpenoids | Liberman-Buchard | | √ | Brownish red if (+), result : green |
| Phenolics | Ferri Chloride (Iron III) | √ | | Green color |
| Flavonoids | Hydrogen Chloride | √ | | Red, yellow or orange |
| Quinones | Natrium Hydroxide (NaOH) | | √ | Green color if (+), result: green |
| Anthocyanins | Hydrogen Chloride<br>Sodium Hydroxide | | √ | Positive if red or red to blue. Result: yellow color |

A reagent ($FeCl_3$) was used to conduct the tannin test, and the presence of a green colour showed that the findings were favourable (**Figure 2a**). The hydrochloric acid (HCl) reagent was used to perform the saponin test, and the presence of foam indicated a positive reaction (**Figure 2b**). Wagner's reagent and Sulphuric Acid ($H_2SO_4$) alkaloids tests yield positive findings when a brownish red colour is present (**Figure 2c**). Using the Liberman-Buchard reagent, a steroid test was performed, and positive findings were indicated by the presence of a green colour (**Figure 2d**). Triterpenoid testing was carried out by using Liberman-Burchard reagent, and the positive reaction will be appear by he forming of green colour as shown in **Figure 2e**

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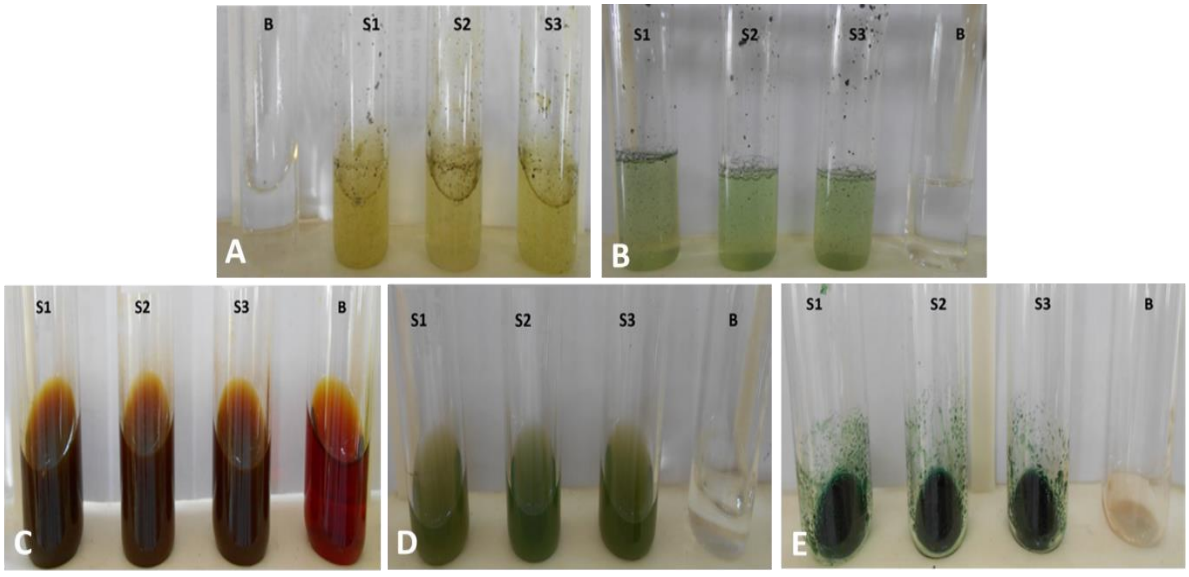


**Figure 2**. The qualitative result of the bioactive compound of *T. ornata* from the Coastal water of Aboru. Tannins (A), Saponins (B), Alkaloids (C), Steroids (D), and Triterpenoids (E). Note: B: blank, S1: replication 1, S2: Replication 2, S3: Replication 3

By introducing 5% $FeCl_3$ solution, a phenolic test was conducted. Green colour formation suggested a successful response (**Figure 3a**). Utilizing of Mg powder and concentrated HCl, a flavonoid assay was conducted and the appearance of green colour indicates the presence of flavonoids (**Figure 3b**). The quinone test is conducted by adding 1 N NaOH, and positive findings are indicated by red, yellow, or orange colours. Yellow colour formation indicates a favourable response (**Figure 3c**).

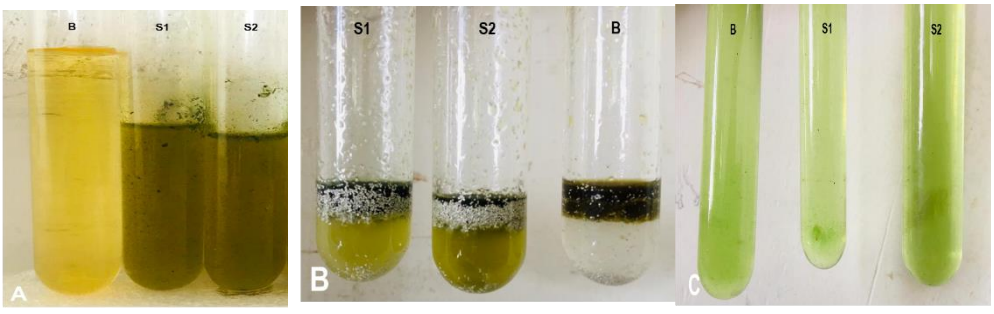


**Figure 3**. Qualitative result of Phenolics (A), Flavonoids (B), dan Quinones (C) of *T. Ornata* from the coastal water of Aboru. Note: B: blank, S1: replication 1, S2: Replication 2

To confirm that samples of *T. ornata* also contained anthocyanins, a test was run. Using two techniques simultaneously for qualitative testing: heating with HCl and adding NaOH second. This qualitative test yielded negative anthocyanin results (**Table 2**), which indicates that the sample did not contain any anthocyanins. Anthocyanins should have produced a red color, but the results were obtained with a yellow hue (**Figure 4**)

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**Table 2.** Qualitative result of Anthocyanins Test of *T. ornata* from the coastal water of Aboru

| No. | Method | Anthocyanins characteristics | Results | Information |
|---|---|---|---|---|
| 1 | Heated with 2M HCl for 2 minutes at 100ºC | Stable red color | Yellow color | - |
| 2 | Dropped with NaOH 2M | Red color change to be green or blue | Yellow color | - |


**Figure 4**. The result of Anthocyanins Test of *T. ornata* from the coastal waters of Aboru

According to Hendrawati and Rahmawati (21) that anthocyanins are amphoteric substances. They are thus capable of reacting with bases or acids. Anthocyanins have a red colour under acidic circumstances and a purple or blue colour under alkaline conditions. The groups connected to the fundamental structure of the bond position were responsible for changes in this colour. As a result of the base condition's reaction with anthocyanin, which results in a type of negatively charged ions, blue colour was expressed in the meantime. This was the precise anthocyanin colour measurement (22).

## CONCLUSION

Based on the findings of this research, it can be concluded that triterpenoids, quinones, and anthocyanins are absent from the brown alga *T. ornata*'s bioactive compounds while tannins, saponins, alkaloids, steroids, phenolics, and flavonoids are present.

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