Phytochemical compounds of *Enhalus acoroides* from Wanci Island (Wakatobi) and Talango Island (Madura) Indonesia

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Abstract. The existence of *Enhalus acoroides* certainly gives important influence to the ecosystem, both as a producer in the food web and as a living habitat. In the last decade, *Enhalus acoroides* was widely used as an object of marine bioprospection research. That research showed potential results as antibacterial, antifungal, even as antifouling. This is a good reason to know the content of phytochemical compounds in *Enhalus acoroides* from two different locations. The purpose of research purpose to determine (1) the crude extract produced by *Enhalus acoroides* from two different locations; and (2) the phytochemical compounds contained in the crude extract of *Enhalus acoroides* from two different locations. This study this research was to used samples collected from Wanci Island (Wakatobi), and Talango Island (Madura), Indonesia. The extraction process and phytochemical test were conducted at the Marine Science Laboratory, FPIK, University of Brawijaya, and lasted for two months, from June 2017 to September 2017. The extraction was done by three solvent, are methanol, ethyl acetate and chloroform. Furthermore, phytochemical test was performed qualitatively. The results provided that the yield produced by *Enhalus acoroides* from Wanci Island, Wakatobi is relatively lower than Talango Island, Madura. *Enhalus acoroides* is also renowned to contain phytochemical compounds of tannins and saponins.

1. Introduction

*Enhalus acoroides* is a type of *Enhalus acoroides* that is easy to find in Indonesia. This species can easily be recognized and identified because it has a different morphology. *Enhalus acoroides* has larger roots, stems and leaves compared to other *Enhalus acoroides* species found in Indonesia [1]. The type of *Enhalus acoroides* leaf is long, hard and stiff like a belt. It can be found in all major islands in Indonesia and even in small islands [2].

The presence of *Enhalus acoroides* certainly has an important influence on the ecosystem, both as a producer in food networks and as a living habitat of biota [3]. Moreover, *Enhalus acoroides* leaf is also renowned to help coral reefs reduce the waves, as well as catch solid sediment particles in the water column. In the last decade, *Enhalus acoroides* is also widely used as a marine bioprospective research object and given potential results as antibacterial, antifungal, even as an antifouling [4, 5, 6].
Previous research showed that environmental conditions would affect the content of bioactive compounds produced by the organism. This condition is a strong reason for the identification of basic information about phytochemical compounds in *Enhalus acoroides* from two different locations. The purpose of this study is to determine (1) the yield produced by *Enhalus acoroides* from two different locations; (2) a class of phytochemical compounds in the yield of *Enhalus acoroides* from two different locations.

2. Methodology
This research began with the collection of fresh *Enhalus acoroides* samples from Wanci Island, Wakatobi and Talango Island, Madura. The samples of fresh *Enhalus acoroides* were obtained were then dried naturally under the sun and extracted at the Marine Science Laboratory, Fisheries and Marine Science Faculty, University of Brawijaya. The *Enhalus acoroides* extract obtained was then used as a test material, namely phytochemical test. There were three sections for processing; there were sampling fresh *Enhalus acoroides*, extraction and evaporation, and the phytochemical test. This research was conducted from September to November 2017.

The tools and materials used in this study were divided into three major sections (table 1), i.e. tools and materials for *Enhalus acoroides* sampling, extraction, and phytochemical test. This research was conducted in several stages, i.e. collection of *Enhalus acoroides* samples, extraction, phytochemical test. The samples of fresh *Enhalus acoroides* leaves were taken directly from their habitat, then cleaned from the biofouling. Then, the leaves of the *Enhalus acoroides* were dried naturally until the *Enhalus acoroides* leaves became constant. For instance, the dried *Enhalus acoroides* leaves were prepared three times as replicates.

| Stages | Tools | Materials |
|--------|-------|-----------|
| 1. Sampling *Enhalus acoroides* | Mask | For instance of *Enhalus acoroides* |
|       | Snorkel | Methanol |
|       | Plastic | Klorofom |
|       | Dive knives | Ethyl acetate |
| 2. Extraction of bioactive materials | Sbath | Sulfuric acid 2N |
|       | Whatman | Reactant meyer |
|       | The scales | Reagents |
|       | | dragendorf |
|       | | Reactant wagner |
| 3. Test Of Phytochemicals | Test tubes | Magnesium |
| a. Alkaloid | Pipette | |
|       | Glass cup | |
| c. Flavonoid | Test tubes | Amyl alcohol |
|       | Pipette | Alcohol |
|       | Glass cup | |
| d. Saponin | Test tubes | HCl2N |
|       | Pipette | |
e. Tannin

The extraction process was carried out by modification of some previous studies [4, 5], in which exemplified *Enhalus acoroides* samples were weighed 40 gram each, then soaked in non-polar solvents, chlorofomes (120 ml) with a ratio of 1:3 (w/v) in a glass bottle. Then, it was macerated for 24 hours. Subsequently, the *Enhalus acoroides* sample solution was filtered using a filter paper. The exemplified *Enhalus acoroides* sample filtrate was then re-soaked with semi-polar solvent, ethyl acetate (120 ml) in a glass bottle, then macerated for 24 hours. Next, the *Enhalus acoroides* sample solution was filtered using filter paper. Exemplared *Enhalus acoroides* sample filtrate was re-soaked with polar solvent, methanol (120 ml) and macerated for 24 hours. After 24 hours, the *Enhalus acoroides* sample solution was filtered using filter paper. After that, *Enhalus acoroides* sampling solution of filtration result was evaporated using Rotary evaporator applied at a temperature of 50 °C so that raw extract of *Enhalus acoroides* in the form of paste was obtained. The raw extract pasta of the *Enhalus acoroides* was weighed in order to know the percentage of rendement.

Phytochemical test is one of qualitative tests for identifying the bioactive compounds that are conceivable within an organism. There are four classes of compounds to be tested: Alkaloids, Flavonoids, Saponins, and Tanin [7, 8, 9]. The quality of the phytochemical compounds in *Enhalus acoroides* extract can be identified by observing the color changes that occur in the extract.

Alkaloid test was performed by adding 1.5 – 2 % HCl into three tubes. Tube 1 solution plus 0.5 ml of dilute acid solution as control, 2 tubes plus 2 - 3 drops of Dragendorff reagent, and tube 3 plus 2 - 3 drops of Mayer reagent. If orange precipitate was formed in tube 2 and yellowish brass precipitate was formed in tube 3, the alkaloids were present. The flavonoid test was performed by dissolving the *Enhalus acoroides* extract in 1 - 2 ml of 50 % hot methanol. Then, Mg metal and 4 - 5 drops of concentrated HCl were added. The test is positive if the solution became red or orange.

The saponin test was done by adding water (1 : 1) into the *Enhalus acoroides* extract, and shaking for 5 minutes. The foam formed which can last for 30 minutes indicates the presence of saponin compounds. The tannin compound was tested by dissolving the *Enhalus acoroides* extract in 1 - 2 ml of water and 2 drops of FeCl₃ solution; the presence of a blackish blue color indicated the presence of a tannin compound error and if the color was blackish, it showed a tannin catechol compound.

### 3. Result and Discussion

The *Enhalus acoroides* extracts produced in this study showed different values between *Enhalus acoroides* taken from Wanci Island (Wakatobi) and Talango Island (Madura). The crude extract of *Enhalus acoroides* collected from Talango Island (Madura) was higher yield than Wanci Island (Wakatobi). These can be seen from the percentage of the crude extract of two *Enhalus acoroides* samples as shown in table 2.

#### Table 2. *Enhalus acoroides* extract yield from two different locations.

| No | Example of *Enhalus acoroides* | Solvents |
|----|---------------------------------|----------|
|    |                                 | Methanol | Ethylacetate | Chloroform |
| 1  | *Enhalus acoroides* in Wanci Island, Wakatobi | 1.125 | 0.25 | 0.55 |
| 2  | *Enhalus acoroides* in Talango Island, Madura | 1.725 | 0.125 | 0.8 |
The yield produced by *Enhalus acoroides* through extraction with methanol solvent was higher when compared to ethyl acetate and chloroform. This could happen because methanol is a polar solvent, which can extract polar compounds in the body of the organism, compared to ethyl acetate and chloroform. Meanwhile, the semi-polar and non-polar compound of *Enhalus acoroides* is less than that of the polar compound. This condition also has been reported in previous research.

The phytochemical qualitative test of the six extracts of *Enhalus acoroides* showed almost the same result, a positive response to the saponin and tannin test (Table 3). A positive response to the saponin test was demonstrated by the appearance and persistence of the foam during the test, in chloroform solvent extraction. Furthermore, a positive response of tannin test was demonstrated by the ethyl acetate extract of *Enhalus acoroides* from Wanci Island (Wakatobi), as well as chloroform extract from two sites.

Chemically, saponins have a wide diversification of structures, and certain saponin compounds with their surfactant properties can cause lysis of the protozoan cell wall, thereby being used for protozoal defaunation [10]. Tannins are generally defined as polyphenol compounds that have a fairly high molecular weight (over 1000) and can form complexes with proteins. Tannin has a biological activity as an antioxidant, so that the tannin will have an effect on the antioxidant activity [9].

| No | Phytochemical compounds | *Enhalus acoroides* in Wanci Island | *Enhalus acoroides* in Talango Island |
|----|-------------------------|----------------------------------|----------------------------------|
|    |                         | Metanol | Ethyl Acetate | Chloroform | Metanol | Ethyl Acetate | Chloroform |
| 1  | Alkaloid                | -       | -             | -          | -       | -             | -          |
| 2  | Flavonoid               | -       | -             | -          | -       | -             | -          |
| 3  | Saponin                 | -       | +             | +          | -       | -             | +          |
| 4  | Tanin                   | -       | +             | +          | -       | -             | +          |

Note: (-): Nothing, (+): Any

Alkaloid and flavonoid test in this study showed a negative value, meaning that there were no alkaloid and flavonoid compounds in the *Enhalus acoroides* extract. This result differs from some previous studies, which reported that *Enhalus acoroides* extracts generally contain flavonoid group chemicals. This is due to the different environmental conditions of *Enhalus acoroides* sampling. Flavonoid compounds become one of interests in bioprospective research. This is because some pure compounds belonging to the flavonoid group have the potential to be used as antifouling. Flavonoid compounds, according to Jensen *et al.* [11], are also able to inhibit the growth of sticking microorganisms, fungi type Schizichytrium aggregatum.

4. Conclusions

*Enhalus acoroides* collected from two different sites have slightly different bioactive potentials. Crude extract of *Enhalus acoroides* from Wanci Island (Wakatobi) is relatively higher yield than that from Talango Island (Madura). Meanwhile, phytochemical tests of both extracts showed the same bioactive compounds, were tannins and saponins.

5. References

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