Effect of Different Solvents On The Phytochemical Compounds of Sargassum sp. From Yogyakarta and East Nusa Tenggara

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Abstract. Sargassum sp. is one of the brown algae species. Two species of Sargassum were collected from Deere coast, Alor East Nusa Tenggara (NTT), and Krokoh beach, Gunung Kidul Yogyakarta. This paper compares the phytochemical screening of two species of Sargassum sp. by using three different polarity solvents. The two species of Sargassum sp. were dried, cut, and ground into powder. Their species were extracted successive using ethanol, ethyl acetate, and n-hexane. Phytochemical screening all of the extracts of Sargassum sp were performed with the spot tests as the standard methods. The ethanol extracts of Sargassum sp. from Yogyakarta and NTT showed the presence of saponins, tannins, terpenoids/steroids, and flavonoids. The ethyl acetate and n-hexane extract from the two species of Sargassum sp. have contents of tannins, terpenoids/steroids, and flavonoids. Tannins and terpenoids/steroids were major compounds in ethyl acetate extract both Sargassum sp. from Yogyakarta and NTT. Results showed that different solvents possibly different phytochemical compounds. Phytochemical of seaweed is influenced by species, habitat, isolation method, and harvest season.

1. Introduction

Recently, the research focus has shifted towards the utilization of renewable natural resources from natural on land to natural marine. Among very abundant reserves of marine resources is seaweed. Carbon dioxide and water are converted into carbohydrates by sunlight in seaweed, which is a macroscopic and multicellular plant. Seaweed is classified into three categories based on the color of thalli, there are red (Rhodophyta), brown (Phaeophyta), and green (Chlorophyta) seaweeds with the number of 6500, 1800, and 1500 known species, respectively[1].

Indonesia is an archipelago country with natural seaweed resources that grow along the coastline and will not run out or renewable. Seaweed cultivation in Indonesia is dominated by the Euchema and Gracilaria genus, while the Sargassum genus grows wild on the shore [2]. Sargassum is a very large genus with 400 species spreads throughout the world. Several 50 species are found in Indonesia and two of them are growing on the Deere coast of NTT and the Krokoh beach Yogyakarta. Sargassum is included in the class Phaeophyceae, family Sargassaceae, and order Fucales which is well known in tropical oceans [3].

Sargassum is a brown macroalga due to the presence of fikosantin, chlorophyll a and b, and xanthophyll contained in plastids. Polysaccharides are the main ingredients of brown seaweed which
have different biological activities. The polysaccharide content of *Sargassum* sp. which has biological activity includes alginate, laminarin, and fucoidan. The structure and content of polysaccharides depend on habitat, isolation method, and harvest season [4]. *Sargassum* also contains protein, ash, water, vitamins, and minerals as a macro and microelements, namely potassium (K), sodium (Na), magnesium (Mg), phosphate (P), iodine (I), and iron (Fe) [5]. Recently, secondary metabolites known as phytochemicals have been studied extensively as sources of medicinal agents. The content of phytochemical compounds of *Sargassum* which is beneficial for health includes alkaloid compounds, flavonoids, phenolics, glycosides, tannins, and terpenoids/steroids which are widely used in medicine and the pharmaceutical industry such as blood coagulation, anticancer, antioxidant, antiviral, antibacterial, antilipidemic and immunomodulation activity [6], [7].

Extraction is the process of transferring the matrix to a different phase of a compound or group of compounds to produce an extract to be analyzed. Maceration is the conventional extraction method commonly used where the samples are soaked for several hours or even days to get the extract. The basic extraction mechanism in the various existing extraction techniques must consider the target compound, the solvent applied, and the properties of the solvent in which the sample will be dissolved. As a general rule, polar solutes dissolve in polar solvents and non-polar solutes dissolved in non-polar solvents. The interaction between the solute and the solvent is determined by the hydrophobicity, the solubility, the vapor pressure, and acid-base properties [8].

In this research, both *Sargassum* from NTT and Yogyakarta have soaked with n-hexane solvent then ethyl acetate, and finally with ethanol solvent. Then, all extracts were screened the phytochemical compounds such as alkaloids, saponins, tannins, flavonoids, and terpenoids/steroids. The screening of phytochemicals was conducted by using the spot tests to indicate the group of secondary metabolites.

2. Methodology

The two species of *Sargassum* sp. were defecated properly with freshwater, sponged up, and spread to dry naturally at room temperature for two weeks. The dried *Sargassum* was ground into a powder and stored in sterilized containers for further steps. The powdered samples of *Sargassum* sp were extracted using sequential maceration with increasing the polarity of pro analysies (p.a) grade solvents of ethanol, ethyl acetate, and n-hexane for 24 hours at the room temperature. Each extract was filtered and concentrated at 40°C temperature under a vacuum. The dark, gummy solid of each extract was used for the screening of secondary metabolites. All extracts were screened phytochemically for saponins, tannins, terpenoids/steroids, flavonoids, and alkaloids using spot tests as standard procedures by Harbone [9].

3. Result and Discussion

The extraction of *sargassum* sp. was carried out in the powdered form. The small sizes of samples can increase surface area and contact with the solvent more effectively during the extraction process. The powder of *Sargassum* sp. from Yogyakarta and NTT have the same visible characteristics as shown in table 1.

| No | Characteristics | Sargassum Yogyakarta | Sargassum NTT |
|----|-----------------|----------------------|---------------|
| 1. | Colour          | Greenish Brown       | Greenish Brown|
| 2. | Odor            | Fishy                | Fishy         |
| 3. | Texture         | Soft                 | Soft          |

Successive extraction was performed with ethanol (polar), ethyl acetate (semi-polar), and n-hexane (nonpolar) solvents to screen the content of phytochemical compounds in each extract. Ethanol has relatively high solubility and is inert so it does not react with other components. Three solvents have a low boiling point so that it is easily evaporated without using high temperatures in the purification process [10]. Each solvent can dissolve compounds with the same polarity. In this study *Sargassum*
sp. was soaked for 24 hours to attract the active compounds of *Sargassum*. The extraction is carried out at room temperature because phytochemical compounds are very susceptible to damage and easily oxidized at high temperatures. The dark and gummy solid was obtained from the extraction with different weights.

Apart from the technique and efficiency of the extraction process, external factors like the solvent, temperature, ratio, solvent contact time, and particle size affect the concentration of the extract compounds produced [8]. Therefore, the best extraction method under optimal conditions must be employed to prepare each particular product. Nevertheless, Cowan (1999) reported that the choice of solvent may affect the extraction efficiency of conventional methods. The solvent selection must pay attention to the polarity factor of the target compound. Azmir et al. (2013) described that molecular affinity between solvents and solutes, use of co-solvents, mass transfer, human toxicity, financial feasibility, and environmental safety must be considered in the selection of solvents for the extraction of bioactive compounds.

The extracts of ethanol, ethyl acetate, and n-hexane from *Sargassum* were screened for phytochemical compounds. One of the functions of the phytochemical compounds or secondary metabolites of seaweed is as a defense against various infectious diseases [11]. The qualitative results of *Sargassum* phytochemical screening were summarized in table 2.

### Table 2. Phytochemical compounds present in the different extract of *Sargassum* sp.

| No | Phytochemical Tested | *Sargassum* Yogyakarta | *Sargassum* NTT |
|----|----------------------|------------------------|-----------------|
|    |                      | n-hexane | Ethyl acetate | ethanol | n-hexane | Ethyl acetate | ethanol |
| 1. | Saponins             | -        | -            | +       | -        | -            | +       |
| 2. | Tannins              | +        | ++           | +       | +        | ++           | +       |
| 3. | Terpenoids/Steroids  | +        | ++           | +       | +        | ++           | +       |
| 4. | Flavonoids           | +        | +            | +       | +        | +            | +       |
| 5. | Alkaloids            | -        | -            | -       | -        | -            | -       |

Description :  
Signs (++) : contain more compound (solid colour)  
Signs (+) : contained compounds (colored)  
Signs (-) : no contained compounds (no color formed) [12].

The ethanolic extracts of *Sargassum* sp. from Yogyakarta and NTT showed the presence of phytochemical compounds such as saponins, tannins, terpenoids/steroids, and flavonoids. While n-hexane and ethyl acetate extracts are presence tannins, terpenoids/steroids, and flavonoids. Alkaloids are absent in all extracts of two species of *Sargassum*. These results exhibited that two *Sargassum* sp have different phytochemical compounds in different solvents. The ethanolic solvent produced an extract that has more complete phytochemical compounds than n-hexane and ethyl acetate solvents. This indicates that ethanol has a higher polarity than others. Several previous studies have shown that polar solvents such as ethanol tend to be more effective at attracting many phytochemical compounds. The content of phytochemical compounds is influenced by the polarity and the type of solvent used for extraction [13]. Tannins and steroids in ethyl acetate extract contain more compounds than ethanol and n-hexane extracts.

Saponins are active compounds surface that is easily detectable through its ability to form foam. The components of the glycoside bonds present in saponins to cause these compounds to tend to be polar [9]. Presence of saponins positive if the extract under test forms 1-10 cm high foam with time intervals ± 10 minutes [14]. Based on the results of phytochemical screening revealed that saponins presence only in ethanolic extract of both *Sargassum* sp. from Yogyakarta and NTT. Saponins are polar compounds, easily extracted by ethanol solvent as a polar solvent. Saponins in *sargassum* sp have functioned as antioxidant effects via inhibiting neurotransmitters of the GABA system, reduced brain levels of adrenalin, and noradrenaline, and decreased rate of cerebral metabolic rate for cerebral blood flow and oxygen [15].
All *Sargassum* extracts also contain tannins which detected by FeCl₃ reagent in the color of blackish-green [14]. Seaweeds extracts are rich in phenolic compounds. Phenolic compounds and its derivative like tannins have hydroxyl groups and aromatic rings that act as the radical scavenging power of these compounds and important to defense mechanisms against bacteria and environmental stress [11]. Tannins have been recognized for their potent antioxidant effect, antimicrobial, anticancer, antidiabetic, anti-hepatotoxic, anti-stress, anti-inflammation, anti-hyperlipidemic, anti-hypertension, and anti-aging [11], [16].

Steroids and terpenoids were detected both in *Sargassum* species from Yogyakarta and NTT extract with glacial acetic acid and sulfuric acid spot test. The steroid has several functions, such as increasing body stamina effect (aphrodisiac) and anti-inflammation, whereas the triterpenoid compound has functioned as the anti-tumor activity [17].

The presence of flavonoids in *Sargassum* sp. from Yogyakarta and NTT shows that the seaweed has activities as an antioxidant, anti-allergic, antimicrobial, anti-inflammatory, and anticancer. Flavonoids as an alternative in medicine because they have beneficial potential in fighting diseases and for human health [11]. Flavonoids can be tested for their presence using Mg and HCl to produce a red color, yellow or orange [9].

Results of phytochemical screening indicated that *Sargassum* species from Yogyakarta and NTT have the potential to be developed as herbal medicine because of the content of secondary metabolites.

### 4. Conclusion

Overall the results of the research can be concluded that the ethanol extract of *Sargassum* sp. Yogyakarta and NTT contain phytochemical compounds like saponins, tannins, terpenoids/steroids, and flavonoids. The n-hexane and ethyl acetate extracts of the *Sargassum* sp. Yogyakarta and NTT showed the presence of tannins, terpenoids/steroids, and flavonoids. The ethanolic extract has more complete phytochemical compounds than n-hexane and ethyl acetate extracts. Moreover, *Sargassum* sp. contains diverse phytochemical compounds in different solvents.

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