ANALYSIS OF TOTAL FLAVONOID LEVELS IN BROWN ALGAE (SARGASSUM SP. AND PADINA SP.) AS ANALGESIC DRUG THERAPY

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INTRODUCTION
Indonesia is a country known for its abundant natural resources and very extensive sea areas. Approximately 78% of the Indonesian territory is covered by water with shallow and deep seas [1]. As an archipelagic country with large areas for seaweed cultivation (11,109 km²), Indonesia is endowed with an abundance of tropical seaweed resources [2]. Several studies have shown that marine organisms, seaweed, and marine algae provide a high source of bioactive secondary metabolites that may be useful in the development of new pharmaceutical agents [3,4]. However, until now, marine algae in coastal areas of Indonesia have been neglected, especially in the pharmacological area [1-3].

Marine algae is classified into several types based on the composition of nutrients, pigments, and chemicals, such as Rhodophyta (red algae), Phaeophyta (brown algae), and Chlorophyta (green algae). This marine algae has been considered safe, non-toxic, easy to find, and its availability is not limited to various fields [3,4]. A number of pharmacological activities have been reported on marine algae, for example, anti-tumor, cytoprotective antioxidants, antihelmintic, anticoagulant, antibacterial, antifungal, and hepatoprotective effect, and inhibiting DNA polymerase and xanthine oxidase [4,5]. A study by Hong et al. using Sargassum fulvellum and Sargassum thunbergii indicated the presence of anti-inflammatory, analgesic, and anti-inflammatory activity in mice [6]. In another study conducted by Thennarasan et al., the analgesic effects of brown algae extract from Lobophora variegata showed significant analgesic activity when used in rats induced by chemical stimulants [7].

Although some studies have shown the analgesic activity of brown algae, until now, there is a little to no information available on the analgesic effect of the Sargassum sp. and Padina sp. of brown algae. Recent studies on Sargassum sp. and Padina sp. have primarily focused on anti-inflammatory and hemostatic effects in wound healing. Based on this fact, this study aims to analyze the flavonoid content of brown algae that serves as an analgesic as well as comparing the types of brown algae (Sargassum sp. and Padina sp.) that have the best analgesic effect.

METHODS
This type of research is experimental laboratory with post-test design with control group design. This research was conducted at Laboratorium Biofarmaka Hasanuddin University Faculty of Pharmacy in May 2017. The population of this research is brown algae that grows in Punaga waters, Takalar Regency, South Sulawesi Province. The samples used are Sargassum sp. and Padina sp. Sampling was done using a convenience sampling method.

Preparation of the extracts was done using the maceration method until dense extracts of Sargassum sp. and Padina sp. were obtained. Total flavonoid measurements were performed in triplicate at three different concentrations: 150 ppm, 300 ppm, and 450 ppm. The total flavonoid content of Sargassum sp. and Padina sp. was determined using colorimetric methods with AlCl₃, reagents and spectrophotometry with a standard blank ratio in the laboratory.

Based on the total flavonoid measurement data, a quercetin calibration curve was made resulting in the equation y=0.078x+0.029 (R²=0.994), where y is the absorbance value and x is the quercetin content. Using the quercetin calibration curve, absorbance measurements of Sargassum sp. and Padina sp. samples were used to determine the total flavonoid levels.

RESULTS
The total flavonoid level in Sargassum sp. and Padina sp. samples was determined after absorbance measurements and reported in Tables 1 and 2, respectively.
Table 1 shows the total flavonoid content in Sargassum sp. samples at three concentrations, each performed in triplicate. The average total flavonoid level in the 150 ppm sample is 1.237±0.158%, in the 300 ppm sample is 1.492±0.168%, and in the 450 ppm sample is 1.553±0.087%.

In this study, the measurement results showed that Padina sp. and Sargassum sp. have effective analgesic activity as a pain reliever based on the content of flavonoids they contain. This is supported in research conducted by Thunrasana et al. who tested the analgesic effects of algae extracts from brown type L. variegata. This study found that the content of flavonoids in algae can reduce pain by reducing prostaglandins [7]. Another study by Simpi et al. found that Sargassum ilicifolium is able to relieve pain by producing acetic acid which allows seaweed to produce its analgesic activity both peripherally and centrally [5].

In the results of this study, it was found that the flavonoid content Padina sp. extracts are higher than in the Sargassum sp. extracts and they can serve as an analgesic. Flavonoids are efficacious as analgesics whose mechanism of action inhibits cyclooxygenase enzyme action [13]. This is supported in research by Asmawati et al. who found that brown algae Padina sp. and Sargassum sp. contain flavonoids with good anti-inflammatory biological activity [14].

Brown algae is a source of bioactive secondary metabolites rich in steroids, flavonoids, glycosides, alkaloids, and insecticides. These active metabolites that have large drug values, and therefore, this herb plant and its products can be used to cure various diseases because it has no side effects compared to pharmaceutical drugs [15]. The findings in this study reinforce claims in the health and medicine industry that seaweed can be used as a solution to various symptoms related to inflammation.

### CONCLUSION

Both Padina sp. and Sargassum sp. have a total flavonoid content that can act as an analgesic drug. Padina sp. is suspected to have more effective analgesic activity than Sargassum sp. in terms of total flavonoid concentration. Since levels of flavonoids measured in this study are total flavonoid levels, further research on the composition of other active substances in Sargassum sp. and Padina sp. which can act as analgesic drugs needs to be done.

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**Table 1: The total flavonoid content of Sargassum sp. using AlCl₃ reagent**

| Concentration | Replication | Absorbance | Total flavonoid level (%) | Average±SD |
|---------------|-------------|------------|---------------------------|-------------|
| 150 ppm       | I           | 0.148      | 1.114                     | 1.237±0.158 |
|               | II          | 0.156      |                           |             |
|               | III         | 0.184      |                           |             |
| 300 ppm       | I           | 0.328      | 1.318                     | 1.492±0.156 |
|               | II          | 0.380      | 1.538                     |             |
|               | III         | 0.399      | 1.620                     |             |
| 450 ppm       | I           | 0.531      | 1.453                     | 1.553±0.087 |
|               | II          | 0.588      | 1.613                     |             |
|               | III         | 0.580      | 1.592                     |             |

SD: Standard deviation

**Table 2: The total flavonoid content of Padina sp. using AlCl₃ reagent**

| Concentration | Replication | Absorbance | Total flavonoid level (%) | Average±SD |
|---------------|-------------|------------|---------------------------|-------------|
| 150 ppm       | I           | 0.273      | 2.167                     | 2.318±0.135 |
|               | II          | 0.303      | 2.429                     |             |
|               | III         | 0.295      | 2.357                     |             |
| 300 ppm       | I           | 0.588      | 2.420                     | 2.376±0.092 |
|               | II          | 0.553      | 2.271                     |             |
| flavonoid     | III         | 0.592      | 2.439                     |             |
| 450 ppm       | I           | 0.876      | 2.429                     | 2.375±0.091 |
|               | II          | 0.820      | 2.270                     |             |
|               | III         | 0.875      | 2.427                     |             |

SD: Standard deviation
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AUTHORS’ CONTRIBUTION

This work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. M. Ruslin, Fuad Husain Akbar, and A. St. Hajrah Yusuf collected the data, analyzed the data, and wrote the introduction, discussion, and the material and method part. Subehan helped in all the laboratory work, performed, and designed the study.

CONFLICTS OF INTEREST

The authors declare no conflict of interests of this study.

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