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

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ABSTRACT

Objective: Brown algae is one of the most interesting phyla that consist of pharmacologically active compounds and have been widely studied in recent years. One of the active compounds contained in brown algae that are known to have an analgesic effect is flavonoids. The aim of this study is to analyze the total amount of flavonoid compound in *Sargassum* sp. and *Padina* sp. as an analgesic drug.

Methods: Samples used in this study were brown algae *Sargassum* sp. and *Padina* sp. taken from the Punaga Ocean, Takalar, South Sulawesi. The samples were extracted using the maceration method. After the extraction procedure, the total flavonoid levels were measured using a quercetin standard curve at maximum frequency.

Results: Based on the measurement results, the total flavonoid levels in Sargassum sp. are 1.428%±sSD 0.168%.

Conclusion: Padina sp. has higher total flavonoid levels compared to Sargassum sp. and can potentially act as an analgesic drug.

Keywords: Analgesics, Brown algae, Flavonoids.

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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, antitumor, cytotoxic antioxidants, anthelmintic, anticoagulant, antibacterial, antifungal, 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 antipyretic, 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±0.156%, and in the 450 ppm sample is 1.553±0.087%.

Table 2 shows the total flavonoid content from *Padina* sp. samples at three concentrations, each performed in triplicate. The average total flavonoid level in the 150 ppm sample is $2.318 \pm 0.135\%$, in the 300 ppm sample is $2.376 \pm 0.092\%$ and in 450 ppm sample is $2.375 \pm 0.091\%$.

Table 3 shows the total flavonoid content in *Sargassum* sp. samples and *Padina* sp. samples at each concentration measured. Table 3 shows that the flavonoid content of the *Padina* sp. is higher than the *Sargassum* sp. at the concentration 150 ppm, 300 ppm, and 450 ppm. This account means that the flavonoid content in the *Padina* sp. is higher than in the *Sargassum* sp.

DISCUSSION

The levels of flavonoids in herbal samples can be determined by various methods. In this study, a 70% ethanol solvent was used in the algae extraction process because it has the ability to find compounds in a wide range of polarities, it is not as toxic as other organic solvents, and it is effective in finding active compounds [8]. A research by Asnani *et al.* found that the method of extraction and the type of solvent used did not affect the flavonoid content [9]. The authors agree with this because the structure of flavonoids has an equal amount of polar and non-polar parts and does not depend on the type of solvent used.

Based on the results of this study, the content of flavonoids in *Sargassum* sp. extract is 1.428%±SD 0.168% while the content of flavonoids in *Padina* sp. is 2.357%±SD 0.025%. These study are in line with the results of phytochemical tests performed by Margaret and Marie, and both studies found the presence of flavonoid compounds contained in brown algae type *Sargassum* sp. and *Padina* sp. [10,11]. Another study by Nurjanah *et al.* found that *Sargassum* sp. and *Eucheuma cottonii* contain active components that are thought to have potential as a raw material for the manufacturing of drugs and cosmetics. These components are thought to be flavonoids, phenols hydroquinone, and triterpenoids [12].

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 Thennarasan *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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Concentration	Replication	Absorbance	Total flavonoid level (%)	Average±SD
150 ppm	I	0.148	1.114	1.237±0.158
	II	0.156	1.182	
	III	0.184	1.415	
300 ppm	Ι	0.328	1.318	1.492±0.156
	II	0.380	1.538	
	III	0.399	1.620	
450 ppm	Ι	0.531	1.453	1.553±0.087
	II	0.588	1.613	
	III	0.580	1.592	

Table 1: The total flavonoid content of Sargassum sp. using $AlCl_3$ reagent

SD: Standard deviation

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	Ι	0.588	2.420	2.376±0.092
* *	II	0.553	2.271	
	III	0.592	2.439	
450 ppm	Ι	0.876	2.429	2.375±0.091
	II	0.820	2.270	
	III	0.875	2.427	

SD: Standard deviation

Table 3: A comparison of mean total flavonoid content between
samples of Sargassum sp. and Padina sp. at each concentration

Concentration	Flavonoid level (%)		
	Sargassum sp.	Padina sp.	
150 ppm	1.237±0.158	2.318±0.135	
300 ppm	1.492±0.156	2.376±0.092	
450 ppm	1.553±0.087	2.375±0.091	

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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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