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ANTIBACTERIAL ACTIVITY AND PHYTOCHEMICAL OF *Caulerpa sertularoides* AND *Padina australis* FROM NAIN ISLAND, NORTH SULAWESI, INDONESIA

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ABSTRACT

Methanol extract of *Caulerpa sertularoides* and *Padina australis* were screened against Gram positive (*Staphylococcus aureus* ATCC 6538) and Gram negative (*Escherichia coli* TCC 25922) bacteria. The methanol extract were further extracted by multiple phase partitioning method using water, ethyl acetate and hexane and also screened against those bacteria. The hexane fraction of *P.australis* showed high antibacterial activity against *S.aureus* (inhibitory zone diameter > 20 mm). Phytochemistry result showed that ethyl acetate fraction from *C. sertularoides* extract has phenolic, flavonoid, steroid, triterpenoid, saponin and tanin. Overall, it can be concluded that the methanol and its fractions extract from both types of marine algae have antibacterial potency against *S. aureus* and *E.coli*.

Keywords: *Caulerpa sertularoides, Padina australis,* Antibacterial, Phytochemicals

INTRODUCTION

Nain Island is protected by barrier reefs which restrains streams from the sea. This water movement in this area facilitates marine algae to grow properly due to even distribution of the the nutrients carried by the water current (**Mudeng , 2007**). *Caulerpa sertularoides* and *Padina australis* are two types of marine algae that thrive in this area. Algae contain secondary metabolites (phytochemicals) utilized in the medical, cosmetics as well as in other industries (**Subtijah, 2002**). Previous study reported that marine algae have antimicrobial activity, including those from Morocco waters, namely *Ulva rigida*, *Ulva lactuca, U olivascens*, *Enteromorpha compressa*, *E.linza*, *E. intestinalis*, *Chaetomorpha linum*, *Caulerpa prolifera*, *Codium dichotomum*. They were reported to have antibacterial activities against *E. coli* ATCC 25922 *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212 (**Zbakh *et al*., 2012**). The aqueous extract of *Caulerpa active rasemosa* has been shown to inhibit *Pseudomonas pavanaceae* and *Pseudomonas syntata* while its methanol extract showed activity against *Pseudomonas denitrificans* and *Pseudomonas syntata* (Izzati, 2007). Previous study demonstrated green marine algae (*Caulerpa sertularoides)* and brown marine algae (*Padina australis)* isolated from Nain Island, North Sulawesi to have antioxidant activity **(Dotulong *et al.*, 2013)**. The purpose of this study was to explore the antibacterial potency of marine algae *Caulerpa sertularoides* and *Padina Australia* from Nain Island, North Sulawesi, Indonesia.

MATERIAL AND METHODS

Materials

Raw materials, *Caulerpa sertularoides* and *Padina australis* algae, were collected from Nain Island, North Minahasa, North Sulawesi, Indonesia. The bacterial strains used in this study were *Staphylococcus aureus* ATCC 6538, and *Esherichia coli* ATCC 25922 was a Licroprep RP-18 (Merck), hexane, ethyl acetate (Merck Co. Ltd) and methanol (Sigma Aldrich Co. Ltd), Muller Hinton Agar from (Merck Co. Ltd), Broth Agar from (Merck Co. Ltd). Eyela brand ovens, NDO-410, for sample drying, Buchi rotary evaporators for evaporation of solvents, autoclave machines HVE-50 Hirayama and Incemator Memmert.

Making Seaweed Flour

The sample was dried in the room for 3 days and in an oven at 40 °C until the weight was reduced 10 times. The dried samples were powdered by blender and further sifted.

Methanol Extraction

Samples were extracted with methanol as described by **Dotulong (2014)**. 200 g samples was macerated with 2 L of technical methanol for 48 h, maserat was separated from the pulp by filtering with whatman filter paper No. 1, pulp was macerated again in the same way as previously described twice, so that ± 6 L of maserat is obtained. Maserat was evaporated with a vaccum rotary evaporator at a temperature of 40 ºC.

Fractionation of methanol extract with hexane, ethyl acetate and water (Harborne, 2006 and Tamat *et al*., 2007).

Methanol extract was partitioned with 200 mL of n-hexane-water mixture (1:1), n-hexane fraction separated and collected in an evaporative flask. The same treatment was repeated 3 times, the n-hexane fraction was collected and evaporated in the rotary evaporator vacuum temperature of 40 ºC until dry. The water fraction was partitioned with ethylacetate three times each of 200 mL, ethyl acetate fraction was separated from the water fraction, then all ethyl acetate fractions were collected and then evaporated in the rotary evaporator vacuum at low temperature and pressure as above until dry. The remaining filtrate is the water fraction, then evaporated in a rotary evaporator vacum at a low temperature and pressure as above until a dry extract (water fraction) is obtained. The final results of this extraction process are each n-hexane fraction (non polar fraction), etil fraction lasetate (semi polar fraction) and and water fraction (polar fraction).

Calculation of Rendement

The yield was calculated by the following formula: $\frac{w_2}{w_1} \times 100\%$ (1)

w_1: initial weight of fresh sample
w_2: final weight of extract/fraction

Phytochemical Analysis

Phytochemical analysis were conducted according to **Tamat *et al.* (2007)**. Analysis was carried out qualitatively on methanol extract and hexane, ethylacetate and water fractions from marine algae *C. sertularoides* dan *Padina australis*. The secondary metabolites analyzed were phenolic, flavonoids, tannins, triterpenoids, saponins and steroids and alkaloids which are responsible for antibacterial activity.

Antibacterial Testing (Stephen, 2005).

A sterile cotton sticks was used to inoculate a microbial culture to the agar evenly. Then filter paper disc (about 6 mm in diameter) containing 50 µL of test

compound at a desired concentration, are paced on the agar surface. The petri dishes are incubated at 37 ℃ for a day. The final step is to make observations by measuring the diameter of inhibition zone.

RESULTS AND DISCUSSION

Yield

Table 1 showed the yield data of seaweed extracts and fractions. This data showed that the yield of *C. sertularoides* methanol extract was 0.52% and 1.015% in *P. Australis* methanol extract. The partitioned fractions of methanol extract showed that the water fraction had the highest yield of. 0.255% in *C.sertularoides* and 0.96% in *P.australis*. This data indicated that more polar compounds are contained in this sample of marine algae. This result is supported by the report of **Santoso *et al*., (2004)** on green marine algae (*Ulva reticulata).* In their work, water extracts had a higher yield (7.24%) in comparison to ethyl acetate extract (2.20%) and chloroform extract (0.20%). They also reported in the same study that green marine algae *Caulerpa lentilifera* methanol (polar) extract had a higher yield compared to ethylacetate (semi polar) and hexane (non polar) extracts.

Table 1 Rendement of *C.sertularoides* and *P.australis* Extract and Fraction

Samples	Average Rendement (%)	
	C.sertularoides	***P.australis***
Methanol Extract	0,520	1,015
Hexane Fraction	0,035	0,020
Ethyl Acetate Fraction	0.235	0,025
Water Fraction	0,255	0,965

Antibacterial Activity

Antibacterial activity of methanol extract, hexane, ethylacetate and water fractions from marine algae *C. sertularoides* and *P. australis* can be seen in Table 2.

Table 2 *C. sertularoides* and *P.australis* Antibacterial Activity

No	Samples	Concentration	*C. sertularoides*		*P.australis*	
			E. coli	***S. aureus***	***E. coli***	***S. aureus***
1	Methanol extract	10	7.5	9.5	7,0	10,0
		20	8.5	10,0	9,0	14,0
		30	9,0	11.5	10.5	17,0
		40	10,0	11,5	12,0	18,0
		Control	0	0	0	0
2	Fraction hexane	10	10.5	10,0	7,0	23,0
		20	12,0	12.5	7.5	24.5
		30	12.5	14,0	7.5	30,0
		40	13,0	14,5	9,0	27.5
		Control	0	0	0	0
3	Ethyl Acetate Fraction	10	8,0	6,0	9.5	7,0
		20	10,0	13.5	10,0	9,0
		30	14,0	13,0	10.5	11,0
		40	14,0	16,0	11.5	10,0
		Control	0	0	0	0
4	Water Fraction	10	8.5	7,0,	9,0	9.5
		20	9,0	8,0	8,0	9.5
		30	10,0	9,0	8.5	10,0
		40	11,0	9,0	10,0	8.5
		Control	0	0	0	0

The data in Tables 2 show that the antibacterial activity increased as we increase the sample concentration. Our study revealed that the higher antibacterial activity was indicated by the increased in the diameter of inhibitory zone. Our study is in line with previous study by **Pelczar and Chan (2005).** They reported that at the concentration of brown algae extract *Sargassum* sp 20; 30; 40; 50; 60; 70; 80; 90 and 100 % had antibacterial activity (inhibitory zone) of 0.8; 1,2; 4; 8; 9; 13; 15.7 and 18.6 mm. The results of our study also showed that *S. aureus* were more sensitive to *C.sertularoides* extract with methanol, *C.sertularoides* hexane fraction and *C.sertularoides* ethylacetate fraction and *P.australis* hexane fraction compared to *E. coli.* On the other hand *E. coli* were more sensitive to *C.sertularoides* water fraction, *P australis* water fraction and *P.australis* ethylacetate fraction compared to *S.aureus* (the difference in the sensitivity of bacteria to a bioactive compound is caused by differences in the structure of the bacterial cell wall. Based on differences in the composition and structure of the cell wall, bacteria are divided into Gram positive bacteria and Gram negative bacteria. It was concluded that this difference in cell wall composition can cause differences in bacterial sensitivity to certain compounds (**Bachtiar *et al*., 2012**). The difference in antibacterial activity of a sample against test bacteria is also due to other factors, namely habitat, sampling time, seaweed growth stage, extraction method, extraction solvent and others (**Adaikalaraj *et al.*, 2012**).

The results also showed that *P. australis* hexane fraction had a strong antibacterial activity against *S. aureus* bacteria with a diameter of inhibitory zone greater than 20 mm at a sample concentration of 10 to 40% (Figure 1). A sample is classified as having strong antibacterial activity if it has a zone of inhibition greater than 20 mm (**Lorian, 1980**). The results of this study also indicated that the potential antibacterial compounds against *S. aureus* from *P.australis* marine algae are non-polar compound since they are found in the n-hexane fraction. This data was supported by the results of previous study by Dotulong et al. (2016) They reported that *Laurencia tronoi*, at sample concentration of 2% of n-hexane fraction had a 9.55 mm inhibitory zone diameter against *S.aureus*

The phytochemical analysis shows that *C.sertularoides* ethyl acetate fraction contains almost all phytochemical components, while hexane fractions of *P. australis* contains triterpenoids and saponins. Both components (triterpenoids and saponins) found in the *P. australi*s hexane fraction have the potential as antibacterials. Triterpenoid compounds in plants function as protectors to resist insects and microbial attacks (**Riyanto *et al*., 2013**). Saponins are compounds that are easily crystallized through acetylation so that they can be purified and further studied, potentially hard or toxic saponins are often called sapotoxins (**Harborne, 2006**).

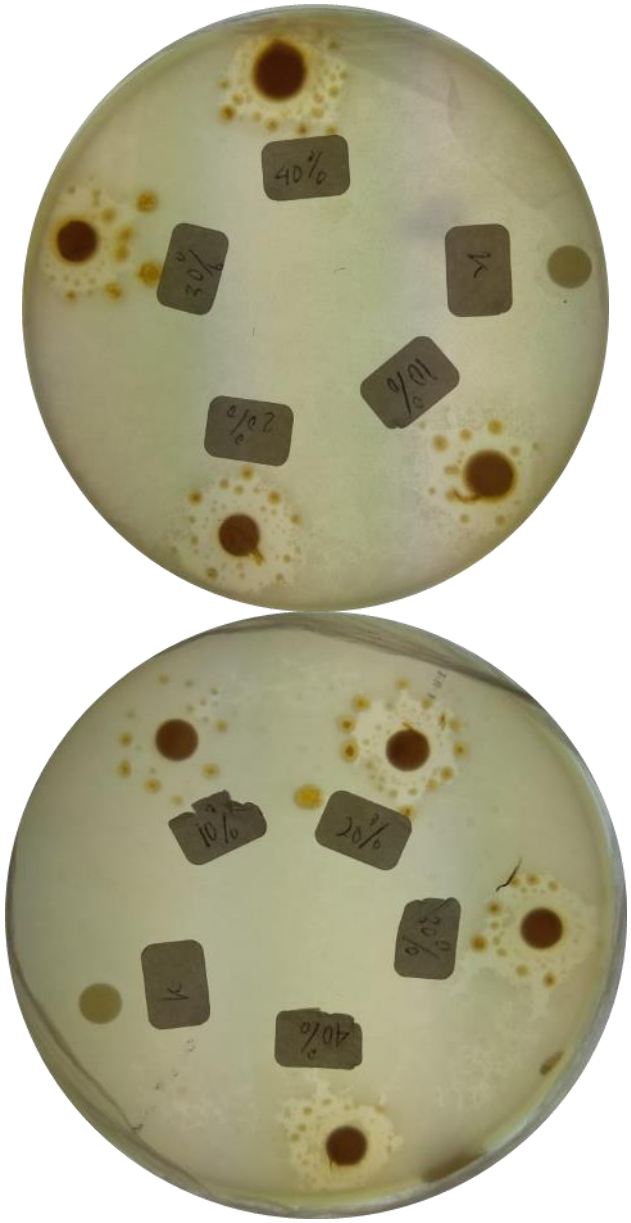

Figure 1 Inhibition zone of n-hexane fraction of *P. australis against S.aureus* at concentration 10, 20, 30 dan 40%

CONCLUSION

S. aureus (gram-positive bacteria) is more sensitive to *C.sertularoides* methanol extract, *C.sertularoides* hexane fraction, *C.sertularoides* ethylacetate fraction and *P.australis* methanol extract compared to *E. coli* (gram-negative bacteria). *E.coli* (gram negative bacteria) is more sensitive to *C.setularoides* water fraction, *P.australis* hexane fraction, *P.australis* ethylacetate fraction, and *P.autralis* water fraction compared to *S.aureus* (gram positive bacteria). Phytochemical analysis results showed that *C.sertularoides* ethylacetate fraction containing almost all phytochemical components, while extracts and other fractions were only a few detected component types. Overall it can be concluded that methanol and fraction-fraction extracts from both types of marine algae have antibacterial activity; the fraction that has the best antibacterial activity is the hexane fraction of *P.australis* against *S.aureus* bacteria because it has a inhibitory zone diameter greater than 20 mm.

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