

# Nutrient Content, Active Compound, and Antibacterial Activity of *Padina australis* against *Aeromonas hydrophilla*

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## ABSTRACT

**Background:** *Padina australis* is one of the brown macroalgae that possess several compounds that can be used for various medicinal properties. **Objective:** This study aims to analyze the nutrient content, active compounds, and antibacterial activity of *Padina australis* against *Aeromonas hydrophilla*. **Methods:** The nutrient content and phytochemical composition of *P. australis* were examined in this study. The antibacterial effect was evaluated using the disc method against *A. hydrophilla*. **Results:** The nutrient content of *P. australis* include 38.5% carbohydrate, 2.07% fat, 13.89% protein, 16.12% water, 33.34% ash, 8.54% total amino acid, and minerals (11.36% calcium, 0.22% iron, and 1.81% potassium). Phytochemical analysis showed that *P. australis* contained phenols, tannin, flavonoid, and steroid. These compound may be responsible for inhibiting *A. hydrophilla*, with an inhibition zone of 10.5 mm for water extract of *P. australis* and 10 mm for methanol extract *P. australis*. **Conclusion:** This study revealed that *P. australis* produced antibacterial effect against *A. hydrophilla* which could be potential for further antimicrobial agent development.

**Key words:** Active compound, Antibacterial activity, *Padina australis*, Nutrient content.

## INTRODUCTION

Macroalgae belong to thallophytes group or plant-like organisms which generally live in coastal areas.<sup>1-3</sup> Macroalgae known as seaweed have significant economic value since they can be utilized as vegetables, traditional medicines, organic fertilizers, and livestock fed.<sup>1,3</sup> Even the phytocoloid compounds extracted from macroalgae as agar, carrageenan, and alginate<sup>3</sup>, it can be used as raw materials of various industries, such as medicine, cosmetics, food, etc. Based on the pigment content, macroalgae are classified as green, red, and brown macroalgae.<sup>4,5</sup> Several studies has been revealed that green, brown and red algae contain different metabolites and have much biological activity such as antiviral, antibacterial and antifungal.<sup>6</sup>

*Padina australis* is one of the brown macroalgae that possess numerous compounds that can be used for various properties, either nutrient content or active compounds. *P. australis* contains 1.05 ± 0.09% protein, 0.58 ± 0.01% fat, 8.78 ± 0.80% carbohydrate, 87.25 ± 0.86% water, 2.34 ± 0.16% ash<sup>7</sup> and minerals as calcium, magnesium, potassium, sodium, copper, zinc, iron<sup>8</sup> that enable to be developed as a food source and livestock fed. *P. australis* also contains various active compounds, such as steroid, terpenoid, flavonoid, tannin, and saponin<sup>2,9,10</sup> that can be used as medicinal drugs.

*P. australis* found abundantly in Indonesia sea and distributed almost all stony coasts including East Nusa Tenggara waters. According to Salosso and Jasmanindar<sup>11</sup>, brown macroalgae recorded in 5 sampling sites of Kupang Bay and distributed in all locations and sampling sites are *P. australis* with up to 80% occurrence frequency. Nevertheless, this

species has not been maximally utilized yet by the community.<sup>10</sup> To optimize the utilization of *P. australis* collected from Kupang Bay, a study on nutrient content, active compounds, and antibacterial activity of *P. australis* were carried out in this paper.

## MATERIALS AND METHODS

### *P. australis* collection

*P. australis* were collected at the lowest tide in Kelapa Lima coastal waters, Kupang Bay, by searching along the coast and taking all encountered *P. australis*. They were put into a plastic bag, cleansed, recorded the fresh weight, air-dried, and then ready for further analyses.

### Chemical composition analysis

Chemical composition analyses of *P. australis* include water, ash, protein, and fat content.<sup>12</sup> Carbohydrate content was determined by difference as follows: 100% - (% water + % fat + % protein + % ash). Amino acid content was determined using a High-Performance Liquid Chromatography (HPLC). Mineral analyses on calcium (Ca), potassium (K), and iron (Fe) were assayed using Atomic Absorption Spectrophotometer (AAS).

### Phytochemical analysis of *P. australis*

Phytochemical investigations of *P. australis* include alkaloid, saponin, flavonoid, tannin, terpenoid, and steroid. Alkaloid was examined using Culvenor-Fitzgerald method, saponin was analyzed using a foam test, tannin was analysed using FeCl<sub>3</sub> and terpenoid and steroid was analyzed using the Lieberman-Burchard method.<sup>13</sup>

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## *P. australis* extraction

Water extraction was done by boiling 10 g of finely ground *P. australis* in 100 ml of distilled water (10%), and incubated for 24 h. The samples have then filtered and stored until further use.<sup>14</sup> Methanol extraction was done by macerating 10 g of finely ground *P. australis* in methanol for 48 h, filtered to separate the sediment and extract, then evaporated, and ready for the antibacterial test.

## Antibacterial activity test

Water and methanol extracts of *P. australis* were examined against *A. hydrophilla*. This examination used disc test method<sup>15</sup> by immersing the sterile disc paper into each extract. After 15–30 min, the disc paper was attached to the TSA media which have been inoculated with *A. hydrophilla*. Measurement of the inhibition zone was carried out after 24 h inoculation at 37°C by observing the presence of clear area formed around the disc paper.

## RESULTS AND DISCUSSION

### Nutrient composition of *P. australis*

Nutrient content of *P. australis* collected from Kelapa Lima coast, Kupang Bay was presented in Figure 1. *P. australis* contained 13.89% protein, lipid 2.66% fat, 38.15% carbohydrate, 11.21% water, and 34.58% ash (Figure 1).

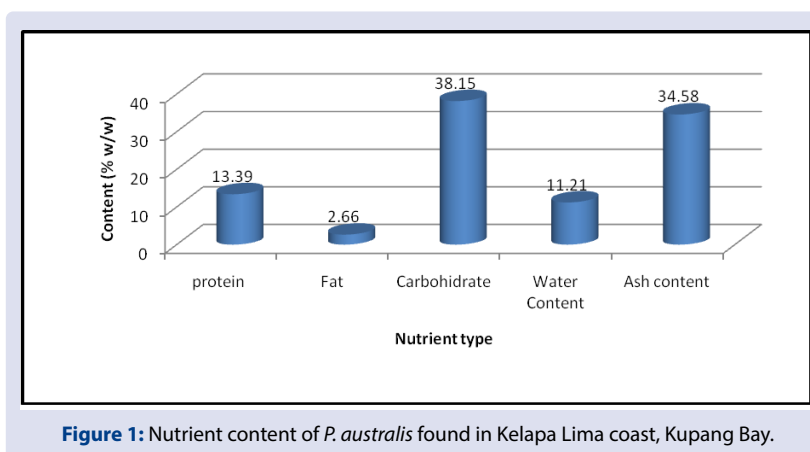


Figure 1: Nutrient content of *P. australis* found in Kelapa Lima coast, Kupang Bay.

Table 1: Amino acid content of *P. australis* found in Kelapa Lima coast, Kupang Bay.

Amino Acids	Content (%w/w)
Aspartic acid	1.16
Glutamic acid	1.32
Serine	0.49
Histidine	0.12
Glycine	0.53
Threonine	0.45
Arginine	0.49
Alanine	0.73
Tyrosine	0.35
Methionine	0.20
Valine	0.62
Phenylalanine	0.55
I-leucine	0.5
Leucine	0.75
Lysine	0.28
Total amino acid	8.54

## The amino acid and mineral content of *P. australis*

*P. australis* containing 15 amino acids in different concentration (Table 1). The highest content were aspartic acid (1.16% w/w) and glutamic acid (1.32 %w/w) and the lowest content were histidine (0.12%w/w) and methionine (0.2 % w/w).

The mineral content of *P. australis* was 10.22% w/w calcium, 1.48% w/w potassium, and 0.125% w/w iron (Figure 2).

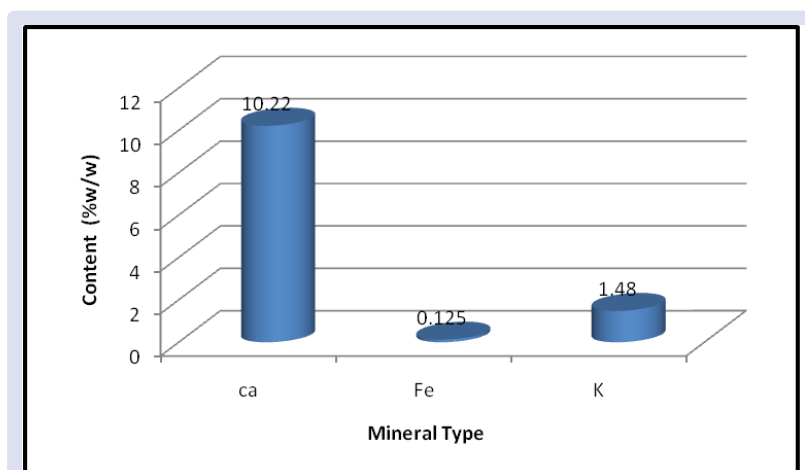
## Active compounds of *P. australis*

Qualitative test of active compounds indicated that *P. australis* contained alkaloid, saponin, flavonoid, tannin steroid, and terpenoid.

## Antibacterial activity of *P. australis*

The antibacterial analysis showed that water and methanol extract of *P. australis* could inhibit the growth of *A. hydrophilla*. It was indicated with the presence of inhibition zones of 10.5 mm for water extract and 10 mm for methanol extract of *P. australis* (Table 3).

This study provided information on the nutrient content of *P. australis*, such as protein, fat, carbohydrate, water, and ash. Protein and fat content of *P. australis* found in Kelapa Lima coast, Kupang Bay, was higher than in Tidung waters, Seribu Islands, only 1.05% protein and 0.58% fat.<sup>7</sup> Compared with other species of macroalgae, *P. minor* found



**Figure 2:** Calcium (Ca), ferrum (Fe), and potassium (K) in *P. australis* found in Kelapa Lima coast, Kupang Bay.

**Table 2:** Active compounds of *P. australis* in Kupang Bay waters.

Compounds	Results	Remarks
Alkaloid	+	White deposit formed
Saponin	+	Foam formation
Flavanoid	+	Yellow color
Tanin	+	Brown deposit formed
Terpenoid	+	Red-yellow color
Steroid	+	Green color formed

Notes: - = Undetected; + = Detected

**Table 3:** Antibacterial test on water extract and methanol extract of *P. australis* against bacteria *A. hydrophilla*

Extract	Inhibition Zone (mm)
Methanol extract	10
Water extract	10.5

in Pahuwato waters, Gorontalo, has only 4.78% protein and 0.52% fat<sup>5</sup>, *P. gymnospora* in India contains only 5.704% protein and 0.02% fat<sup>16</sup>, and *P. tetrastomatica* contains 10.5% protein and 1.14% fat.<sup>17</sup> These differences could result from dissimilarity in harvest age and weather condition at the rearing period.<sup>7</sup>

Furthermore, carbohydrate content in *P. australis* was higher than in Tidung island, Kupang Bay, only 8.78%<sup>7</sup> and in Southeast Coast of India, 14.73%<sup>18</sup>, but lower than carbohydrate content in *P. gymnospora* from Sabah Malaysia, 84.54%<sup>19</sup> and from Tamilnadu, India, 118.14%<sup>16</sup>, and than that in *P. minor* found in Pahuwato waters, Gorontalo, 41.88%.<sup>5</sup> The interspecific difference in carbohydrate content reflects that nutrients of macroalgae could be affected by species and habitat.

The water content of *P. australis* from Kelapa Lima coastal waters, Kupang Bay, was 11.21%, and it is different from that reported by Fitriya<sup>20</sup>, only 6.4%, and Maharany *et al.*<sup>7</sup>, 87.25%. This difference could be influenced by light intensity and temperature at the drying process. The drying method affect the proximate content of *Sargassum polycystum* (brown macroalgae), including water content. Ash content of *P. australis* was high (34.58%), if compared with *P. australis* collected in Tedung Island, Seribu islands, only 2.3%.<sup>7</sup> It is also different from other species, such as *P. minor*, 30.53%<sup>5</sup>, and *P. tetrastomatica*, 27%.<sup>17</sup> Higher ash content was found in *P. gymnospora*, 45.04%.<sup>19</sup> Ash content in a material could be related with number mineral components.<sup>21</sup> The mineral content of macroalga could be affected by the processing method as well.<sup>22</sup> Furthermore, the level of each mineral component is

determined by species, physiological factor, geographic condition, wave frequency, and the method used in mineralization.<sup>23</sup>

*P. australis* collected in Kupang Bay waters contained 15 amino acids with the highest content in aspartic acid, 1.16%, and glutamic acid, 1.32%, and the lowest in histidine, 0.12, and methionine, 0.20%. These results are not different from those in *P. gymnospora* from Tamildanu, India, with the highest in aspartic acid, 12.7%, and glutamic acid, 13.9%, and the lowest in histidine, 2.7% and methionine, 1.5%, despite indifferent amount. However, Shanmuganathan and Devi<sup>16</sup> found that *P. gymnospora*, in India, had different content of amino acid with the highest in glycine (0.605) and tyrosine (0.504) and the lowest in arginine (0.103). Protein content variations in macroalgae could influence the amino acid content.

The highest mineral content of *P. australis* was calcium (10.22% w/w), followed by potassium (1.48% w/w) and the lowest was iron (0.125% w/w). A similar result is also reported by Manteu *et al.*<sup>5</sup>, which the highest mineral content of *P. minor* was calcium (32.91 mg/g), potassium (26.9 mg/g), and the lowest was iron (1.00 mg/g). Also, Shanmuganathan and Devi<sup>16</sup> found that *P. gymnospora* had the highest mineral content in calcium, 156.2 mg 100 g DM<sup>-1</sup>, then potassium, 122.3 mg 100 g DM<sup>-1</sup>, and the lowest in iron, 6.78 mg 100 g DM<sup>-1</sup>. Although the highest mineral content is found in the same mineral for the three *Padina* species from different localities, the concentrations are different. It means that the mineral content of *Padina* sp. is affected by species and habitat.

Phytochemical analysis indicated that *P. australis* contained several compounds such as alkaloid, saponin, flavanoid, tannin, steroid, and terpenoid. The present findings are slightly different from the previous study<sup>19</sup> that found only steroid, terpenoid, polyphenol, and saponin. *P. tetrastromatica* was reported holding alkaloid, terpenoid, steroid, phenol, and flavonoid, but did not contain saponin.<sup>24</sup> *P. australis* contains flavanoid, tannin, and saponin<sup>2</sup>, but Maharany<sup>7</sup> found flavonoid, phenol hydroquinone, triterpenoid, tannin, and saponin in the same species. Even though the bioactive compounds have different content, all the active compounds of *Padina* sp. can be used for pharmaceuticals properties such as inhibit the growth of the pathogenic microorganisms.

The antibacterial activity of *P. australis* has been proved to have the ability to inhibit the growth of *A. hydrophilla* in either methanol extract with an inhibition zone of 10 mm or water extract with the inhibition zone of 10.5 mm (Table 3). The ability of antibacterial activity of *P. australis* against *Vibrio harveyi* in fish was also reported by Gazali and Saputra<sup>2</sup> with an inhibition zone of 12.55 mm at the concentration of 60%. Salosso et al.<sup>9</sup> also showed that the antibacterial activity of *P. australis* against *V. alginoliticus* as well with inhibition zone of 22 mm in acetone extract.

The antibacterial activity of *Padina* spp against pathogenic bacteria in human has also been proved by several previous researchers. Al-Enazi et al.<sup>25</sup> revealed that the antibacterial activity of *P. pavonica* against *Actinobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aururinosus*, *Basilus subtilis*, *Staphylococcus aureus*, *S. epidermis*, and *Streptococcus phygenes*. Haryani et al.<sup>26</sup> also showed the antibacterial activity of *P. australis* against *Vibrio cholera* and *Salmonella thypii*. Other antibacterial studies were also reported by Kemer et al.<sup>3</sup> on *P. australis* from Nain island, North Sulawesi, against *Yersinia enterocolitica* and *Proteus stuarti*, Nuzul et al.<sup>10</sup> on *Padina* sp, from Sorido coast, Biak, against *Staphylococcus aureus* and *Shigella dysenteriae*, and Maheswari et al.<sup>24</sup> on *P. tetrastromatica* from Tamil Nadu, India, against *Salmonella typhi*, *Vibrio cholera*, *Shigella flexneri*, and *Proteus mirabilis*.

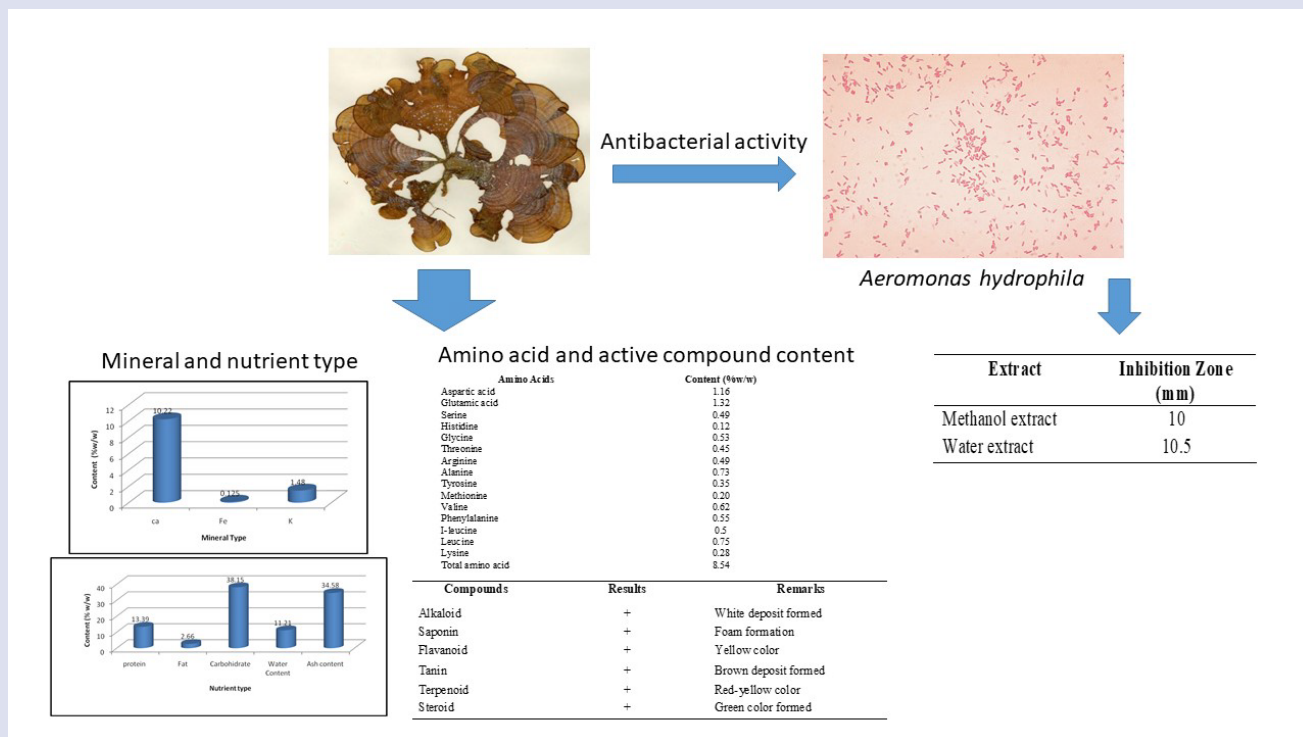
## CONCLUSION

This study revealed that *P. australis* produced antibacterial effect against *A. hydrophilla* which could be potential for further development.

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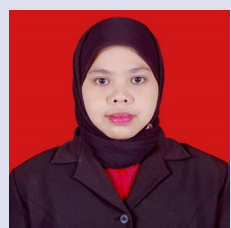
## GRAPHICAL ABSTRACT



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