*Fisheries Journal*, 16 (2), 739-748 (2026)
http://doi.org/10.29303/jp.v16i2.2252

# PHYTOCHEMICAL SCREENING AND ANTIOXIDANT ACTIVITY OF *Padina australis* EXTRACT FROM SUMBERKIMA VILLAGE

Skrining Fitokimia dan Aktivitas Antioksidan Ekstrak *Padina australis* Dari Desa Sumberkima

**Geeta Adsari Barus*, I Nyoman Dodik Prasetia, Made Dwipa Kusuma Maharani, Gressty Sari Br Sitepu**

Department of Marine Biology and Fisheries, Faculty of Mathematics and Natural Sciences, Ganesha University of Education

*Udayana Street, Singaraja, Buleleng Regency, Bali, Indonesia*

*Corresponding Author: geeta@student.undiksha.ac.id

(Received March 11th 2026; Accepted April 25th 2026)

## ABSTRACT

*Padina australis* is known to contain various bioactive compounds. This study examines the potential of Padina australis from Sumberkima Village, which has not been widely explored. The main objective of this research is to conduct phytochemical screening to identify the secondary metabolite profile and determine the level of antioxidant activity through the determination of the $IC_{50}$ value, in order to provide a scientific data foundation for developing the potential of this seaweed, especially in the aquaculture sector. Sample extraction was performed using the maceration method with distilled water as the solvent and heating on a hot plate. The analyzed parameters were phytochemical content, which included quantitative tests for phenols, flavonoids, and tannins, and qualitative tests for saponins, alkaloids, and terpenoids, as well as antioxidant activity using the DPPH method. Data analysis was conducted descriptively to classify the strength of the antioxidant activity and the presence of phytochemical compounds. The quantitative phytochemical test results showed that the P. australis extract contained total phenols of 176.98 mg/100g, flavonoids of 101.49 mg/100g, and tannins of 5.31 mg/100g. Meanwhile, the qualitative tests showed negative results for saponins, alkaloids, and terpenoids. The antioxidant activity test results showed a very high IC50 value of 6213.2823 ppm, indicating that the antioxidant activity level of this extract is classified as very weak or inactive. This low antioxidant activity is suspected to be due to the combination of using distilled water as a solvent and an extraction method that is less effective in optimally extracting potent bioactive compounds. Therefore, further research with solvent variations or the application of modern extraction techniques is recommended.

Keywords: Antioxidant, Distilled water, DPPH, *Padina australis*, Phytochemicals

## ABSTRAK

*Padina australis* diketahui mengandung berbagai senyawa bioaktif. Penelitian ini mengkaji potensi *Padina australis* asal Desa Sumberkima yang belum banyak dieksplorasi. Tujuan utama penelitian adalah melakukan skrining fitokimia untuk mengidentifikasi profil metabolit

sekunder serta mengetahui tingkat aktivitas antioksidan melalui penentuan nilai $IC_{50,}$ guna menyediakan landasan data ilmiah bagi pengembangan potensi rumput laut ini, terutama dalam bidang akuakultur. Ekstraksi sampel menggunakan metode maserasi dengan pelarut akuades dan pemanasan menggunakan *hot plate*. Parameter yang dianalisis adalah kandungan fitokimia yang mencakup uji kuantitatif untuk fenol, flavonoid, dan tanin dan uji kualitatif saponin, alkaloid, dan terpenoid, serta aktivitas antioksidan dengan menggunakan metode DPPH. Analisis data dilakukan secara deskriptif untuk mengklasifikasikan tingkat kekuatan antioksidan dan keberadaan senyawa fitokimia. Hasil uji kuantitatif fitokimia menunjukkan bahwa ekstrak *P. australis* mengandung total fenol sebanyak 176,98 mg/100g, flavonoid 101,49 mg/100g, dan tanin 5,31 mg/100g. Sementara itu, uji kualitatif menunjukkan hasil negatif untuk kandungan saponin, alkaloid, dan terpenoid. Hasil uji aktivitas antioksidan menunjukkan nilai $IC_{50}$ yang sangat tinggi, yaitu 6213,2823 ppm, yang mengindikasikan bahwa tingkat aktivitas antioksidan ekstrak ini tergolong sangat lemah atau tidak aktif. Rendahnya aktivitas antioksidan tersebut diduga akibat kombinasi penggunaan pelarut akuades dan metode ekstraksi yang kurang efektif dalam mengekstraksi senyawa bioaktif poten secara optimal. Oleh karena itu, disarankan penelitian lanjutan dengan variasi pelarut atau penerapan teknik ektraksi modern.

Kata kunci: Antioksidan, Akuades, DPPH, Fitokimia, *Padina australis*,

## INTRODUCTION

Indonesia has abundant marine biodiversity, especially seaweed, which is a strategic asset with high economic potential for the global market (Sofiyanti et al., 2025). Bali Province, especially Sumberkima Village, is considered a prospective location for the development of aquaculture, in line with Prasetia et al. (2024) who revealed that the area has water quality that meets optimal standards for the growth of marine biota. The water temperature conditions in Sumberkima Village were recorded in the range of 28.7–29.2 °C and are still included in the optimal limit and the salinity value ranged from 32.3–32.5 ppt which reflects normal sea water conditions, while the pH value was in the range of 8.7–9.3 and DO was between 0.03 and 0.08, indicating inadequate conditions to support the sustainability of marine biota (Prasetia et al., 2025). According to Mikami et al. (2021), algae possess flexible physiological adaptation capabilities to environmental variations, where favorable conditions optimize primary metabolism for growth and biomass formation, while environmental stress can induce a shift in metabolic pathways toward secondary metabolite synthesis as a cellular protection mechanism. The water quality in Sumberkima Village makes this area a prospective area for further exploration of the potential use of seaweed in sustainable aquaculture development.

Brown seaweeds (Phaeophyceae) are known to possess significant antioxidant activity and diverse secondary metabolite content (Diachanty et al., 2017). One brown seaweed known to be rich in secondary metabolites is Padina autralis. The growth of P. australis is strongly influenced by environmental factors such as substrate, temperature, salinity, and light intensity, which ultimately determine the profile of primary and secondary metabolites produced (Moruk et al., 2024). Previous studies have shown that P. australis extract can enhance nonspecific immune responses in gourami (Purbomarto et al., 2019) and African catfish (Muntasiroh et al., 2020), as well as increase resistance to Aeromonas hydrophila infection in carp (Sheikhzadeh et al., 2022). This potential is supported by its high phytochemical content and antioxidant capacity (Permatasari et al., 2024), as well as antibacterial activity against several pathogenic bacteria (Klomjit et al., 2021).

Although the bioactive potential of P. australis has been extensively studied, its phytochemical profile and antioxidant activity vary significantly due to external factors, including geographic location and habitat conditions (Moruk et al., 2024). Furthermore, the

extraction method and type of solvent used also determine the yield and composition of the resulting compounds (Kasitowati et al., 2021). Currently, there is a significant information gap: there are no scientific reports specifically on the phytochemical profile and antioxidant activity of P. australis from the waters of Sumberkima Village, Bali. This is crucial given that environmental variability in waters can produce unique secondary metabolite profiles (Safia et al., 2020).

Figure 1: *Padina australis*
(Source: Personal documentation)

This research has practical urgency in identifying the potential of *P. australis* from the waters of Sumberkima Village using an extraction method that considers safety aspects in its application. The use of distilled water as a solvent was chosen due to its polar, economical, and non-toxic properties, making the resulting extract safe for direct application as a supplement or immunostimulant in cultivation systems, unlike organic solvents that risk leaving residues. Identification of the biochemical characteristics of this local population provides a fundamental foundation for transforming *P. australis* from wild macroalgae into a source of economically valuable functional raw materials.

The objectives of this research were to describe the phytochemical compounds, including flavonoids, tannins, phenols, saponins, alkaloids, and terpenoids, and to determine the antioxidant activity ($IC_{50}$) of *P. australis* extracts from the waters of Sumberkima Village. The primary contribution of the research results is to provide baseline data on the effectiveness of distilled water in extracting bioactive compounds from *P. australis*, which is expected to serve as a reference for its development and utilization in aquaculture.

## RESEARCH METHODS

The study was conducted from March to August 2025. *P. australis* samples were obtained from a cultivation area in the waters of Sumberkima Village, Gerokgak District, Buleleng Regency, Bali Province. The sample preparation and initial extraction stages were carried out at the Fisheries Biotechnology Laboratory, Ganesha University of Education. The extract concentration process was carried out at the Forensic Laboratory of Udayana University, while phytochemical and antioxidant activity tests were carried out at the Integrated Service Laboratory of the Faculty of Agricultural Technology, Udayana University.

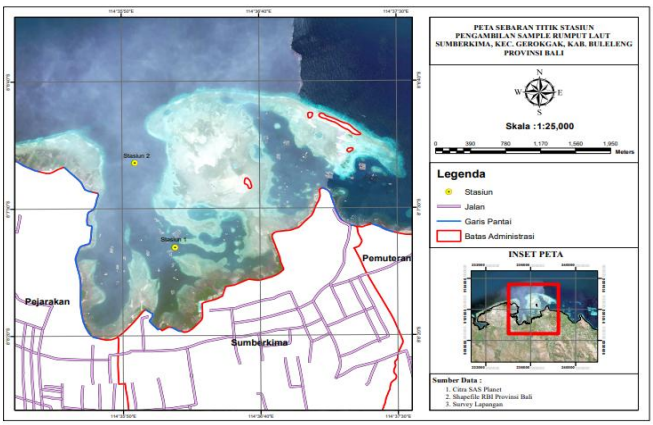


Figure 2. Padina australis Sampling Location
(Source: Personal documentation)

This study aimed to identify the phytochemical profile and evaluate the antioxidant activity of *P. australis* extracts from the waters of Sumberkima Village, Bali, using a descriptive exploratory method. Phytochemical screening was performed quantitatively for total phenol, total flavonoid, and tannin content, as well as the $IC_{50}$ value for antioxidant activity, and qualitatively for the presence of saponins, alkaloids, and terpenoids. Antioxidant activity was tested using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method.

*P. australis* samples were collected by purposive sampling, with the criteria being intact, undamaged, or torn thallus, and having reached mature size. Sample preparation began by washing fresh samples with seawater, then draining them, and air-drying them in a shaded area. The dried crude drug was ground using a blender, sieved to obtain a homogeneous powder, and stored in an airtight container prior to extraction.

The extraction process used a maceration method with distilled water. Fifty grams of P. australis powder was dissolved in 500 ml of distilled water at a ratio of 1:10 w/v for 24 hours. The extract solution was then heated on a hotplate at 80°C for 30 minutes. After returning to room temperature, the solution was filtered through Whatman Grade 4 filter paper. The resulting filtrate was concentrated using a rotary evaporator at 60°C until a thick paste was formed.

Phytochemical screening, including analysis of phenol, flavonoid, and tannin levels, as well as qualitative testing of saponins, alkaloids, terpenoids, and steroids, was conducted in the Laboratory of the Faculty of Agricultural Technology (FTP), Udayana University, by laboratory staff in accordance with the laboratory's Standard Operating Procedures (SOP). Total phenol content was analyzed using the Folin–Ciocalteu spectrophotometry method with gallic acid as a standard, and absorbance measurements were performed at a wavelength of 760 nm. Determination of total flavonoid levels was also carried out spectrophotometrically using quercetin as a standard with an absorbance reading at 415 nm and expressed as mg/100 g QE (Quercetin Equivalent). Meanwhile, tannin levels were measured using a spectrophotometric method with the Folin–Denish reagent, with an absorbance at 725 nm and calculations based on the tannic acid standard. Identification of saponins was carried out through the foam formation method by dissolving the sample in 70% ethanol, then adding distilled water and shaking; the formation of stable foam indicates a positive result. The alkaloid test was carried out by dissolving the sample in 70% ethanol, adding 2N HCl, and heating, where the formation of an orange to brick-red precipitate indicates the presence of alkaloids. Terpenoid and steroid tests used 70% ethanol and chloroform as solvents. The triterpenoid test is carried out by adding acetic anhydride and concentrated $H_2SO_4$ which produces a reddish color as a positive

indicator, while the steroid test is carried out by adding concentrated $H_2SO_4$ slowly through the tube wall so that a red ring forms at the solution interface as a sign of the presence of steroids.

Antioxidant activity was determined using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method to obtain the $IC_{50}$ value. All tests were conducted by laboratory technicians at the Udayana University Agricultural Technology Laboratory. Samples were first extracted using methanol, then homogenized and centrifuged to obtain filtrates. These filtrates were prepared in several concentrations and reacted with DPPH solution. Next, absorbance values were measured using a spectrophotometer at a wavelength of 517 nm. The obtained absorbance data were used to calculate the percentage of free radical inhibition and determine the $IC_{50}$ value, which is the sample concentration capable of inhibiting 50% of free radical activity.

Data obtained from the test results were analyzed descriptively, quantitatively, and qualitatively. Qualitative phytochemical test data (saponins, alkaloids, terpenoids) were interpreted descriptively based on the presence or absence of physical changes (color, sediment, or foam), which were indicated as positive (+) or negative (-). Quantitative data includes the levels of phenols, flavonoids, tannins, and antioxidant capacity determined based on the Inhibitory Concentration 50 ($IC_{50}$) value, which is the concentration of extract capable of inhibiting 50% of free radicals. The $IC_{50}$ value obtained is then classified according to its strength level referring to the antioxidant classification scale according to Fatmawati et al. (2023), namely very strong if it has an IC50 < 50 µg/mL (< 50 ppm), strong in the range of 51–100 µg/mL (51–100 ppm), moderate at 101–250 µg/mL (101–250 ppm), weak at 251–500 µg/mL (251–500 ppm), and very weak if the IC50 value is > 500 µg/mL (> 500 ppm).

## RESULT

### PHYTOCHEMICAL SCREENING RESULTS OF *P. australis*

Phytochemical analysis was conducted using two approaches: quantitative and qualitative tests to detect the presence of secondary metabolite compounds. The quantitative test results (Table 1) indicate that *P. australis* extract contains phenols, flavonoids, and tannins. The results of the phenol, flavonoid, and tannin content tests are shown in the following table:
Table 1: Quantitative Phytochemical Test Results of P. australis Extract

| No. | Compound | Total Content (mg/100g) |
|---|---|---|
| 1 | Phenol | 176,98 |
| 2 | Flavonoid | 101,49 |
| 3 | Tannin | 5,31 |

In contrast, in the qualitative test (Table 2), the *P. australis* extract showed negative results in the saponin, alkaloid, and terpenoid compound tests, where no physical changes were found indicating the presence of these compounds in the extract.

Table 2 Results of Qualitative Phytochemical Tests of P. australis Extract

| No. | Compound | Positive Indicators | Test Results |
|---|---|---|---|
| 1 | Saponins | Formation of stable foam or froth on the surface of the solution. | ( - ) Negative |
| 2 | Alkaloids | Formation of an orange to brick-red precipitate. | ( - ) Negative |
| 3 | Terpenoids | Formation of a reddish color or a red ring on the solution layer. | ( - ) Negative |

## ANTIOXIDANT ACTIVITY TEST RESULTS

Antioxidant activity testing was conducted to determine the extract's ability to scavenge DPPH free radicals. The strength of antioxidant activity was determined based on the $IC_{50}$ (Inhibitory Concentration 50) value, which is the concentration of extract required to inhibit 50% of DPPH free radicals.

Table 3. Results of Antioxidant Activity Test of P. australis Extract

| No. | Sampel | Test Method | IC50 value (ppm) | Activity Categories |
|---|---|---|---|---|
| 1 | *Padina australis* | DPPH | 6213,2823 | >500 (Very weak) |

Based on the data in Table 3, the $IC_{50}$ value obtained was 6213.2823 ppm. This value far exceeds the 500 ppm limit, so the extract's antioxidant activity is categorized as very weak or inactive.

# DISCUSSION

## PHYTOCHEMICAL CONTENT ANALYSIS OF *P. australis*

Based on the results of phytochemical analysis, *Padina australis* extract obtained from the waters of Sumberkima Village was proven to contain several main bioactive compound groups, namely phenols, flavonoids, and tannins. Among the three, phenolic compounds showed the highest abundance level with a value of 176.98 mg/100g, followed by flavonoids at 101.49 mg/100g, while tannins were detected in a much lower concentration, namely 5.31 mg/100g. Meanwhile, qualitative testing did not show the presence of saponins, alkaloids, or terpenoids in the tested extract. This is indicated by the absence of foam or stable bubbles (saponin negative), the absence of orange to brick-red deposits (alkaloid negative), and the absence of reddish color or red rings in the solution layer (terpenoid negative). This content pattern indicates that the *P. australis* extract is dominated by polar secondary metabolites that have the potential to contribute to biological activity, especially as antioxidant agents. These findings are consistent with previous reports on the phytochemical profile of *P. australis* from several waters in Indonesia. A study by Nurrahman et al. (2020) reported the presence of alkaloids, flavonoids, steroids, terpenoids, saponins, and tannins in *P. australis* from the Poteran Islands, Madura, while Djafar et al. (2024) specifically revealed the presence of alkaloids, flavonoids, steroids, saponins, and tannins in the same species from North Sulawesi waters. Furthermore, Hudaifah et al. (2020) also confirmed the presence of various bioactive components, including alkaloids, triterpenoids, tannins, and saponins, in *P. australis* from Sumber Kencono village, Banyuwangi Regency, East Java, further strengthening the view that this brown algae is a potential source of secondary metabolites.

The high levels of phenols detected in this study are of significant relevance, given that phenolic compounds in brown algae are known to act as reducing agents and free radical scavengers. Furthermore, the presence of flavonoids in *P. australis* extract also suggests broad biological implications. These compounds not only play a role in free radical scavenging mechanisms but are also reported to possess other significant biological activities. A study by Saputra et al. (2024) reported that ethanol extract of *P. australis* exhibited cytotoxic potential against HeLa cancer cells. Furthermore, a study by Julyasih et al. (2020) confirmed that phenolic and flavonoid compounds in red and green algae inhibited the growth of *Escherichia coli*, indicating that flavonoids play an important role in antibacterial mechanisms. This finding aligns with Julyasih & Purnawati (2023), who confirmed that several types of green algae contain phenolic compounds and their derivatives (flavonoids) that have the potential to inhibit the growth of *Escherichia coli*.

Tannins were only detected in limited quantities, but their presence remains biologically significant. Tannins are known to interact with proteins and dissolve in microbial walls, thus contributing to antibacterial activity. This is consistent with Widjaya et al. (2021), who reported positive inhibitory activity of *P. australis* extract containing several of these compounds against *Propionibacterium acnes* bacteria. Thus, even at relatively low levels, tannins still have the potential to support the extract's overall bioactivity.

The undetected saponins, alkaloids, and terpenoids in this study cannot be interpreted as an absolute absence of these compounds in *P. australis* from Sumberkima Village. The secondary metabolite profile of algae is highly dynamic and influenced by complex interactions between internal factors and aquatic environmental factors. Variations in growth stages, physiological conditions, and environmental parameters such as light intensity, temperature, salinity, and nutrient availability are known to influence secondary metabolite biosynthesis (Lopes et al., 2024). Furthermore, the choice of aquadest as a solvent and the application of the maceration method with heating in this study are suspected to limit compound extraction. The choice of aquadest as a solvent is based on its highly polar nature and its role as a universal solvent for extracting hydrophilic compounds (Abubakar & Haque, 2020). Secondary metabolite compounds such as flavonoids, phenols, saponins, and tannins, which are polar in nature, tend to be more soluble in polar solvents, while alkaloids are semi-polar and therefore soluble in both polar and nonpolar solvents, and terpenoids are generally nonpolar and therefore more suitable for extraction with nonpolar solvents (Muawanah et al., 2023). Therefore, the use of the maceration method with pure water is suspected to be less than optimal in extracting all of these secondary metabolite compounds. Therefore, further research with a more specific extraction approach is needed to evaluate the potential presence of other bioactive compound groups in *P. australis* from Sumberkima Village.

## ANALYSIS OF ANTIOXIDANT ACTIVITY LEVEL OF Padina australis EXTRACT

The antioxidant activity of Padina australis extract was tested using the DPPH method, with the $IC_{50}$ parameter as an indicator of the extract's ability to quench free radicals. This method is widely used for initial screening of antioxidant activity due to its simple and efficient procedure (Martiningsih et al., 2016). The test results showed an $IC_{50}$ value of 6213.2823 ppm, based on the classification of Fatmawati et al. (2023), an $IC_{50}$ value above 500 ppm is categorized as very weak, so the tested extract has very limited ability to ward off DPPH free radicals. The low antioxidant activity is correlated with the phytochemical profile of the extract. Although phenolic compounds (176.98 mg/100g), flavonoids (101.49 mg/100g), and tannins (5.31 mg/100g) were detected, antioxidant effectiveness is determined not only by the presence of these compounds, but also by their concentration, chemical structure, and synergistic interactions between metabolites (Loho et al., 2021). Phenolic and flavonoid compounds are potent natural antioxidants (Junopia et al., 2020), but the extracted flavonoids are thought to be in the glycosidic form, which has lower antioxidant activity, and the tannin content is very minimal, thus limiting their contribution to total antioxidant capacity (Moniung et al., 2022). Furthermore, the undetected alkaloids, saponins, and terpenoids, also known to possess antioxidant activity, contributed to the low extract potency.

The discrepancy between the results of this study and several reports reporting high antioxidant activity in *P. australis* is thought to be primarily due to differences in extraction methods. The antioxidant activity of an extract is significantly influenced by the type of solvent and extraction technique used (Muderawan et al., 2022). A study by Martiningsih et al. (2021) showed that variations in extraction methods can produce significant differences in phytochemical content and antioxidant activity. Therefore, the use of distilled water in this study is suspected to be the main limiting factor, requiring further research with optimized solvents and extraction methods to increase the antioxidant activity of *P. australis* extract.

## CONCLUSION

This study shows that *Padina australis* extract from the waters of Sumberkima Village contains the main phytochemical compounds phenol 176.98 mg/100g, flavonoid 101.49 mg/100g, tannin 5.31 mg/100g, while saponins, alkaloids, and terpenoids were not detected using the extraction method used.

Based on antioxidant activity testing using the DPPH method, the *P. australis* extract showed a very high $IC_{50}$ value (6213.2823 ppm), thus categorizing it as having very weak antioxidant activity. This indicates that the presence of secondary metabolites, which act as the primary antioxidants in the extract, is not yet able to provide effective free radical scavenging capacity, either due to limited concentration, the chemical structure of the compounds, or suboptimal extraction of other potential antioxidant compounds.

## ACKNOWLEDGMENTS

This research was supported by the RIIM LPDP and BRIN Grant, grant number 64/IV/KS/05/2023, entitled "Minawisata Development Model Based on Sustainable Fisheries Cultivation in Sumberkima Village, Buleleng, Bali." We thank Ganesha University of Education, Singaraja, the Buleleng Reef Restoration Foundation, and the Sumberkima Bay Community Empowerment Group (PKMASWAS) for their support and collaboration in this program.

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