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PHYTOCHEMICAL SCREENING AND TOXICITY OF BROWN MACROALGAE *PADINA AUSTRALIS* OF PASIAGAN, BONGAO, TAWI-TAWI

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ABSTRACT

Phytochemical screening of various marine species is essential for uncovering and recognizing varied potentials in pharmacognosy that may yield significant health benefits. The toxicity characteristics of the species will enhance subsequent investigations into diverse medicinal applications. This study aimed to determine the bioactive compounds in the brown macroalgae *Padina australis* from Pasiagan, Bongao, Tawi-Tawi and assess their toxicity. The results showed that *Padina australis* had a variety of active secondary metabolites, such as alkaloids, flavonoids, saponins, phenols, tannins, triterpenoids, and steroids, whereas, cardiac glycosides were not present. The toxicity test indicated a lethal concentration 50 (LC_{50}) value of 1288.25 µg/ml, categorizing it as non-toxic. These results suggest that *Padina australis* from Pasiagan, Bongao, Tawi-Tawi, with its notable bioactive potential, could be beneficial for future pharmaceutical applications and may also have potential as food sustenance due to its low toxicity. Further research is recommended to explore the full spectrum of its medicinal and nutritional benefits.

KEYWORDS: *Seaweeds, brown algae, phytochemical, toxicity Philippines.*

INTRODUCTION

The ocean, hosting the largest concentration of species on the planet, emerged as a focal point of scientific inquiry in the mid-20th century. Since that time, a significant number of new substances with pharmacological potential have been documented (Altmann, 2017). The brown alga *Padina australis* Hauck is an algal species that is affiliated to the family Dictyotaceae, order Dictyotales and class Phaeophyceae. Among the different species from the genus *Padina, P. australis* is considered as one of the most widespread species that dwells in the coastal zones and of temperate and tropical marine areas worldwide (Čagalj et al., 2021). The genus Padina includes 75 species in the algae database, with 54 species recognized as taxonomically accepted according to the literature (Guiry and Guiry, 2020). Within this species, 15 can be located in the Philippines (Ang et al., 2013).

Padina australis is characterized by its flat, thin, and soft structure, reaching heights of 15-20 cm. The color exhibits a light brown hue, with areas of whitish brown resulting from minor calcification. The blade's cross section reveals a consistent thickness of 2 cells throughout its entirety. The sorus lacks an indusium covering. The plants thrive on rocks, gravel, or dead coral in mid intertidal to subtidal zones along moderately wave-exposed shorelines (JIRCAS, 2012), akin to the locations where the same species were documented in Pasiagan, Bongao, Tawi-Tawi.

Macroalgae are accessible, safe, and cost-effective, and their bioactive properties, which positively influence human health, have garnered significant interest. Padina serves multiple purposes, including its application as food, fodder, and bio-fertilizer. The brown alga is recognized for its various beneficial properties, including antimicrobial, insecticidal, antioxidant, antibiotic, anti-inflammatory, hypo-allergenic, hepatoprotective, and antidiabetic activities (Ansari et al., 2019).

The phytochemical screening is recognized as an effective method for identifying bioactive compounds in macroalgae (Jati et al., 2019). In the interim, one approach to assess toxic materials involves evaluating the toxicity of Artemia salina leach shrimp larvae, commonly referred to as the Brine Shrimp Lethality Test. This approach is frequently employed for the preliminary evaluation of active compounds found in plant extracts due to its cost-effectiveness, speed, simplicity (requiring no aseptic conditions), and reliability (Pohan et al., 2023). This research seeks to determine the phytochemical constituents and toxicity levels present in the methanolic extract of *Padina australis* of Pasiagan, Bongao, Tawi-Tawi, serving as a foundation for future applications and studies.

MATERIALS AND METHODS

The algae that were used in this study were collected last September, 2024 in the coastal water of Pasiagan which is a barangay in the municipality of Bongao, in the province of Tawi-Tawi. Its population as determined by the 2020 Census was 3,786. This represented 3.26% of the total population of Bongao. Geographically, Pasiagan is located at 5° 1' North, 119° 45' East in the island of Bongao. Elevation at these coordinates is estimated at 6.6 meters or 21.7 feet above the mean sea level. The phytochemical analysis was performed in accordance with the established protocols detailed by Harborne in 1973. The evaluation of the algal extract's toxicity was carried out using the brine shrimp lethality (BSLT) assay, which is a recognized standard for initial toxicity assessment.

RESULTS AND DISCUSSION

Phytochemical Screening

Table 1: Phytochemical screening of *Padina australis.*

Phytochemicals	*Padina austalis*
Alkaloids	+
Flavonoids	+
Phenols	+
Tannins	+
Saponins	+
Triterpenoids	+
Steroids	+
Cardiac Glycosides	-

Where; + present, -absent

Table 1 summarized the phytochemical composition of the brown algae *Padina australis.* The results show the presence of alkaloids, flavonoids, phenols, tannins, saponins, triterpenoids, and steroids and the absence of cardiac glycosides. The results obtained are in accordance with the study of Haryani *et al.* (2014) and Maharany *et al*., (2017), which also identified the presence of secondary metabolite compounds.

Alkaloid: Alkaloid was determined through Wagner test and yielded a favorable result after the addition of 0.5 g of the sample with 2 ml of hydrochloric acid (2N). The formation of a light brown-to-red precipitate indicates the presence of alkaloid. Alkaloids are chemical substances characterized by the presence of basic nitrogen atoms, typically originating from amino acids. The majority of alkaloids are colorless and crystalline (Ghaliaoui *et al.*, 2024). Marine alkaloids originating from marine organisms are described in the literature, presenting different biological and pharmacological activities (Alarif *et al.*, 2019). Therapeutically, alkaloids are particularly well known as anaesthetics, cardio protective and anti-inflammatory agents. Well-known alkaloids used in clinical settings include morphine, strychnine, quinine, ephedrine, and nicotine (Marripati, 2023). Suryati *et al.* (2017) indicated that the biological activity of alkaloid compounds is attributable to the presence of nitrogen-containing alkaline groups. Contact between the base group and the bacteria will elicit a reaction with the amino acid molecules from the cell wall and the bacterial DNA, which constitutes the primary component of the cell nucleus, the hub for regulating all cellular processes.

Flavonoids: Flavonoids were identified by adding two to three drops of sodium hydroxide to a 2 ml extract, which initially displayed a vibrant yellow color. Upon the addition of a few drops of diluted hydrochloric acid, the solution turned colorless, reflecting the reaction seen in *Padina australis*. Diverse flavonoid varieties substantially influence immune system functionality and inflammatory cell activity. The therapeutic effects of flavonoids are predicated on their inhibitory actions regarding prostaglandin in both the central and peripheral nerve systems. Prostaglandin is synthesized from arachidonic acid by cyclooxygenase-1 and -2, and it is crucial in mediating pain and inflammation. Flavonoid chemicals derived from brown algae possess the potential for application as analgesic and anti-inflammatory agents (Tajrin *et al.*, 2020).

Phenols: The presence of phenolics was verified through the ferric chloride test, where 2.5 ml of 5% $FeCl_3$ was added, resulting in the formation of a red-brown precipitate, which signifies the presence of phenols. The phenolics are secondary metabolites defined as aromatic benzene ring compounds possessing one or more hydroxyl groups bonded directly to an aromatic ring, including their functional derivatives (Mekinić, 2019). Moreover, *P. australis* Hauck from brown algae is familiar as an active antibacterial, because of the presence of phenolic compounds and tannins.

Tannins: Tannins, akin to phenolics, were assessed through the ferric chloride test, in which the formation of a blue-black or greenish-black precipitate indicates their presence. Padina sp. also contains other bioactive substances, such as tannins. Tannins are classified into two categories: condensed tannins and hydrolysed tannins (Makatamba et al., 2020). Tannin is an astringent that can precipitate blood proteins such as thrombin. Thrombin converts fibrinogen into a set of fibrin fibres in the wound to stop bleeding (Galang *et al.*, 2015) Research conducted by Tirtawijaya (2015) demonstrates the ability of tannins to induce protein clumping. Tannins that interact with proteins create water-insoluble copolymers, which may lead to the shrinkage of skin pores, hardening of the skin, and impede diffusion as well as light bleeding. Furthermore, *Padina sp*. is regarded as having superior anti-bleeding properties compared to *Sargassum sp*., as indicated by the total tannin concentration in the study conducted by Fauzi *et al.*, (2018).

Saponin: Saponin test was conducted to identify the presence of saponin by agitating a 2.5ml extracted sample with distilled water and monitoring for the development of stable foam during the procedure involving Padina australis. Dikkala et al. (2023) describe saponins as secondary metabolites characterized by their heat-soluble, amphiphilic, glycosidic nature, which are naturally present in various plant parts, including leaves, flowers, and fruits. These compounds are triterpenoids derived from steroidal aglycones that are connected to oligosaccharide moieties, and they have extensive applications in the pharmaceutical industry. Saponins represent a category of secondary metabolites found in marine plants, specifically in seaweeds. Saponins find widespread application in the cosmetics, pharmaceuticals, food, and agricultural sectors, attributed to their diverse biological and physiochemical properties (Feroz, 2018).

Triterpenoids: The triterpenoid tests utilized the Salkowski method, wherein the sample was introduced to chloroform and subsequently filtered. After the filtration process, 3-4 drops of concentrated sulfuric acid were introduced to the filtrate. The mixture was agitated and subsequently allowed to remain undisturbed. The presence of triterpenoids indicated the emergence of a yellow color in the lower layer. Triterpenoids are the most abundant secondary metabolites present in marine organisms, such as marine sponges, sea cucumbers, marine algae and marine-derived fungi. A large number of triterpenoids are known to exhibit cytotoxicity against the variety of tumor cells, as well as anticancer efficacy in preclinical animal models (Li et al., 2013). Triterpenoids and their derivatives possess a wide range of biological effects including hepatoprotective, hypoglycemic, immunomodulatory, anti-inflammatory, antioxidant, and antitumor activities (Zhou *et al.*, 2017).

Sterols: To identify the steroids, the Liberman-Burchard test was employed, involving the filtration of a 2.5 ml sample mixed with 5 ml of glacial acetic acid. After the filtration process, 2 ml of acetic acid was introduced to the filtrate. The formation of a blue-green ring indicates the presence of steroids. Steroids are compounds that are commonly present in a range of plant species, typically existing as sterols (Suryelita et al., 2017). Sterols represent a category of lipids that are commonly found in both macro- and micro-seaweeds. These compounds are triterpenoid derivatives that consist of six isoprene units. According to Gross *et al.*, (2015), sterols are an important group of steroid which are present in high quantities in seaweeds. Many health effects have been stated regarding the vital biological capacity of sterols such as anti-inflammatory, anti-diabetic, antioxidant and anti-cancer. Fucosterol is the most abundant type of steroid in the Phaeophyta division (Payghami *et al.*, 2015).

Cardiac Glycosides: The Keller-Killiani test was conducted to determine cardiac glycosides. One ml of glacial acetic acid, one ml of 5% FeCl3, and one ml of concentrated sulfuric acid were added to 2.5 ml of the sample. The absence of cardiac glycoside was indicated by the lack of green-blue color development in the sample. Despite the adverse prevalence of cardiac glycosides, it is noteworthy that glycosides were found in certain brown algae. In the study of Sudha and Balasundaram (2018) one of the secondary metabolites present in *Padina pavonica* is glycosides. Furthermore, the phytochemical analysis in the study conducted by Thamizharasan (2018), yielded positive results for glycosides. Glycosides are compounds consisting of a sugar molecule attached to a functional group through a glycosidic bond. A variety of functional group molecules, such as flavonoids, phenolics, steroids, thiols, and nitriles, are present as glycosides in food (Zeece, 2020). Conversely, cardiac glycosides are a class of organic compounds that increase the output force of the heart and increase its rate of contractions by acting on the cellular sodium-potassium ATPase pump (Fu *et al*, 2019).

Brine Shrimp Lethality Assay

Table 2: Brine shrimp lethality of *Padina australis.*

Concentration	% Mortality	LC_{50}
500	20	
1000	30	
1500	50	1288.25
2000	70	
2500	80	

Alongside phytochemical screening, toxicity test was performed on the methanol extract. Toxicity tests are carried out to determine the safety level of an extract. Brine Shrimp Lethality Test (BSLT) method utilize *Artemia salina* larvae for toxicity test purposes and is expressed in the Lethal Concentration 50 (LC_{50}) value. The advantages of using these larvae is its high sensitivity to sample, easy cultivation, shorter life cycle (Panjaitan et al., 2023).

Analysis of toxicity of *Padina australis* was done by doing LC_{50}. Moreover, a compound can be toxic if in short time, it can kill 50% of A. salina larvae (Khadijah et al., 2021). The *Padina australis* from Pasiagan, Bongao, Tawi-Tawi methanol extract's Probit Analysis results revealed an LC_{50} value of 1288.25 μg/ml indicating non-toxic. For various uses, *Padina australis* may be safe and sustainable. Similarly, a study by Khadijah et al., (2021) found that *Padina australis* from Puntondo Coast has a toxicity result of 6344.54 ppm, indicating its use as a functional food and its low toxicity due to the absence of saponin. In contrast, Haryani et al. (2019) found that Padina sp. has an LC_{50} value of 177.83 ppm, whereas Panjaitan (2023) reported an LC_{50} value of 204.325 ppm for the methanol extract generated through maceration. Harayani and Panjaitan's toxicity assessments suggest the potential use of *Padina australis* in the development of medical products.

CONCLUSION

The study's findings suggest that the secondary metabolites in *Padina australis* from Pasiagan, Bongao, Tawi-Tawi offer promising opportunities for future pharmaceutical applications. Furthermore, the LC_{50} value of these metabolites suggests that they may have relevance in the field of food sustenance. Moreover, a variety of factors, including the collecting site, influence the toxicity of *Padina australis*, underscoring the need for further research into the secondary metabolites.

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