# Phytochemical profiling of *Eucheuma denticulatum* (N.L. Burman) Collins and Hervey, 1917 and *Padina minor* (Yamada,1925) seaweeds for compounds of potential biomedical and pharmaceutical applications

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**Abstract:** Seaweeds' are known for their economic importance and bioactive compounds with biomedical potentials to address some medical conditions. This study supports the need for an inventory of seaweeds' bioactive constituents and their use through phytochemical profiling of the red seaweed *Eucheuma denticulatum* and brown seaweed *Padina minor* in Cebu, Philippines. The methods of extraction used were ethanolic and aqueous, which are decoction, cold infusion, and soaking. Extracts were subjected to tests for the presence of alkaloids, carbohydrates, proteins, flavonoids, cardiac glycosides, phenols, saponins, steroids, tannins, terpenoids, and sterols. Both decoction and cold infusion methods of *E. denticulatum* aqueous extract (EAE-d and EAE-ci) were positive in most of the bioactive compounds except in sterols, the majority were positive results except steroids and sterols in soaking method (EAE-s), and four compounds (saponins, proteins, sterols, and steroids) were negative in ethanolic extract (EEE). All tested phytoconstituents were present, and the majority appeared very strong in all aqueous extracts of *P.minor* (PAE-d, PAE-ci, and PAE-s), while only sterols were negative in ethanolic extracts (PEE). All the aqueous extracts showed a higher number of phytoconstituents compared to ethanolic extracts. This phytochemical screening study revealed the richness of *E. denticulatum* and *P. minor* in bioactive compounds. Identified compounds have biomedical and pharmacological potential applications but are underexplored on the country's seaweed species. Further screening and specific studies are recommended to test the rest of the genus and brown, green, and red algae.

**Keywords:** red seaweed; brown seaweed; ethanolic extract; aqueous extract; bioactive compounds

## 1. INTRODUCTION

One of the Philippines ' abundant natural resources is marine algae, with a broad spectrum of therapeutic properties. Seaweed-derived bioactive products become a source of healthy attributes as different studies unlock the pharmacological potentials. Some of the seaweeds' biomedical potentials are potent antioxidant, antitumor, immunostimulatory, anti-inflammatory, pulmonary fibrosis anticoagulant or antithrombotic, lipid-lowering, antiviral, antibacterial, anti-protozoan, hyperplasia prevention, gastrointestinal, regenerative, and nano-medicine applications (Patel, 2012), which are credited to their abundance in bioactive compounds such as polysaccharides, polyunsaturated fatty acids, pigments, polyphenols, minerals, and plant growth hormones. In addition, specific phytoconstituents like flavonoids, alkaloids, galactan, fucoidan, alginate, laminarin, lectins, chlorophylls, carotenoids, and proteins make a significant contribution to the world's battle against pathogens and severe medical conditions.

The appropriate choice of extraction method is one of the significant factors to consider in phytochemical screening and to ensure the highest biological activity and maximize phytoconstituents' number. In the same way, the chemical changes during drug preparation are considered highly important in the manufacturing of nutraceuticals (Rasheed *et al.*, 2018). Thus, there are choices of solvents like methanol, ethanol, acetone, n-hexane, ethyl acetate, chloroform, and water, but the optimal solvent is dependent on the kind and parts of the plant to use and compound to determine (Deyab *et al.*, 2016; Jegan *et al.*, 2019; Ponnanikajamideen *et al.,* 2014; Rajeshkumar, 2016).

Several authors claimed that different kinds, locations, extraction methods, and solvents were factors to consider for the differences in terms of phytoconstituents (Abdullah *et al.,* 2018; Arsianti *et al.*, 2018; Machmudah *et al.,* 2017; Nurjana *et al.,* 2017; Prasasty *et al.,* 2019; Putri *et al.,* 2019). Seaweed species studied were *Eucheuma cotonii and* brown seaweeds such as *Dictyota dichotoma, Padina gymnospora, and P. tetrastromatica.* The phytochemical studies from different authors analyzed *E. cotonii* and used various solvents, provided different sets of positive compounds. The phytochemical analysis on *Padina australis* identified phenols, tannin, flavonoids, and steroids (Salosso et al., 2020). *P. tetrastromatica* was found positive on fucoidan and other bioactive compounds but not tannins ( Rajeshkumar, 2017). In the near a thousand recorded species of seaweeds, only less than half are of economic value, and the country is talking only 5% of its significance (Trono, 1999). These confirm the claim that seaweeds are underexplored.

There are limited studies on the phytochemical analysis among seaweed samples in the Philippines, even if we are among the leading countries in seaweed farming and various genus and species. Thus, raising the need to conduct specific studies to develop an inventory of biomedical compounds and maximize biomedical and pharmacological utilization. This study *aimed to carry out phytochemical profiling* of red seaweed *E. denticulatum* and brown seaweed *P. minor* using ethanolic extracts and aqueous extraction methods of decoction, cold infusion and soaking. Identifying phytochemical constituents of seaweed species widen and open up possible solutions to some of the diseases that continuously bring public threat. Thus, the study supports the United Nation's sustainable development goal to promote good health and well-being. Furthermore, considering the maximum potentials of seaweeds is another way to value one of our richest marine resources, which is coupled with proper conservation and sustainable development.

## 2. METHODOLOGY

### *2.1 Seaweed Collection and Preparation*

The samples of red seaweed *E. denticulatum and* brown seaweed *P. minor* were collected from the shallow coast of Olango island with geographical coordinates at 10˚ 21' 37" North, 124˚ 04' 58" East Pangan-an, Lapu-Lapu City, Cebu, the Philippines. Samples were prepared and air-dried for seven days (Deyab, 2016), small parts were preserved and packed for identification. The brown seaweed samples were classified and verified based on their morpho-anatomical characteristics by the herbarium curator in the University of San Carlos as *Padina minor* Yamada 1925, while the red seaweed samples were *Eucheuma denticulatum* (N.L. Burman) Collins and Hervey 1917.

### *2.2 Seaweed Aqueous Extracts Preparation*

Three aqueous-extraction methods have used the decoction, cold infusion, and soaking adapted and modified from Jebasingh *et al.* (2015) and Pise & Sabale (2010). In the decoction method of *E. denticulatum* and *P. minor* aqueous extract (EAE-d and PAE-d), 50g of dried and powdered samples were macerated in 150 mL distilled water at 60° Celsius for 10 minutes, which is the same in cold infusion ((EAE-ci and PAE-ci) except that powdered material was immersed entirely in cold distilled water and allowed to stand for 24 hours. This process is modified from the procedure used by the United Nations Industrial Development Organization *et al.* (2008). Maceration is almost the same in the soaking method (EAE-s and PEA-s), except that fresh samples were used to preserve the seaweeds' active phytochemical constituents. After extraction in all the processes, all collected supernatant was filtered and stored at 4˚C until dosing.

### *2.3 Seaweed Ethanolic Extracts Preparation*

The pure carrageenan ethanolic extracts used the dried and powdered samples, macerated in 95%

ethanol for at least 72 hours, and filtered. A rotary evaporator was used with 100 rpm and 40 ˚C temperature to remove alcohol, collect ethanolic extract (EEE and PEE), and stored it at 4˚C until the phytochemical screening process.

*2.4 Phytochemical Screening Process* (*Procedures used were mainly adapted from Guevara, 2005*). Commercially available chemicals and reagents were used as technical and reagent grade. Therefore, no further purification was done before the experimentation.

Chemical tests were conducted on aqueous, and ethanolic seaweed extracts from decoction, cold infusion, soaking, and ethanolic maceration to detect the presence of different phytoconstituents.

*2.5.1 Alkaloids' Test (Wagner's reagent).* Small amount of extract was treated with 3-5drops of Wagner's reagent [1.27g of iodine and 2g of potassium iodide in 100 mL of water] and observed for a reddish-brown precipitate.

2.5.2 Test for Carbohydrates (Molisch's test). Molisch's reagent were dropped to the 2mL portion of the various extracts, followed by 2mL of concentrated Sulphuric acid (H2SO4) down the side. Allow the mixture stand for two to three minutes and observe for the indication of positive result which is red or dull violet color at the interphase of the two layers.

*2.5.3 Test for Cardiac glycosides (Keller Kelliani's test).* Exactly five mL of each extract was treated with 2mL of glacial acetic acid in a test tube, a drop of ferric chloride solution, and carefully underlaid with 1mL concentrated sulphuric acid. A brown ring at the interface for deoxy sugar characteristic of cardenolides, violet ring while in the acetic acid layer, and the greenish ring may form.

*2.5.4 Test for Flavonoids (Alkaline reagent test).* Two mL of extracts were treated with few drops of 20% sodium hydroxide solution. The intense yellow color, which was colorless on the addition of dilute hydrochloric acid, indicates flavonoids. As an alternative, a portion of an aqueous sodium hydroxide solution was added to the extracts. The blue to violet color has anthocyanins, yellow color for flavones, and yellow to orange for Flavonoids. (Ponnanikajamideen *et al*, 2014).

*2.5.5 Test for Phenols (Ferric chloride test).* A fraction of the extracts were treated with aqueous 5% ferric chloride, and a positive result of bluish-black has phenol.

*2.5.6 Test for Proteins (1% ninhydrin solution in acetone).* Two mL of filtrate was treated with 2-5 drops of ninhydrin solution in a boiling water bath for 1-2 minutes and observed for purple color.

*2.5.7 Test for Saponins (Foam test).* To 2mL of the extract was added 8 mL of water, shaken vigorously for 15 minutes, and observed for the persistent foam to confirm the presence of saponins.

*2.5.8 Test for Sterols (Liebermann-Burchard test).* One ml of extract treated with drops of chloroform, acetic anhydride, concentrated H2SO4 and observed for dark pink or red color.

*2.5.9 Test for Steroids.* In 0.2 mL of extract, 2 mL of acetic acid was added, and the solution was cooled in ice, followed by the addition of concentrated $H_2SO_4$. A violet to blue or bluish-green indicated the presence of a steroidal ring.

*2.5.10 Test for Tannins (Braymer's test).* An amount of 2 mL of extract was treated with 10% alcoholic ferric chloride solution and observed for a blue or greenish color solution.

*2.5.11 Test for Terpenoids (Salkowki's test).* An amount of 1 mL chloroform was added to 2ml of each extract, followed by a few drops of concentrated sulphuric acid. A reddish-brown precipitate produced immediately indicated the presence of terpenoids.

## *2.6. Biosafety/ Biorisk Clearance*

This study ensured a proper procedure for the safety and security of any valuable biological material, handling chemicals and reagents, safety protocols in the laboratory, and proper waste disposal to avoid hazardous effects. As a result, this has an ethical certificate from the Institutional Biorisk Committee of Cebu Technological University with a protocol number of 2020-MA-001.

# 3. RESULTS AND DISCUSSION

In the phytochemical screening using seaweed extracts, EAE-d showed positive results on alkaloids, carbohydrates, phenols, flavonoids, tannins, terpenoids, saponins, proteins, cardiac glycosides, lipids like steroids, and negative on sterols (Table 1). All tests turned positive in PAE-d, including sterols (Table 2). A high concentration of phenolic compounds was found in extracts from the decoction method and high in brown seaweeds (Godlewska *et al.*, 2016; Burtin, 2003). Brewing an infusion or a decoction from the plant parts are still the most widely applied extraction procedures for preparing herbal teas, currently used in traditional medicine practice (Rasheed *et al.*, 2018).

Table 1: Results of Phytochemical screening of seaweeds (*E. denticulatum)* using different extraction methods

| Constituents | *EAE-d* | *EAE - ci* | *EAE-s* | *EEE* |
|---|---|---|---|---|
| Alkaloids | +++ | +++ | ++ | +++ |
| Carbohydrate | ++ | + | +++ | ++ |
| Phenols | + | + | + | + |
| Flavonoids | ++ | ++ | ++ | +++ |
| Tannins | + | + | + | + |
| Terpenoids | + | + | + | + |
| Saponins | ++ | ++ | ++ | - |
| Proteins | +++ | +++ | +++ | - |
| Lipids | | | | |
| Sterols | - | - | - | - |
| Steroids | + | + | - | - |
| Cardiac Glycosides | +++ | + | ++ | +++ |

*+ weak ++ strong +++ very strong*

Like in decoction, EAE-ci almost all were positive on tested phytoconstituents, and sterol was negative while the extract from EAE-s was negative in both steroid and sterols (Table 1). In table 2, all compounds tested positive in PAE-ci and PAE-s. In a study where the impact of extraction methods was compared quantitatively, the cold infusion was optimal in preserving anthocyanins and recovering organic acids (Rasheed *et al.,* 2018). Soaking and decoction methods were used to study plant growth bio-stimulants and were found to have similar macro and microelement concentrations (Godlewska *et al.*, 2016). Water was then believed to be a suitable solvent for the isolation of phenolic compounds, and the methods are environmentally friendly. The EEE has shown the following phytoconstituents: alkaloids, carbohydrates, phenols, flavonoids, tannins, terpenoids, and cardiac glycosides. However, it's negative on the content of protein, saponins, sterols, and steroids.

Table 2: Results of Phytochemical screening of seaweeds (*P. minor)* using different extraction methods

| Constituents | *PAE-d* | *PAE - ci* | *PAE-s* | *PEE* |
|---|---|---|---|---|
| Alkaloids | +++ | +++ | +++ | +++ |
| Carbohydrate | + | ++ | + | +++ |
| Phenols | ++ | +++ | +++ | +++ |
| Flavonoids | +++ | +++ | +++ | +++ |
| Tannins | +++ | +++ | +++ | +++ |
| Terpenoids | +++ | +++ | +++ | ++ |
| Saponins | +++ | ++ | ++ | + |
| Proteins | +++ | ++ | +++ | +++ |
| Lipids | | | | |
| Sterols | ++ | ++ | ++ | - |
| Steroids | + | + | + | + |
| Cardiac Glycosides | ++ | + | + | + |

*+ weak ++ strong +++ very strong*

Phytochemical screening on brown seaweed *P.minor* showed a higher more number of bioactive compounds. Among the eleven tests conducted, only sterols were negative in the PEE, and all were revealed positive in the three aqueous extracts PAE-d, PAE-ci, and PAE-s with phytoconstituents such as alkaloids, carbohydrate, phenols, flavonoids, tannins terpenoids, saponins, proteins, sterols, steroids, and cardiac glycosides. There is a very strong presence of alkaloids in all aqueous and ethanolic extracts of both *E. denticulatum* and *P. minor.* The same very strong compounds from carbohydrate content of EAE-s and PEE; cardiac glycosides of EAE-d and EEE; flavonoids in EEE and all PAE and PEE; protein content of EAE's, PAE-d, PAE-s, and PEE; saponins of PAE-d; tannins and terpenoids of all PAEs.

The phytoconstituents phenolic compounds found in brown algae and some red algae are integral structural components of cell walls that possess good secondary ecological functions like protection from ultraviolet radiation, reproductive role in algal reproduction, and protective mechanism against biotic factors. The major phenolic group of brown algae, phlorotannin, has a vast array of bioactivities such as antioxidant, anti-inflammatory, anticancer, and antidiabetic (Kim & Himaya, 2011). In addition, the cell wall of brown algae is rich in fucoidan and laminarin, while carrageenan is in red algae. The sulfated polysaccharides like fucoidan, laminarin, and carrageenan are beneficial biological activities potential as anticoagulant, antiviral, antioxidative, anticancer, and immunomodulating activities (Wijesekara *et al*., 2011). Thus, Brown and red seaweeds' high content of fucan, fucoidan, and carrageenan possess multiple biological activities (Patel, 2018).

Another important compound that is highly abundant in red and brown seaweeds is an alkaloid, and their sub constituents *carpaine* present in the leaves responsible for the seaweeds anti-thrombocytopenic activity Zunjar *et al.,* 2016). Furthermore, the flavonols and flavonoids have stimulant effects on blood cell production, supporting the seaweeds' potential works as antitumors and immunomodulatory activities (Singh). Brown seaweeds in methanolic and ethanolic solvents have compounds phenols and flavonoids, while tannins, phenols, flavonoids, alkaloids, and coumarin when all seaweeds were tested. Phytochemical constituents of seaweeds revealed different results for the composition dependent on its kind and growth location due to differences in water temperature, salinity, light, nutrients, and other environmental factors.

## 4.CONCLUSIONS

The present phytochemical screening on *E. denticulatum* and *P. minor* revealed its richness in bioactive compounds such as alkaloids, carbohydrates, phenols, flavonoids, tannins, terpenoids, saponins, proteins, steroids, cardiac glycosides. However, sterols were only found in PAE. Seaweed bioactive compounds have biomedical and pharmacological potential use like antioxidant, analgesic, anti-inflammatory, anticoagulant, anti-hypercholesterolemia, lipid-lowering, antiviral, antibacterial, anti-protozoan, hyperplasia prevention, gastrointestinal, regenerative, anticancer, and nano-medicine applications but underexplored on the seaweed species in the country. Therefore, further screening is recommended on the same seaweeds in other areas in the Philippines and the rest of the genus and brown, green, and red algae. This helps in the battle against evolving pathogens and increasing medical concerns in the country and worldwide.

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