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PHYTOCHEMICAL SCREENING AND PROXIMATE COMPOSITION OF THE SEAGRASS*HALODULE PINIFOLIA* OF THE COASTAL WATERS OF CARMEN, AGUSAN DEL NORTE, PHILIPPINES

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ABSTRACT

Phytochemical screening provides basic information about the medicinal importance of the plant extract and are lead compound for drug discoveries and knowledge of the chemical composition of seagrass is important both for the assessment of the nutritional value of marine invertebrate or vertebrate herbivores and for the evaluation of potential sources of protein, carbohydrates, and lipids for commercial use or for possible human consumption. Phytochemical and proximate analyses were determined on the seagrass *Halodule pinifolia* using standard methods on a dry basis. The phytochemicals detected in the methanolic extracts of *Halodule pinifolia* were alkaloids, flavonoids, saponins, phenols, tannins, triterpenoids, and steroids. The results of the proximate composition showed that *Halodule pinifolia* contained 10.06% crude protein, 0.33% crude lipid, 58.30% total carbohydrate contents and 31.31% ash content. The results indicated that the seagrass *Halodule pinifolia* contained different active secondary metabolites along with significant bioactive potential that might be helpful for the future pharmaceutical applications and are good potential sources of highly nutritious feed stuff.

KEYWORDS: Seagrass, Tropical, phytochemical, proximate, Philippines.

INTRODUCTION

Many communities have lived adjacent to seagrass meadows for centuries, recognizing how these meadows provide people with livelihoods, food-security, and medicines. Healthy seagrasses are bioindicators for a healthy community in the marine ecosystem. Overall, there are only seventy-two (72) species of seagrasses in the world(Short, 2011). Seagrass occupies only less than two percent of the world's ocean. Mostof the population has decreased faster in the recent year, and on the brink of extinction because of human activities, commercialization, and industrialization.

Seagrasses are a rich source of structurally novel and biologically active metabolites which they produce in order to sustain the extreme environmental conditions prevailing under sea. These metabolites are not explored to their utmost potential. Many seagrass species are found to have antibacterial, antifungal, antiviral activities. Secondary metabolites extracted from *Enhalus acoroides* exhibit antibacterial, anti-larval, anti feedant activity (Qi *et al.*, 2008). Antibacterial agents exist in *Cymodocea serrulata, Halophila ovalis, Zostera capensis* (Kumar *et al.*, 2008) and *Halodule pinifolia* (Umamaheshwari *et al.*, 2009). *Thalassia testudinum* were shown to inhibit the growth of thraustochytrid (zoosporic fungus) Schizochytrium aggregatum. Some researchers reported increase in phenolic acid content in *Zostera marina* and *Thalassia testudinum* during pathogen infection (Vergeer and Develi, 1997; Steele *et al.*, 2005). Extracts from *Halophila decipiens, Thalasia testudinum, Ruppia maritima and Halodule wrightii* have inhibitory effects on fungal pathogen *Lindra thalassiae*, *Fusarium* sp 2 and 3 (Ross *et al.*, 2007). The extracts of Caribbean seagrass *Thalassia testudinum* inhibits of HIV integrase and thus contains lead potential metabolites against HIV (Rowley *et al.*, 2002; Neelima *et al*. 2015). Seagrasses are also good potential sources of highly nutritious feed stuff.

Halodule pinifolia is a seagrass that can be found elsewhere on the 7640 tropical islands in the Philippines. It is a small seagrass with slender rhizomes, up to 1.5 mm in diameter; internodes usually 0.5-2.0 cm long; simple or laxly branched roots, 2 to several per node, short, erect shoots consisting of 2-4 leaves borne at each node. Leaf blades linear, 4-15 cm long, not more than 1.5 mm wide. Leaf tips obtuse with few irregular serrations. Leaf margins entire; conspicuous midrib furcate at the tip. The basal portions of leaves are enclosed by sheaths, 3 cm long. Ovate scales on each node not persistent at maturity. The general purpose of this study was to characterize the seagrass *Halodule pinifolia* in terms of their secondary metabolites profile and nutritive values.

MATERIALS AND METHODS

The seagrasses that were used in this study were collected last June, 2019 in the coastal waters of Carmen. Officially, the Municipality of Carmen is a town in the province of Agusan Del Norte in Caraga Region (Region XIII) of the Philippines. Geographically, Carmen is located at 9°00′N 125°16′E. According to the Philippine Statistics Authority, the municipality has a land area of 311.02 sq.km. (120.09 sq. mi.) constituting 11.39% of the 2,730.24-square-kilometer- (1,054.15 sq. mi.) total area of Agusan del Norte. Carmen is strategically located in the Western Agusan Corridor. It is bounded on the north by the Butuan Bay, south by Buenavista, east by Nasipit and west by Misamis Oriental. The predominant seagrasses in the area are *Halodule pinifolia*, *Enhalus acoroides* and *Thalassia hemprichii.* The phytochemical screenings in this study were determined using the different standard chemical tests of (Kaur & Arora, 2009). Proximate analysis was done to determine the crude protein, fat, ash, and total carbohydrate contents of the samples that were carried out in triplicates on a dry basis according to standard methods (AOAC, 2005). Carbohydrate content was estimated based on the net difference between the other nutrients and the total percentage composition.

RESULTS AND DISCUSSION

The result of the phytochemical composition of the seagrass *Halodule pinifolia is* summarized in Table 1. The results of the phytochemical screening of *Halodule pinifolia* methanolic extract show the absence of cardiac glycosides and coumarin, while alkaloids, flavonoids, saponins, phenolics, tannins, steroids and triterpenoids were present. The qualitative phytochemical screening of *Halodule pinifolia* was in agreement with the work of Saranya *et al*., (2017).

Seagrass is a rich source of alkaloids substances (Vergeer et al., 1995) secondary metabolites, particularly phenol, fatty acids, and sterols (McMillan, 1983).

The alkaloid derivative ever isolated from a seagrass species was a flavonoidal alkaloid (Subhashini *et al*., 2013). Alkaloids are generated by various living organisms especially, by higher plants where about 10 to 25% of these containalkaloids. The flavoalkanoid that is present in the seagrass are found to contain flavonoid as the basic structure and the element nitrogen-containing constituent, which is an alkaloid, and because of the diverse composition and wide-ranging pharmacological capabilities, it might be an essential venue for discovering new drugs. There are several records that potential therapeutic indications of alkaloids are beneficial for cancer and viral infections, inflammation and immune modulation, neurological and psychiatric conditions, and diabetes.

Table 1: Phytochemical screening of Halodule pinifolia.

Phytochemicals	***Halodule pinifolia***
Alkaloids	+
Tannins	+
Phenols	+
Flavonoids	+
Saponins	+
Steroids	+
Triterpenoids	+
Coumarin	-
Cardiac Glycosides	-

Where; + present, - absent

In general, the seagrass *Halodule pinifolia* is abundant in flavonoids. Cannac *et al.*, (2009) identified flavonoid glycosides and acyl derivative after hydrolysis of the flavonoid aglycone in the seagrass *Posidonia oceanica* leaves. Flavonoids are essential pigments for producing the colors needed to attract pollinating insects. Flavonoids are free radical scavengers that can work against oxidative cell damage and have substantial anticancer activities, and that might instigate mechanisms that destroy cancer cells and prevent tumor invasion. These were due to their ability to neutralize and quench radicals. This is also because of the presence of conjugated ring structures and carboxylic groups which have been proven to retard lipid peroxidation and due to their redox properties.

Seagrasses are rich in tannins. Tannin cells are specialized for the production of phenolic compounds, which play defensive roles against herbivores and microorganisms. Athiperumalsami *et al*., (2008) reported the amount of tannin in the *H. ovalis* and was found to be in the average level of 2.940–9.800 mg/g. Twelve seagrass genera, and 29 species in the Potamogetonaceae have been found to contain tannin cells attached to their leaves. Species of *Posidoniaceae: Posidonia; Cymodoceoideae: Halodule, Syringodium, Cymodocea*, *Thalassodendron*, *Amphibolis* and two genera in the *Hydrocharitaceae (Enhalus acoroides* and *Thalassia hemprichii*) contained condensed tannins with the Rf values and color reactions. While species of Potamogetonaceae *(Zosteroideae: Zostera, Phyllospadix, Heterozostera)* are not denoted by tannin cells in the leaves (McMillan, 1984). Tannins are used in the medication of cancer and inflamed or ulcerated tissues.

Seagrasses are abundant with phenolic compounds. Significantly, phenolic compounds play an essential role in the inhibition of microbial growth, amphipod grazing, and in the resistance to the so-called waste-disease. Yuvaraj *et al.*, (2011) reported a high phenol content (9.44±0.02mg/g) in the seagrass *H. ovalis* collected from the Chunnambar estuary. The amounts of phenol investigated appeared to be more than reported in pulses. Ragupathi *et al*., (2013b) observed that *H. pinifolia* recorded high phenolic (21.64 mg g 1), tannin (17.12 mg g 1), and vitamin E (34.49 mg g 1) content. The presence of polyphenols in most of the seagrasses also helps to preserve the fatty acids. Polyphenols are known for their ability to prevent fatty acids from oxidative decay and provide defensive armor against the oxidative stress of oxidizing agents and free radicals. Along with, the phenolic compound portrays a multitude supplemental functions in a plant's life cycle like structural roles in various supporting or protective tissues, defense mechanisms, as attractants of different organisms for pollinators and seed-dispersing animals, and as allelopathic agents, protection against radiation and signal agent in the interactions between plants and their environment.

Two steroids, p-hydroxy benzoic acid, and 4-4 dihydroxy benzophenone were isolated from the seagrass *Thalassia hemprichii*, collected from the Saudi Red Sea coast (Qi *et al*., 2008). Seagrass *Halodule uninervis* was analyzed for its phytochemistry contents. The samples were collected from the coast of Lampung. The result showed the content of phytochemical compounds of methanol extract seagrass are flavonoids, alkaloids, steroids, and phenols (Baehaki *et al*., 2017). Steroids play critical roles in a number of disorders, including malignancies like prostate cancer, where steroid production inside and outside the tumor promotes cancer cell aggressiveness.

It was reported that the ethanol ethanolic extract of the seagrass *S. isoetifolium* has a variety of phytochemicals such as carbohydrates, flavonoids, alkaloids, tannins, saponins, and glycosides. Aswathi *et al*., (2012b) reported phytochemicals such as cardiac glycosides, saponins, resins, proteins, acidic compounds, reducing sugars, tannins, terpenoids, and alkaloids in the extract of seagrass *C. rotundata* using ethanol and methanol as solvent. Qualitative test of phytochemicals from methanol extracts of seagrass including *Halodule pinifolia* showed positive activity with phytoconstituents such as saponins, resins, proteins, carbohydrates, glycosides, acidic compounds, reducing sugars, cardiac glycosides, phenols and alkaloids. Saponins are believed to react with the cholesterol-rich membranes of cancer cells, Moreover, saponins in medicinal plants are responsible for most biological effects of cell growth and division in humans and have an inhibitory effect on inflammation.

Terpenoids were manifested in numerous studies performed on the isolation and characterization of novel and potent anti-inflammatory compounds like, terpenes and diterpenes (Terencio et al., 1998). Together with eight known biochemical compounds, two types of meroterpenoids have been secluded from the seagrass-derived fungus *Pestalotiopsis sp.* There are also new compounds from the seagrass that were isolated, like the structural class of diarylheptanoids, a new meroterpenoid, and the first briarane diterpene and only the second analog of this kind with a tricyclic skeleton. Briarane diterpene is the first brominated briarane diterpene metabolite. These newfound biochemical compounds were found to have weak to strong activities against multidrug-resistant (MDR) and methicillin-resistant bacterial strains, which could be a new source needed to various MDR strains. Triterpenoids possess rich chemistry and pharmacology (e.g., cholesterol) with several pentacyclic, and new hope for more biochemical compounds with antibiotic activity in seagrasses will be discovered. Different clinical trials of triterpenoids have been tested to determine its effectiveness on humans in preventing diseases like chronic disorders, and the possible mechanisms responsible for the observed therapeutic actions (Gil et al., 2015).

Several studies showed the presence of cardiac glycosides in seagrass. In the phytochemical analysis of seagrass *Cymodocea rotundata* using ethanol and methanolalcohol as solvents, the result showed positive with various biochemical compounds like tannins, saponins, resins, proteins, acidic compounds, reducing sugars, terpenoids, cardiac glycosides and alkaloids. In contrast, phenols, steroids, catechols, and flavonoids showed negative results (Aswathi *et al.*, 2012). Cardiac glycosides are phytochemicals composed of an aglycone that is related to steroid hormones that can be found in different plants and are very toxic. Bufadienolides are more connected with cardio activity. Various cardiac glycoside-containing plants have been recognized as toxic to veterinary animals and humans. The other group aglycones of cardiac glycosides are the cardenolides.

Phytochemical analysis of an aqueous methanolic extract of six species of seagrasses revealed the presence of coumarins, flavonoids, phenols, quinones, saponins, sterol, sugars, and terpenoids (Kannan *et al*., 2013). But in marine ecosystems, most of the coumarins isolated were usually from marine-derived fungus like *Aspergillus similanensi* from the sponge-associated fungus. Also, four new metabolites, including an isocoumarin, have been isolated from the seagrass-derived fungus *Pestalotiopsis* species. Another isocoumarin compound was isolated frommarine-derived fungus associated with seagrass. These show that most coumarins in the marine ecosystem are derived from associated fungus.

Table 2: Proximate analysis of Halodule pinifolia.

Proximate composition	*Halodule pinifolia (%)*
Crude protein Content	10.06 ± 0.28
Crude Lipid Content	0.33 ± 0.11
Total Carbohydrate content	58.30 ± 1.05
Ash content	31.31 ± 0.94

Values are means ± SD for 3 determinations.

The results for the proximate composition analyses are shown in Table 2. The crude protein in this study was determined using the Kjeldahl method on a dried basis. The seagrass *Halodule pinifolia* contained of 10.06% crude protein. The results of this study is in line with the Comparative proximate composition values for different species of the the seagrasses of Aketa & Kawamura, 2001; Dawes & Guiry, 1992; Shams *et al*., 2013; Rengasamy *et al*., 2013; Dawes, 1986; Dawes, 1990; Ndidi *et al*., 2014. The whole plant was taken for the analysis in this research. There were broad variations in the biochemical composition of the different seagrass. The different variations in the biochemical composition of the seagrass were due to the geographical locations, changes in the climate, environmental factors, and many others. The variations in the composition is mostly connected to the profuse effusive systems of the seagrass species. The thick rhizomes seagrass species like *Cymodocea rotundata*, *Cymodocea serrulata*, *Enhalus acoroides* and *Thalassia hemprichii* have higher protein content. The protein content of seagrass also depends on the availability of the nutrients in its surrounding environment. An indication that there is a clear-cut spatial variation in the biochemical content of seagrass species.

The crude lipid (dry basis) of *Halodule pinifolia* was determined using a semi-continuous extraction, particularly the Soxhlet method. Based on the Soxhlet method, as presented in Table 2, it shows very low lipid content with 0.33%. The results were consistent with the study conducted by Pradheedba *et al*., (2010) on the eight species of seagrasses, including *Halodule pinofolia.* They reported that the lipid content of seagrass leaves and rhizomes varied from 0.01 to 3.2% and 0.03 to 4.1%, respectively. Lipid content of all seagrass was generally low (Erik Coria-Monter & Elizabeth Durán-Campos, 2015). Lipids are important in the diet as energy sources ofessential fatty acids and fat-soluble vitamins. They also contribute satiety and palatability to the diet. A low lipid diet is good for the health.

The ash content of the seagrass was determined using a dry ashing method. Table 2 shows *Halodule pinifolia* contained crude lipid of 31.31%. The results of this study is in line with the Comparative proximate composition values for different species of the the seagrasses of Aketa & Kawamura, 2001; Dawes & Guiry, 1992; Shams *et al*., 2013; Rengasamy *et al*., 2013; Dawes, 1986; Dawes, 1990; Ndidi *et al*., 2014. Other seagrasses recorded high ash content values like *Thalassia testudinum* 40% and *Thalassia halodule wrightii* 30%. The high ash content of the seagrasses indicates the presence of a surmountable diverse mineral components.

Total carbohydrate content of the seagrass *Halodule pinifolia* was calculated as the residue by difference from total lipid, protein, and ash values. The results in Table 2 shows the total carbohydrates content of the seagrass *Halodule pinifolia* with 58.30%. Montano *et al*., (1999) reported that high carbohydrate content value for *Enhalus acoroides* 72.4% and low carbohydrate content value of *Thalassia hemprichii* 26.63%. Generally, carbohydrates content values in most seagrass species are high, like *Zostera capricorni* 60.0% and Z*ostera marina* 50.9%. Soluble products of sugar and other soluble carbohydrates include glucose and fructose is the dominant storage carbohydrates in most seagrasses. The results suggest that seagrasses are excellent dietary supplements and sources of nutrients for human consumption. Given these high nutritional levels, seagrasses could also be used as food for farmed animals, and fish.

CONCLUSION

The sseagrass in this study manifested the presence of a wide array of secondary compounds. A wide variation in the secondary metabolite composition of the marine angiosperms is observed within, and between genera. The different active secondary metabolites along with significant bioactive potential might be helpful for the future pharmaceutical applications considering the diverse pharmacological uses of the seagrass in different parts of the world. The chemical contents reported in this study, particularly the high protein, ash, carbohydrate contents and low lipid content would appear to make the seagrasses as good dietary supplement and source of nutrients for human consumption. Given these high nutritional levels, this seagrass species could also be used as feed stuff.

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