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Phytochemical screening and proximate composition of the sea grasses *Enhalus acoroides* and *Thalassia hemprichii* in the Coastal Waters of Carmen, Agusan Del Norte, Philippines

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Abstract

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. While phytochemical screening provides basic information about the medicinal importance of the plant extract and are lead compound for drug discoveries. Proximate composition and phytochemical analyses were determined on the seagrass *Enhalus acoroides* and *Thalassia hemprichii* using standard methods on a dry basis. The result of the proximate composition showed that *Enhalus acoroides* contained 9.35% crude protein, 0.07% crude lipid, 54.94% carbohydrate contents and 35.64% ash. While *Thalassia hemprichii* contained 14.98% crude protein, 0.17% crude lipid, 52.87% carbohydrate contents and 31.98% ash. The phytochemicals detected in the methanolic extracts of *Enhalus acoroides* were alkaloids, flavonoids, saponins, phenols, tannins, and steroids. While for *Thalassia hemprichii* were alkaloids, flavonoids, saponins, phenols, tannins, triterpenoids and steroids. The results indicate that seagrass *Enhalus acoroides* and *Thalassia hemprichii* are good potential sources of highly nutritious feed stuff and the different active secondary metabolites along with significant bioactive potential might be helpful for the future pharmaceutical applications.

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Introduction

Sea grasses are submerged flowering plants, found mostly along the coastline, covering an estimated global area of 300,000-600,000 km². It is believed that, sea grasses originated on land and later went to adapt to underwater life. These underwater flowering plants, which have been with us since the age of the dinosaurs, have long been known to have antimicrobial properties (Amos, 2017). There are 72 known species of sea grass, of which 10 are at risk of extinction and 3 are endangered (Short, 2011). Sea grasses have declined in the area by about 29% since the beginning of the twentieth century, at an annual rate of about 1.5% and faster in recent years, replaced with unvegetated mud and sand soils (Fourqurean *et al.*, 2012).

The Philippines has the second highest seagrass biodiversity in the world. Seagrass species were found from 529 sites in the Philippines. In relation to sea grass as a resource in need of protection, its status as such is yet largely unknown, becoming a focus of scientific inquiry only in the last 30 years and as an object of conservation, only in the last 15 years. The coastal nature of Philippine demography, in addition to numerous development facilities, has caused eutrophication of marine waters, which, along with habitat loss, is a major long-term threat to sea grass ecosystems. Some advancement in sea grass research was made locally that are useful steps to reverse seagrass habitat losses. This study was conducted to determine selected nutritional and phytochemical properties of the sea grass *Enhalus acoroides* and *Thalassia hemprichii* in the coastal waters of Carmen, Agusan Del Norte, Philippines.

Materials and methods

The sea grasses that were used in this study were collected last July 24, 2017 in the coastal waters of Carmen, officially the Municipality of Carmen is a municipality in the province of Agusan Del Norte in the Caraga (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 sqkm(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 *Enhalus acoroides* and *Thalassia hemprichii*. Proximate analysis was done to determine the crude protein, fat, ash, and total carbohydrate contents of the samples was carried out in triplicates on a dry basis according to standard methods (AOAC, 2006). Carbohydrate content was estimated based on the net difference between the other nutrients and the total percentage composition. The phytochemical screenings in this study were determined using the different standard chemical tests of (Kaur & Arora, 2009).

Results and discussion

The results for the proximate composition analyses are shown in Table 1.

Table 1. Proximate analysis of sea grass.

Proximate Composition	Thalassia hemprichii (%)	Enhalus acoroides (%)
Crude Protein	14.98 ± 02.28	9.35 ± 0.17
Crude Lipid	0.14 ± 0.06	0.07 ± 0.06
Carbohydrate	52.17 ± 1.019	54.94 ± 0.18
Ash content	31.98 ± 0.78	35.64 ± 0.34

Values are means ± SD for 3 determinations.

The results of proximate analyses for *Thalassia hemprichii* and *Enhalus acoroides* contained crude protein values of 14.98% and 9.35% respectively. The results were consistent with the results of the study of Montano *et al.* (1999) with reported crude protein values of *Thalassia hemprichii* 15.87% and *Enhalus* *acoroides* 8.8%. Kannan and Kannan (2002) have reported also that the maximum protein content in *Enhalus acoroides* was 15.93 mg/g.

Lipid values of *Thalassia hemprichii* recorded 0.17% and 0.07% for *Enhalus acoroides*. The results were consistent with the study made by Pradheedba *et al.* (2010) on the eight species of sea grass including *Thalassia hemprichii* and *Enhalus acoroides*. 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.

Carbohydrate values showed *Thalassia hemprichii* contained 52.87% and 54.14% for *Enhalus acoroides*. Montano *et al*., (1999) reported high carbohydrate content value for Enhalus acoroides 72.4% and low carbohydrate content value of *Thalassia hemprichii* 26.63%. Generally carbohydrate content values in most sea grass species are high like *Zostera capriconi* 60.00% and *zostera marina* 50.90%.

The results showed that the ash content of *Thalassia hemprichii* contained 31.98% and *Enhalus acoroides* recorded 35.64%. The result was in line with the study of Yamamuro and Chirapart (2004), who reported on the ash content percentage on the leaf sheath of *Enhalus acoroides* 28.9% and *Thalassia hemprichii* 32.05%.

The higher amount of protein is essential for animal growth and increased milk production (Tangka, 2003). Plant proteins are a source of food nutrient, especially for the less privileged population in developing countries. Proteins are one of the macromolecule and it is an alternate energy source when other energy sources are in short supply. They are building block units and food protein is needed to make vital hormones, important brain chemicals, antibodies, digestive enzymes, and necessary elements in the manufacture of DNA. Some proteins are involved in structural support, while others are involved in bodily movement, or in defense against germs (Bailey, 2008). The presence of high protein levels in sea grasses indicates that they may have increased value as a food or that a protein base bioactive compound may be isolated in the future (Pradheeba *et al*., 2010).

Lipid content in sea grasses was not a striking feature as it showed only very little difference between the species. Generally the lipid content of sea grass is very low. Dietary fat increases the palatability of food by absorbing and retaining flavors (Antia *et al*., 2006). But a low dietary fat is good for the health.

The dominant storage carbohydrates in most sea grasses are the soluble product of sucrose and other soluble carbohydrate includes glucose and fructose. Carbohydrates are essential for the maintenance of life in both plants and animals and also provide raw materials for many industries (Ebun-Oluwa & Alade, 2007). Carbohydrates produced by plants are one of the three main energy sources in food, along with protein and fat. Glucose is also used by animal cells in the production of other substances needed for growth (Westman, 2002).

Generally the ash content of sea grass is high. The ash content is a reflection of the mineral contents preserved in the plants. This suggests a high deposit of mineral elements in sea grasses. The high ash content would appear to make sea grasses good dietary supplement and source of nutrients for human consumption.

The result of the phytochemical composition of the sea grasses *Thalassia hemprichii* and *Enhalus acoroides* are summarized in Table 2. The results of the phytochemical screening of *Thalassia hemprichii* 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 *Thalassia hemprichii* was in agreement with the work of Saranya *et al*., (2017). While the result of *Enhalus acoroides* shows the absence of coumarin, triterpenoids and cardiac glycosides, while alkaloids, flavonoids, saponins, phenolics, tannins and steroids were present.

Table 2. Phytochemical screening of seagrass.

Phytochemicals	*Thalassia hemprichii*	*Enhalus acoroides*
Alkaloids	+	+
Tannins	+	+
Phenols	+	+
Flavanoids	+	+
Saponins	+	+
Steroids	+	+
Triterpenoids	+	-
Coumarin	-	-
Cardiac Glycosides	-	-

Where; + present - absent.

The qualitative phytochemical screening of *Enhalus acoroides* was in agreement with the works of Vanitha *et al.*, (2017).

The only alkaloid derivative ever isolated from a sea grass species was a flavonoidal alkaloid (Thangaradjou *et al.*, 2013). Alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites.

This probably endows this group of agents, its antimicrobial activity. Several alkaloid containing medicinal plants are reported to have been used by the early man as pain relievers, as recreational stimulants or in religious ceremonies to enter a psychological state to achieve communication with ancestors or God (Heinrich *et al.*, 2005; Gurib-Fakin, 2005).

Seagrasses are rich in phenolic substances, including phenolic acids, sulphated phenolic acids, flavones, condensed tannins and lignins, but not hydrolyzable tannins. Plants that have tannins as their component are astringent in nature and are used for the treatment of intestinal disorders such as diarrhea and dysentery (Bajai, 2001), thus supporting the reasons why sea grass can be positioned among medicinal plants used for the treatment of microbial infection. Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues (Okwu & Emineke, 2006; Li *et al.*, 2003; Adegboye *et al.*, 2008).

The phenolic acids that predominate in the seagrass also occur widely in land plants, but gallic acid was detected in a greater percentage of seagrass (Zapata *et al.*, 2019). Various bioactivities of phenolic compounds are responsible for their chemo preventive properties (e.g., antioxidant, anticarcinogenic, or antimutagenic and anti-inflammatory effects) and also contribute to their inducing apoptosis by arresting cell cycle, regulating carcinogen metabolism and ontogenesis expression, inhibiting DNA binding and cell adhesion, migration, proliferation or differentiation, and blocking signaling pathways.

The presence of sulfated flavones was indicated in *Halophila, Thalassia* and *Zostera* species. Four flavones namely, luteolin, apigenin, luteolin-3-glucoronide and luteolin-4-glucoronide were identifided from ethanol extract of air dried *Enhalus acoroides* from South Chinaa Sea. The number of tannin cells (supposed to produce the phenolic compounds) largely increased in the adult and intermediate leaves. It accelerates its production of secondary metabolites to limit invasion of the beds. This also suggests the presence of phenols as defense mechanisms in seagrass prevent the micro- and macroalgal epiphytes (Thangaradjou *et al.*, 2013). It has been reported that flavonoids are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities (Pourmorad *et al.*, 2006; Ugwu *et al.*, 2013) and they might induce mechanism that affects cancer cells and inhibit tumor invasion (Rafat *et al.*, 2008). These activities could be attributed to their ability to neutralize and quench radicals (Ugwu *et al.*, 2013; Pourmorad et *al.*, 2006; Omale and Okafor, 2008). It can also be due to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Rice-Evans *et al.*, 1995).

Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability (Roa *et al.*, 1995). Saponins have the property of precipitating and coagulating red blood cells (Yadav & Agarwala, 2011). Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al.*, 2000; Okwu, 2004). Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation (Just *et al.*, 1998; Okwu & Emineke, 2006, Liu & Henkel, 2002).
Steroids and their metabolites often function as signaling molecules (the most notable examples are steroid hormones), and steroids and phospholipids are components of cell membranes. Steroids such as cholesterol decrease membrane fluidity. Similar to lipids, steroids are highly concentrated energy stores. However, they are not typically sources of energy; in mammals, they are normally metabolized and excreted. 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.

There are three main triterpene families: oleane, ursane, and lupane triterpenes. The main triterpenoids found in the oleane family are oleanolic acid, erythrodiol, and β-amyrin; in the ursane family are ursolic acid and uvaol; and in the lupane family are lupeol, betulin, and betulinic acid. Triterpenoids possess a rich chemistry and pharmacology (e.g. cholesterol) with several pentacyclic, motifs. Lupane, oleanane and ursane show particular promise as anti-cancer agents.

Coumarins are colorless, crystalline and natural, a simple description for a group of compounds with multiple therapeutic effects, found throughout the seagrass. Coumarin is used in the pharmaceutical industry as a precursor reagent in the synthesis of a number of synthetic anticoagulant pharmaceuticals similar to dicoumarol, the notable ones being warfarin (brand name Coumadin) and some even more potent rodenticides that work by the same anticoagulant mechanism. 4-hydroxycoumarins are a type of vitamin K antagonist.

Cardiac glycosides, which are highly toxic and found in a number of plants, are usually phytochemicals consisting of an aglycone (structurally related to steroid hormones) linked to one or more sugar molecules. Cardiac glycosides are an important class of naturally occurring drugs whose actions help in the treatment of congestive heart failure (Yukari *et al.*, 1995). Seagrass is used for the treatment of cardiac infections along with other ailments such as cough, and chest pain.

Conclusion

The chemical contents reported in this study, in particular the high protein, ash, carbohydrate; low lipid content would appear to make these sea grasses good dietary supplement and source of nutrients for human consumption. Given these high nutritional levels, this sea grass species could also be used as feed stuff. Also, 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.

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