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PHYTOCHEMICAL SCREENING AND LC_{50} OF *ENHALUS ACOROIDES* OF PASIAGAN BONGAO, TAWI-TAWI, PHILIPPINES

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ABSTRACT

Phytochemical screening revealed the constituents of the plant extracts and their bioactive agents such as alkaloids, flavonoids, saponins, and tannins indicate potential medicinal benefits and ecological significance. Understanding the toxic properties of seagrass is vital for its use in pharmaceutical applications. This research was aimed to determine the phytochemical component of Seagrass (*Enhalus. acoroides)* using the standard methods on a dry basis and its toxicity using the Brine Shrimp Lethality Test (BSLT) method. The phytochemicals detected in the methanolic extracts of *Enhalus acoroides* were alkaloids, flavonoids, phenols, tannins, saponins and steroids while triterpenoids and cardiac glycosides were not present. The results of toxicity testing showed that seagrass *Enhalus acoroides* had a toxicity level of 575.44 ppm which means toxic. The findings suggest that *Enhalus acoroides* seagrass could be an excellent source for further analysis and anticancer formulations. Additionally, its various active secondary metabolites, which possess notable bioactive properties, could be beneficial for future Medicinal Applications.

KEYWORDS: *Phytochemical Screening, Toxicity Test, Enhalus Acoroides, Philippines.*

INTRODUCTION

Seagrass, as foundational marine plants, play a crucial role in the ecosystem by providing habitat, food, and nursery grounds for a multitude of marine organisms. Among these seagrasses, *Enhalus acoroides* stands out for its ecological significance and potential medicinal properties. This marine plant, commonly found in tropical and subtropical regions, has been the focus of various studies due to its rich phytochemical profile and its potential applications in pharmaceutical field, which acts as an antibacterial (Nur et al., 2021). In Thailand, carminatives are utilized to enhance blood and lymphatic circulation (Klangprapun et al., 2018). Seagrass is submerged vascular plants that can form condensed vegetative communities in shallow marine environments, estuaries with high salt content, constant puddles, and areas with open water at low tide. They are also found on sand substrates, muddy sand, mud, and coral shards (Kurniawan et al. 2014).

Seagrasses are known for their diverse range of bioactive compounds, which are essential to their ecological roles and have great potential for various uses. Seagrasses have been used in traditional folk medicine for the treatment of various ailments, including digestive disorders, inflammatory conditions, and skin diseases. Moreover, extracts from different seagrasses have been shown to possess a variety of pharmacological activities, such as anticancer, anti-inflammatory, and antimicrobial. In the study of Prajoko et al in vitro investigation revealed new evidence that Seagrass *Enhalus acoroides*-ethanol (EAE) is highly potent in fighting breast cancer. These properties of seagrasses are attributed to the presence of a wide array of secondary metabolites Wehbe et al., (2024).

Seagrass thrives most abundantly in tropical oceans, making them the most productive region for this type of vegetation. Southeast Asia is renowned fortis rich seagrass diversity, hosting 24 out of the 60 identified seagrass species. These species thrive in the warm waters of Indonesia, the Philippines, Thailand, and Vietnam El Shaffai et al., (2023).

In the Philippines, the coastal areas and estuaries of Bongao, Tawi-Tawi are highly productive, faunally rich, and ecologically important habitat, a host extensive beds of *Enhalus acoroides* (61.32%) indicating that seagrass biodiversity found in this area is still healthy (Fatima et al., 2018).

Despite its abundance, there remains a gap in comprehensive studies that explore its phytochemical composition and toxicity levels. Understanding these aspects is essential not only for potential medicinal applications but also for ensuring safe and sustainable

use of this marine resources. It forms vast, and extensive seagrass beds that sheltered and provide source of food to thousands of flora and faunal species. Seagrasses thus, constitute a major link of food chain. (Fatima et al, 2018)

Phytochemical screening is essential for identifying bioactive compounds in plants, such as alkaloids, flavonoids, saponins, and tannins, which have therapeutic effects like antioxidant, antimicrobial, and anti-inflammatory properties. Discovering these compounds in Enhalus acoroides could highlight its medicinal potential and aid in creating natural health products. Additionally, determining the LC50 (lethal concentration) of Enhalus acoroides extracts is crucial for understanding its toxicity, indicating the concentration that causes lethality in 50% of a test population. This helps ensure safe usage levels in various applications. This study aims to assess the phytochemical properties and LC50 of Enhalus acoroides from Pasiagan, Bongao, Tawi-Tawi, Philippines, providing insights into its bioactive compounds and safety for potential medicinal and ecological applications.

MATERIALS AD METHODS

The sea grasses that were used in this study were collected last September 28, 2024 in the coastal waters of Pasiagan, one of the village in the Municipality of Bongao, province of Tawi-Tawi in Bangsamoro Autonomous Region in Muslim Mindanao (BARMM), Philippines. Pasiagan is situated at approximately 5.0085, 119.7508 (4º 53’ 57” N,119º 55’ 30” E), in the island of Bongao. It is nearby Sowang-Kagang, as well as the village of Nalil, Bongao, Tawi-Tawi. Elevation at these coordinates is estimated at 6.6 meters or 21.7 feet above mean sea level. The phytochemical test was conducted based on standard methods (Harborne, 1973) and its toxicity level using Brine Shrimp Lethality Test (BSLT) method.

RESULT AND DISCUSSION

The results of phytochemical test of the extract *Enhalus acoroides* were summarized in Table 1. The results revealed the presence of alkaloids, flavonoids, phenols, tannins, saponins and steroids, however, triterpenoids and cardiac glycosides were not present. The results were similar with the study by Tangon et al., (2019) which reported that the methanolic extract of *Enhalus Acoroides* seagrass contained the bioactive compound alkaloids, tannins, flavonoids, saponins, phenols, and steroids while triterpenoids and cardiac glycosides were absent. As shown in Table 1. this study revealed that *Enhalus acoroides is* absence of Triterpenoids and Cardiac glycosides. Similarly, in the study of Bharatharathna et al., (2019) and Ameen et al., (2024) that Triterpenoids and Cardiac glycosides were not also present in *Enhalus acoroides* but this result differs from some previous studies, which reported that *Enhalus acoroides* extracts generally contain triterpenoids and Glycosides chemicals (Ahmed et al, 2022). This is due to the different environmental conditions of *Enhalus acoroides* sampling. The presence of various bioactive compounds justifies the use of the whole plants for treating various ailments. Some of the bioactive secondary metabolites identified may become commercially important phytopharmaceuticals (Murugeswaran et al, 2022).

Table 1: Phytochemical Screening of Enhalus acoroides.

Phytochemicals *Enhalus Acoroides*	
Alkaloids	+
Flavonoids	+
Phenols	+
Tannins	+
Saponins	+
Triterpenoids	-
Steroids	+
Cardiac glycoside	-

Where; + present, - absent

Alkaloids presence was evaluated qualitatively using Wagner's Test. The appearance of a brown or reddish-brown precipitate indicated the presence of alkaloids. Alkaloids exhibit wide variety of pharmacological properties such as antibacterial, antimalarial, antihypertensive and anticancer activity. In the study of Ahmed et al., 2022 reveals that the extract of *Enhalus acoroides* has anticancer property in HepG2 and MCF-7 cell line. Hydro alcoholic extract of *Enhalus acoroides* possess various bioactive compounds responsible for various medical activities. Several anticancer medicines derived from marine species are reported to be effective. Alkaloids also block oxidative processes that can lead to cancer initiation. This method is mediated by a reduction in the peroxidation enzymes Lipooksigenase (LOX) and Xanthine Oxidase Cyclooxygenase (COX), which delays the cell.

Flavonoids were detected by introducing a couple of drops of sodium hydroxide to the extracts, which initially developed a vibrant yellow hue. This color gradually faded to colorless upon the addition of a few drops of diluted hydrochloric acid. Flavonoids are a large group of plant polyphenols widely distributed in nature and contained in human food. Recent reportsof antiviral, anti-fungal, antioxidant, anti-inflammatory, antiallergenic, antithrombic, anticarcinogenic, hepatoprotective, and cytotoxic activities of flavonoids have generated interest in studies of flavonoid-containing plants. The presence of flavonoid chemical compounds in the extract of seagrass *Enhalus acoroides* in the research of Dewi et al., (2018) shows that it has the potential to be a natural chemical antifouling, antibacterial, antifungal, and other pharmaceutical raw materials. Flavonoid compounds are found in seagrass. In the study conducted by Tuapattinaya, et al., (2019), it was found that the coastal waters of Rutong and Waai have the highest average flavonoid levels in the leaves of *Enhalus acoroides*, measuring 3.5697. Phytochemical analysis for the presence of secondary metabolite constituents in the

specimen indicated that flavonoid compounds were found in the extracts. The mechanism of action of flavonoid compounds as antimicrobials is to inhibit the nucleic acid synthesis and damage the membrane, causing the release of intracellular compounds Anand et al., (2019).

Phenols was confirmed by employing ferric chloride tests. The appearance of a red-brown precipitate indicated the presence of phenols. Seagrass were reported as a valuable source of phenolic compounds and are of ecological importance (El Shaffai, et al., 2023). As it must thrive in high-stress environments, they are rich in phenolic compounds that confer the plant with self-defense mechanisms. The presence of phenols in the methanol extract of *Enhalus acoroides* is a significant finding, as phenolic compounds are known for their strong antioxidant properties and supports the traditional use of this seagrass in folk medicine for treating various ailments (Tangon et al., 2019 and Pharmawati et al., 2020). These compounds play a crucial role in neutralizing free radicals, thereby reducing oxidative stress and potentially preventing various diseases and suggests its potential use as a natural antioxidant source (Ahmed et al., 2022).

Tannins similar to phenolics were assessed using the ferric chloride test. The formation of a blue-black or greenish-black precipitate indicated the presence of tannins. Tannins, known for their astringent properties, have been used traditionally to treat wounds and inflamed mucous membranes. Tannins have reported as antibacterial, antifungal, and antiviral compounds by preventing enzymatic activities and inhibiting the synthesis of nucleic acids (Huang et al., 2024). Additionally, tannins primarily strengthen the plant cell wall, making it almost impenetrable to harmful pathogens. The methanol extract of *Enhalus acoroides* contained phenols, steroid, and tannin (Menajang, et al. 2019). Tannins inhibit the S phase or synthesis of the cell cycle. The cell will carry out DNA synthesis and chromosomal replication during the S phase. Saponins prevent Bcl-2 from forming. Bcl-2 is an anti-apoptotic protein that promotes cell division (Widiastuti et al., 2019). The enriched therapeutic phytochemical compounds such as phenols, flavonoids, and terpenoids may be responsible for anticancer activity of HEEA.

Saponins was identified by vigorously shaking the extracted sample after adding distilled water, and then observing the formation of a stable foam. Saponins are a diverse group of naturally occurring plant secondary metabolites present in a wide range of foods ranging from grains, pulses, and green leaves to sea creatures. Saponins possess additional bioactivities that make them valuable in the pharmaceutical industry as anti-inflammatory, antimicrobial, antiviral, and antiparasitic. Their ability to form stable complexes with drugs further expands their potential in drug delivery systems. (Timilsena et al., 2023) Saponins also find applications in pharmaceuticals and other allied industries (Barbosa., 2014).

Triterpenoid was tests using the Salkowski test, showed the presence of a yellow color in the lower layer. Triterpenoids are versatile secondary metabolites with a diverse array of physiological activities, possessing valuable pharmacological effects and influencing the growth and development of plants. As more triterpenoids in cereals are unearthed and characterized, their biological roles in plant growth and development are gaining recognition. Triterpenoids are studied for their anti-inflammatory, hepatoprotective, analgesic, antimicrobial, antimycotic, virostatic, immunomodulatory and tonic effects. They are used in the prevention and treatment of hepatitis, 412 J. Med. Plant. Res. parasitic and protozoal infections and for their cytostatic effects (Lu et al., 2024). Recent research has uncovered that triterpenoids from the roots of certain plants play a role in modulating microbial communities within and around the roots, contributing to enhancing healthy plant growth. For instance, the triterpenoids excreted and synthesized by Arabidopsis thaliana roots can enhance the adaptability of roots by regulating metabolic soil networks (Huang et al., 2019).

Steroids were also present in the extract, to determine its presence, Liebermann-Burchard test was utilized. According to Setyoningrum et al., (2020) that the phytochemical analysis of *Enhalus acoroides* extract revealed various bioactive components, including steroids, and have some biological activity. The steroids are used as a primary ingredient in the development of drugs (immunostimulants or immunomodulators (Sari et al., 2020). Steroid compounds show the highest activity in the generation of intracellular ROS (Siddiqui et al., 2018). Studying steroids in seagrasses can lead to the discovery of new bioactive compounds that could be used in pharmaceuticals and other applications.

Cardiac glycosides were performed through the Keller-Killiani test. A 2.5 ml sample was mixed with 1 ml of glacial acetic acid, 1 ml of 5% $FeCl_3$, and 1 ml of concentrated sulfuric acid. The absence of cardiac glycosides was confirmed by the lack of green-blue color development in the sample. Cardiac glycosides are natural sterols and constitute a group of secondary metabolites isolated from plants. Today, cardiac glycosides represent the most diversified naturally derived compounds strongly recommended for the treatment of various cancers. Cardiac glycosides are of particular interest because of their potential as drug repurposing candidates and are used in various human ailments Karas et al., (2020).

Brine Shrimp Lethality Assay

Table 2: Brine shrimp lethality of *Enhalus Acoroides.*

Concentration	%Mortality	LC_{50}
500 ppm	10	
1000 ppm	10	
1500 ppm	30	575.44
2000 ppm	30	
2500 ppm	40	

Besides phytochemical screening, a toxicity test was performed on the methanolic extract of *Enhalus acoroides.* Brine shrimp lethality test (BSLT) method used *Artemia salina* leach to test the toxicity and was expressed with lethal concentration (LC50) value. Brine Shrimp Lethality Test provides baseline information for the anticancer potential or safety of bioactive compounds in samples (Premarathna et al. 2020). There are alternative methods for testing a sample's toxicity, but BSLT is the most convenient (Naidu et al., 2014).

As shown in Table 1, the observed nauplii mortality percentages after a 24-hour period in varying methanolic extract are as follows: 10% at 500 ppm, 10% at 1000 ppm, 30% at 1500 ppm, 30% at 2000 ppm and 40% at 2500 ppm. The results of this study indicate that seagrass *Enhalus acoroides* methanol extract has an LC_{50} value of 575.44 ppm which could be categorized as moderately toxic. Similarly, in the study conducted by Bareta et al., (2024) indicates that seagrass *Enhalus acoroides* extract contained alkaloids, flavonoids, tannins and steroids has an LC_{50} value of 126.77 ppm which means toxic. As added by Cordova et al., (2022), their study on the toxicity level of *Enhalus acoroides* after 24-hour revealed a high toxicity with the LC50 value of 4.5 ppm.

There are a few studies of the toxicity activity of seagrass, e.g., Kannan et al. (2014) recorded lesser toxicity in *Enhalus acoroides* compared to other seagrass species. In another investigation by Orno et al. (2020), different plant parts (e.g., leaf, stem, and rhizome) of *Enhalus acoroides* were found to inhibit the growth of *Artemia salina* nauplii, categorizing them as non-toxic and moderately toxic. The findings on the toxicity gained by Orno and Cordova suggested that *Enhalus acoroides* has the potential for anticancer formulations and other medicinal purposes.

CONCLUSIONS

The finding of this study revealed that *Enhalus acoroides* methanol extract had bioactivity potentials. Extracts at concentrations of 500 ppm, 1000 ppm, 1500 ppm, 2000 ppm, and 2500 ppm exhibit moderate toxicity to *Artemia salina* larvae, as evidenced by LC_{50} values of 575.44 ppm, demonstrating the effectiveness of the BSLT method. Further research is necessary to identify and isolate the active compounds with anticancer properties to fully leverage the medicinal benefits of *Enhalus acoroides*. The diverse active secondary metabolites, coupled with their significant bioactive potential, could be valuable for future pharmaceutical applications, given the wide-ranging pharmacological uses of this seagrass globally.

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