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SECONDARY METABOLITES AND PROBIT ANALYSIS OF MANGROVE *RHIZOPHORA MUCRONATA* FROM BONGAO TAWI-TAWI, PHILIPPINES

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ABSTRACT
Phytochemical screening involves the systematic extraction and identification of bioactive compounds in plant and other species; aims to determine their chemical constituents and potential medicinal properties. Phytochemical analysis was conducted through the aid of a standard test. This study aimed to analyse the secondary metabolites of *Rhizophora mucronata* and evaluate their bioactivity through Probit Analysis and determination of LC_{50} values. The phytochemicals detected in the methanol extracts of *R. mucronata* revealed a diverse profile of secondary metabolites, including the presence of alkaloids, flavonoids, phenols, tannins, saponins, triterpenoids, steroids and cardiac glycosides. Probit Analysis was used in this study to determine the lethal concentration of *R. mucronata* extracts against brine shrimp (*Artemia salina*). It yielded to a non-toxic result: LC_{50} value 1905.46 µg/ml. The findings of this study contribute to a better understanding of the chemical composition and biological activities of this species. Further, the identified secondary metabolites and their associated bioactivities hold potential for the development of natural products with applications in medicine and environmental remediation.

KEYWORDS: *Mangrove, Toxicity, Pythochemical screening, methanol extraction, Brine Shrimp Lethality Assay (BSLT)*

INTRODUCTION

Mangroves are salt and flood-tolerant trees and shrubs that grow abundantly in estuaries, intertidal zone and coastal shorelines of the tropics and subtopics. They play pivotal role in our ecosystem and provide services such as storm protection, and habitat for juvenile species. Mangroves are one of the most carbon-rich forests in the tropical ecosystem, more so than tropical rainforest (Buitre, M.J.C., et al., 2019).

Tawi-Tawi, a province located at the southernmost tip of the Philippines, is home to a significant expanse of mangrove forests. *R. mucrunata*, commonly known as the red mangrove; one of the most abundant and ecologically important mangrove species found in Tawi-Tawi. It is also known as *Bakawan-babae* in Filipino; an erect evergreen tree, reaching 20-25 meter with dark black bark, horizontally fissured. Has both aerial and stilt roots growing from lower branches. Broadly elliptic to slightly oblong leathery leaves measuring 11-23 cm and 5 -23 cm, opposite arrangement. A single-seeded fruit and germinates while still on tree. Additionally, *R. mucronata* is a cosmopolitan present all over the world that can tolerate 90 ppt of salinity ranges 3-5 to 17.5ppt (Santini et al., 2015). It has a higher tolerance and accumulative properties of heavy metals than any other mangroves (MacFarlene and Burchett, 2000).

Apart from their ecological benefits, red mangrove has an array of pharmacological capacities (Awuku-Sowah et al., 2022). It has the potential properties: anti-bacterial, anti-viral, and anti-fungal (Setyawan et al., 2019).

Furthermore, these compounds found have various beneficial biological activities, such as antioxidant, anti-inflammatory, and anti-cancer (Sudhir et al., 2022).

Phytochemical screening involves the systematic extraction and identification of bioactive compounds in plant and other species; aims to determine their chemical constituents and potential medicinal properties.

Phytochemical screening plays a vital role in identifying bioactive compounds in plants, which are crucial for medicinal application. Additionally, phytochemical screening aids in the discovery of novel compounds with potential health benefits, thereby fostering the advancement of natural product-based drug discovery (Harborne, 1998). Thus the information gained in this screening is essential for the ongoing research into plant-derived therapies and the sustainable use in medicinal plants.

The present research work carried out to investigate the phytochemical constituents and toxicity levels present in

the methanol extract of *R. mucronata*, which is potential for the development of natural products with applications in medicine and environmental remediation.

MATERIAL AND METHODS

The *R. mucronata* leaves collected in this study were colledted in the coastal area of Barangay Pagasinan, Sanga-Sanga, Bongao Tawi Tawi last September 2024. Meanwhile during the conduct of the study, about 5g of fresh red mangrove (*R. mucronata)* leaves were thoroughly rinsed with distilled water to remove any contaminants. After cleaning, the leaves were spread out in a single layer on a clean, fry surface to air-dry for a period of 7 days. Once completely dried, the leaves were brittle and easily crumbled. Then, the dried leaves were cut into small pieces using scissor. The dried small pieces of leaves were ground into a fine powder using a blender. The resulting powder was stored in a tightly sealed container at room temperature.

Methanol Extraction

One hundred grams (100g) of dried, powdered red mangrove (R. *mucronata)* leaves were transferred into a clean, dry 500ml Beaker. Methanol, a polar organic solvent known for its ability to extract a wide range of bioactive compounds from plant material, was added to the beaker in a volume of 200ml. The mixture was then being gently swirled to ensure complete wetting of the plant material by the methanol. The mixture was set aside and allowed to undisturbed for a period of 24 hours. During this time, the methanol gradually penetrated the plant cells, extracting the desired compounds. It was mixed thoroughly to ensure complete contact between the solvent and the plant material. After the extraction period, the mixture was filtered using a Whatman filter paper and placed in a glass funnel. The filtrate, containing the extracted compounds dissolved in methanol, was collected in a clean, labelled Erlenmeyer flask. The plant residue remaining on the filter paper was discarded.

RESULTS AND DISCUSSIONS

Phytochemical Screening

Table 1: Phytochemical Screening of *R.mucronata.*

Phytochemicals	*R.mucronata*
Alkaloid	+
Flavanoid	+
Phenolics	+
tannins	
Saponins	+
Triterpenoids	+
Steroids	+
Cardiac glycosides	+

Where; + present, - absent

Table 1 summarized the Phytochemical constituents of *R.mucronata*. The result show presence of alkaloids, flavonoids, tannins, Steroids and cardiac glycoside. However, there is a trace result of phenolics, saponins and triterpenoids. This qualitative phytochemical screening result was also in agreement to the work of (Rami *et al.,* 2012).

Alkaloids. Alkaloid was determined through Wagner's Test which yielded to a positive result. This was done after a 0.5g of the sample was dissolved in 2 ml of 2N hydrochloric acid. The resulting solution was then filtered to remove any insoluble particles. Four drops of Wagner's reagent were added to 2 ml of the filtered solution. The result showed a brown or reddish-brown precipitate indicating the presence of alkaloids. This comformed with the study of Sharma *et al.,* (2015) that there is a strong presence of alkaloids in *R. mucronata*.

Alkaloids play a significant role in plants by protecting them from predators and regulating their growth it has an anaesthetic, cardio protective and anti-inflammatory agents (Heinrich et al., 2021). Significantly, alkaloids have a wide variety of pharmacological appliances in the therapeutic area such as analgesic, antiasthmatic, anticancer, antihypertensive, antipyretic and antihyperglycemic effects. Further, Alkaoids have potential therapeutic effects in treating psychiatric disorders focusing on their ability to modulate neurotransmitter system like serotonin, dopamine, and norepinephrine (Kanter et al., 2004).

Flavonoids. Flavanoids were identified by adding 2 ml extract then 10% of sodium hydroxide solution is added to the extract. An initial deep yellow color developed, indicating the formation of a colored complex with the flavonoids. A few drops of dilute hydrochloric acid were added to the yellow solution, then in a few seconds, the color gradually fades and became colorless, that signifies the presence of flavonoids.

Flavanoids are among the most commonly found phenolic compounds in fruits and vegetables since they play important role on color and taste, synthesis of enzymes and vitamins and minimizing lipid peroxidation effects (Vuolo et al., 2019).

Therapeutically, the presence of flavonoids has important effects on plant biochemistry and physiology as antioxidants, enzymes inhibitor, precursors of toxic substances and they are also recognized to possess anti-imflammatory, anti-oxidant, anti-allergic and anticarcinogenic activities. This result is congruent with journal of Prabhu V. V and Guruvayoorappan, (2012). Further the long term consumption of foods rich in flavonoids offer health benefits to individuals with early-death risk factors (Bondonno et al., 2019)

Phenols and Tannins. The presence of phenolic compounds and tannins in a sample can be qualitatively determined using the ferric chloride test. 5ml of the sample solution is prepared. Then, 2.5ml of 5% ferric chloride ($FeCl_3$) solution is added to the sample. After

that, it formed a red-brown precipitate that indicates the presence of phenols.

Phenols belong to broad group of chemical substances having aromatic rings in hydroxyl group. Phenols are widely distributed into plant kingdom, mainly fruits and vegetables (Vuolo et al., 2019). It is similar to alcohol but form stronger hydrogen bonds.

Therapeutically, phenols derived from various natural sources which are linked to antioxidant, anti-inflammatory, anti-allergic, anti-carcinogenic, antihypertensive, cardio protective, anti-arthritic and antimicrobial activities (Rauha et al., 2000). Because of their potential health benefits, natural antioxidants are considered to be a better alternative than the synthetic ones (Fu et al., 2010)

Meanwhile, it formed greenish-black precipitate which signals the presence of tannins. Tannins are polyphenolic compounds known for their antioxidant, antimicrobial, and anti-inflammatory in the study of Zulaika, et al., (2019), Tannins have a therapeutic relevance, including their use in traditional medicine for treating gastrointestinal disorders, wounds, and infections. It also highlights the potential of tannins in modern pharmaceutical applications, particularly in the development of natural remedies with fewer side effects. properties. It also contributes to its therapeutic characteristics, including astringent, anti-diabetic, anti-rhematoid, and hypotensive propeties (Gurib-Fakim and Brendler, 2004).

Saponins. The presence of saponins was determined using the olive oil test. 2.5ml of the solution was mixed with 5 ml of distilled water. Then it was vigorously shaken until a stable foam formed. Three drops of olive were then added to the solution and shaken again. The formation of an emulsion indicated the presence of saponins.

Saponins is characterize by its glycosidic structure, which is responsible for their surface-active properties. Therapeutically, saponins highlight its anti-infalamatory, antioxidant and anticancer and immunomodulatory properties (Sulatan et al., 2012).

Thus, it has a potential in the development of pharmaceutical formulations in their ability to enhance the absorption of bioactive compounds in the body.

Triterpenoids. The Salkowski Test was used to determine the presence of triterpenoids. 2.5ml of the sample solution was taken, then 5 ml of chloroform was added to the sample and the mixture was filtered. Then, another 4 drops of concentrated sulphuric acid were added to the filtrate. The mixture was shaken and left for a minute undisturbed. After a few minutes, lower layer of the test tube turned into yellow, this indicates the presence of triterpenoids.

According to (Li et al, 2013), triterpenoids are the most abundant secondary metabolites present in marine organsims, such as marine sponges, sea cucumbers, marine algae and marine-derived fungi. A large number of triterpenoids are known to exhibit cytotoxicity against a variety of tumor cells, as well as anticancer efficacy in preclinical animal models. The structural features and the potential use of triterpenoids of marine origin to be used in the pharmaceutical industry as potential anti-cancer drugs leads.

Steroids. The Liebermann-Burchard test was utilize in determining the presence of steroids. 2.5ml of the sample solution was taken. Then, the sample was filtered. 2.5ml of acetic acid and 1 ml of concentrated sulphuric acid were added to the filtrate. After a few seconds, a formation of blue-green ring indicated the presence of steroids.

Steroids are a class of organic compounds characterized by a core structure of four interconnected carbon rings, typically classified into corticosteroids and anabolic steroids.

Significantly, (Khan et al., 2017) conducted a thorough phytochemical analysis of *R. mucronata* focusing on its steroid content among other bioactive compounds. The researchers identified a significant presence of steroids which they linked to various therapeutic benefits, including anti-inflammatory and analgesic properties. The findings suggest that the steroid in *R. mucronata* could enhance its use in traditional medicine, supporting claims of its effectiveness in treating ailments related to inflammations and pain.

Cardiac Glycosides. The Keller-Kiliani test was used to determine the presence of cardiac glycosides. 2.5ml of the sample was prepared, then 1ml of glacial acetic acid, 1ml of 5% ferric chloride ($FeCl_3$), and 1 ml of concentrated sulphuric acid were added to the sample. After that, a green-blue color appeared and which indicates the presence of cardiac glycosides.

Cardiac glycosides are a group of naturally occurring compounds, primarily derived from plants such as Digitalis species, it is also characterized by their steroidal structure with a lactone ring.

The presence of the cardiac glycosides in this sample is also in agreement with the study of (Shaid et al., 2018); conducted a detailed phytochemical evaluation of *R. mucronata*, identifying several bioactive compounds including cardiac glycoside.

The presence of these compounds was linked to a potential cardiovascular benefits suggesting that *R. mucronata* could play a role in managing heart-related conditions. The authors highlighted the importance of these glycosides in traditional medicine, emphasizing their therapeutic properties that may enhance cardiac

function and support heart health, thereby warranting further research into their mechanism of action.

Larva Preparation of *Artemia salina*

The Brine Shrimp Larva (BSL) method employed in this experiment is based on the Meyer method (1982), with certain modifications as described by Purnama et al. (2021). *Artemia salina*, commonly known as brine shrimp, was used as the test organism. Brine Shrimp eggs were placed in improvised plastic hatching vessels containing seawater from Beachside, Tubig Boh, Bongao, Tawi-Tawi. The hatching vessels were equipped with an aerator to provide a constant supply of oxygen to the water and placed under low-intensity lighting. After a hatching period of 48 hours, the nauplii (larval stage of brine shrimp) had emerged from the eggs. The hatched nauplii were carefully transferred to a separate container using a pipette to avoid damaging them. These nauplii were then used for subsequent toxicity testing experiment.

Brine Shrimp Lethality Assay

Table 2: Brine Shrimp lethality of *R. mucronata.*

Concentration	**%Mortality**	**LC_{50}**
500	10	
1000	10	
1500	50	
2000	60	
2500	60	1905.46

Meanwhile, after determining the bioactive compounds through the phytochemical screening, toxicity test. Toxicity test is use to assess the harmful effects of a substance such as a chemical drug, environmental pollutant on living things. Brine Shrimp lethality (BSLT) method utilize Artemia salina larvae was utilize in this test which tis expressed in the Lethal Concertation 50 (LC_{50}). Artemia salina commonly known as brine shrimp is widely used in toxicity testing due to its sensitivity to a variety of toxicants, eases of cultivation, and short life cycle; ideal for assessing acute toxicity of chemicals, pharmaceuticals, and environmental pollutants.

The obtained mortality data were analysed using Probit Analysis and LC_{50} calculations with the aid of an Excel program. An extract is considered active if the calculated LC_{50} value is less than or equal to 1000$\mu g/ml$.

According to Meyer (1982), the minimum concentration that can state that the extract has activity that is causing the death of 50% of shrimp larvae is $LC_{50} \leq 1000\mu g/ml$. In this study, it was shown that the percentage of the mortality rate of *A. salina* was directly proportional to the increase in the concentration of the extract.

The LC_{50} value is calculated using the linear regression equation and the antilog of the x value corresponding to 50% mortality. In the table above, it shows that the LC_{50} is 1905.461 $\mu g/ml$. This substance is classified as non-toxic because of the LC_{50} value is greater than 1000 ppm. A substance with an LC_{50} greater than 1000 ppm is generally considered non-toxic to aquatic organisms.

Overall, the BSLT results indicate that the dried-leaves of *R. mucronata* tested is relatively safe for aquatic organisms.

CONCLUSION

The study concludes that *R. mucronata* contains a variety of secondary metabolites, on the Brine Shrimp Lethality Test (BSLT), the LC_{50} values for methanol extract is 1905.460718 $\mu g/ml$ which is non-toxic. The findings of this study contribute suggest to a better understanding of the chemical composition and biological activities of this species. The identified secondary metabolites and their associated bioactivities hold potential for the development of natural products with applications in medicine and environmental remediation.indicate that this leaves extract were categorized as non-toxic, suggesting its potential for safe use in various application.

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