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PHYTOCHEMICAL SCREENING AND BRINE SHRIMP LETHALITY TEST (BSLT) OF THE METHANOLIC EXTRACT OF SYZYGIUM AQUEUM OF BONGAO,TAWI-TAWI, PHILIPPINES

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ABSTRACT

Phytochemical screening is essential for identifying various bioactive plant compounds that contribute to drug development, health benefits, and scientific research while Brine Shrimp Lethality Test (BSLT) is a simple cost-effective method to assess toxicity and potential bioactivity of plant extract and other substances. This study investigates the phytochemical composition and toxicity of *Syzygium aqueum* leaves, commonly known as watery rose apple, collected from Capitol Hill, Bongao, Tawi-Tawi. The leaves were subjected to a comprehensive phytochemical screening, revealing the presence of several bioactive compounds, including alkaloids, flavonoids, tannins, and saponins. These compounds were recognized for their potential antioxidant, antimicrobial, and anti-inflammatory properties, aligning with the traditional uses of *S. aqueum* in herbal medicine. To assess the toxicity, the Brine Shrimp Lethality Test (BSLT) was employed, revealing a lethal concentration LC_{50} of 794.33µg/ml which is toxic. These results laid the groundwork for further investigations into the therapeutic applications of *S. aqueum* and highlight the necessity for additional studies to isolate individual compounds and evaluate their specific biological activities. The findings suggested that the extract possessed promising pharmacological potential with a relatively safe profile, supporting its continued use in traditional medicine.

KEYWORDS: *Syzygium aqueum*, Phytochemical screening**,** Toxicity, Flower.

INTRODUCTION

Plants have long been recognized as rich sources of bioactive compounds with significant pharmacological potential. Among these, *Syzygium aqueum*, commonly known as watery rose apple, is a plant species belonging to the family Myrtaceae and native to Southeast Asia. Known for its edible fruits, *S. aqueum* has been traditionally used in folk medicine across various cultures.

The leaves bark, and fruits of different *Syzygium* species have shown antimicrobial, antioxidant, anti-inflammatory, and even anticancer properties, largely due to the presence of phytochemicals such as flavonoids, tannins, alkaloids, and saponins (Kumala *et al.*, 2020; Ubhenin *et al.*, 2023).

Phytochemicals are secondary metabolites that play a vital role in plants' adaptation to environmental stresses, but they also have significant therapeutic relevance in humans. Flavonoids and tannins, for example, are known for their antioxidant effects, while alkaloids often exhibit antimicrobial and cytotoxic properties (Matthews *et al.*, 2022; Vickery & Vickery, 1981). Studying the phytochemical profile of plants can therefore provide insight into their medicinal value, as well as guide the development of plant-based therapies. Given the ethnobotanical use of *S. aqueum* in traditional medicine, especially in Southeast Asian communities, investigating its chemical composition and biological activity is essential.

The brine shrimp lethality test (BSLT) is a widely used bioassay to evaluate the general cytotoxicity of plant extracts. Originally developed by Meyer *et al.* (1982), the BSLT uses *Artemia salina* (brine shrimp) larvae as model organisms to test for toxicity, which can be a preliminary indicator of anticancer potential. The assay's simplicity, cost-effectiveness, and reliability make it an attractive choice for screening plant extracts for cytotoxic compounds (Meyer et al., 1982; Hendrawati, 2009).

This study aimed to conduct a phytochemical screening and brine shrimp lethality test on *S. aqueum* leaves collected from Capitol Hill, Bongao, Tawi-Tawi. By identifying key phytochemicals and assessing toxicity, this research contributes to the broader understanding of *S. aqueum*'s medicinal potential and safety, especially in traditional and natural medicine settings.

MATERIALS and METHODS

Sample Collection: The sample collection of *Syzygium aqueum* leaves was conducted with careful attention to environmental and seasonal factors to preserve phytochemical integrity. Mature, healthy leaves were selected from established trees located at Capitol Hill, Bongao, Tawi-Tawi, during the early hours of the morning to reduce the risk of photodegradation and oxidative stress that could affect sensitive compounds. Collection took place during the dry season to prevent any excess moisture that could interfere with subsequent sample preparation and analysis. Each leaf was handpicked to avoid contamination from other plant species, using sterilized gloves and scissors to minimize potential contamination.

The collected leaves were immediately placed in sterile, moisture-resistant polyethylene bags and labeled with essential information, including date, location, and environmental conditions at the time of collection. These samples were then carefully transported to the Integrated Science Laboratory at Mindanao State University-Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), located at Sanga-Sanga, Bongao, Tawi-Tawi, where they were promptly stored in a cool, dark environment to preserve their biochemical properties until further processing. This approach ensured that the collected samples were of high quality which represented the native phytochemical profile of *S. aqueum* in the collection area.

Sample Preparation: Fresh leaves of *Syzygium aqueum* were collected from Capitol Hill, Bongao, Tawi-Tawi. After collection, the leaves were washed thoroughly with distilled water to remove impurities and air-dried in a shaded area for 7 days to preserve their bioactive compounds. Once completely dried, the leaves were finely ground into powder using a laboratory grinder, ensuring consistency in the sample for subsequent extraction and analysis.

Methanolic Extraction: Methanolic extraction was performed to obtain the bioactive compounds from the powdered leaflets, following standard phytochemical procedures. For this, approximately 50 grams of powdered leaves were soaked in 200 mL of methanol for 72 hours, with occasional shaking to facilitate the extraction of phytochemicals. The mixture was then filtered using Whatman filter paper separating the liquid extract from the plant residue. The resulting filtrate was concentrated using a rotary evaporator under reduced pressure to yield a semi-solid methanolic extract, which was stored at 4°C until further phytochemical analysis.

Phytochemical Screening: The prepared methanol extract was subjected to phytochemical screening to detect the presence of various bioactive constituents, including alkaloids, flavonoids, tannins, saponins, and terpenoids. Reagents and chemicals used for the phytochemical tests were obtained from the Integrated Science Laboratory at Mindanao State University-Tawi-Tawi College of Technology and Oceanography (MSU-TCTO), located in Sanga-Sanga, Bongao, Tawi-Tawi, ensuring standardized testing conditions and reagent purity.

Phytochemical screening of the *Syzygium aqueum* methanol leaf extract was performed to identify bioactive compounds. Each test was carefully conducted using standardized protocols to ensure the reliability of the results, as described below.

RESULTS and DISCUSSION

Table 1: Phytochemical Screening of Syzygium aqueum.

Phytochemicals	Syzygium aqueum
Alkaloids	+
Flavonoids	+
Phenols	+
Tannins	+
Saponins	+
Triterpenoids	+
Cardiac Glycosides	+
Steroids	+

Where; + present, - absent

Test for Alkaloids: Wagner's test was used to detect the presence of alkaloids. 2 mL of the methanol extract was combined with 1–2 drops of Wagner's reagent, a solution made from iodine and potassium iodide. The mixture was gently shaken, and the appearance of a reddish-brown precipitate indicated a positive result. This reaction occurred as the reagent bound with alkaloids to form a characteristic precipitate. Many alkaloids have potent pharmacological properties which make them valuable in medicine. Alkaloids are well known for their wide range of therapeutic properties, and including anesthetic effects (Rajput *et al.,* 2022).

Test for Flavonoids: Shihoda' test was employed for the alkaline reagent to identify flavonoids. 2 mL of the methanolic extract was treated with a few drops of 2% sodium hydroxide solution. The appearance of an intense yellow color suggested the presence of flavonoids. Upon adding a few drops of dilute hydrochloric acid, the yellow color disappeared, further confirming the

presence of flavonoid compounds, as they exhibit color changes in response to pH alterations. Flavonoids are plant compounds with antioxidant and anti-inflammatory properties that linked to various health benefits. According to S. Ahmed *et al.,* (2020) flavonoids are important group of naturally occurring polyphenolic compounds having an antioxidant, anti-inflammatory, antidiabetic, antiallergic activities, while some other flavonoid compounds exhibit potential antiviral activity.

Test for Phenols and Tannins: The Ferric Chloride test was used for the detection of phenolics and tannins. In this test, 2 mL of the methanolic extract was mixed with a few drops of 5% ferric chloride solution. A deep blue or greenish-black color was observed, indicating the presence of phenolic and tannin compounds. This color change results from the formation of complexes between phenolic and tannin compounds with ferric ions. Phenols are versatile compounds used in various industries, while tannins are plant polyphenols used in training, winemaking, and medicine. Methanol extract of *S. aqueum* leaves contained a few phenolic compounds such as caffeic acid; Sobeh, Mahmoud, *et al***.,** 2018, 2018). Tannins are polyphenolic biomolecules that have antioxidant, antimicrobial, antinutritional, anticancer, cardioprotective properties (Babar *et al.,* 2019).

Test for Saponins: Saponins were detected using the Froth test. In this procedure, 2 mL of the methanol extract was vigorously shaken with 5 mL of distilled water in a test tube for approximately 5 minutes. The formation of stable, frothy foam that lasted for at least 10 minutes indicated the presence of saponins, which are known to reduce surface tension and create stable bubbles. Saponins are plant compounds with surface-active properties, used in soaps, medicines, and are linked to various health benefits. Saponins possess several biological properties such as anti-oxidant, anti-tumor, hypocholesterolemic, hypoglycemic, and anti-inflammatory activities. Saponins are also associated with defense mechanism in plants related to their anti-microbial, anti-fungal, anti-parasitic and molluscicidal effects Shen *et al*., 2017.

Test for Triterpenoids: The Salkowski test was conducted to detect triterpenoids. In a clean test tube, 2 mL of the methanolic extract was mixed with 2 mL of chloroform, followed by the careful addition of 2 mL of concentrated sulfuric acid down the side of the test tube. There was reddish-brown ring at the interface between the chloroform and sulfuric acid layers confirmed that there is a presence of triterpenoids.

Terpenoids are a diverse group of organic compounds found in plants that are responsible for various flavors, fragrances and colors, and have potential medicinal properties. Triterpenoids have a hepatoprotective, anti-inflammatory, antimicrobial, and immunomodulatory activities (Akkol, Çankaya, *et al.,* 2021).

Test for Steroids: The Liebermann-Burchard test was used to identify steroids. In this test, 1 mL of the methanolic extract was mixed with 2 mL of acetic anhydride. After gently shaking, 2 drops of concentrated sulfuric acid were added. A color change from pink to blue-green indicated the presence of steroidal compounds, which undergo oxidation to produce this distinct color. Steroids are crucial for growth, development, and reproduction. Saponin have been used to treat a variety of ailments, including alcoholism, inflammation, infection, pre- and post-menopausal symptoms, cerebrovascular and cardiovascular diseases, such as hypertension and coronary heart disease, cataract, dementia, gastritis, prophylaxis, ultraviolet damage, gastric ulcer, duodenal ulcer, etc. Anabolic-androgenic steroids are the man-made derivatives of the male sex hormone testosterone. Physiologically, elevations in testosterone concentrations stimulate protein synthesis resulting in improvements in muscle size, body mass and strength (Bhasin *et al*., 1996; 2001).

Test for Cardiac Glycosides: The Keller-Kiliani test was used to detect cardiac glycosides. For this test, 2 mL of the methanolic extract was mixed with 1 mL of glacial acetic acid containing a drop of ferric chloride. Concentrated sulfuric acid was carefully added down the side of the test tube to form a layer. The appearance of a brown ring at the interface and a violet ring below indicated the presence of glycosides. Cardiac glycosides are potent compounds that can increase the force of heartcontractions and slow heart rate which make them valuable in treating heart failure. Cardiac Glycosides compounds in many plants preserve chemicals as inactive form of glycosides (Ali Reza *et al.,* 2021).

Brine Shrimp Lethality Test (BSLT): The toxicity potential of the *S. aqueum* extract was evaluated using the BSLT, as described by Meyer et al. (1982). **Artemia salina** eggs were incubated in artificial seawater for 24 hours under light to hatch into larvae (nauplii). The assay was carried out in serial dilution at concentrations of 2,500ppm, 2,000ppm, 1,500ppm, 1,000ppm, to 500ppm µg/mL of the methanol extract.

The phytochemical analysis of *Syzygium aqueum* leaves revealed the presence of several important bioactive compounds. Alkaloids, detected by an orange precipitate, are associated with a range of pharmacological properties, including antimicrobial and anticancer effects (Desai et al., 2022). The yellow color indicating flavonoids suggests the leaflets have potential antioxidant properties that could protect against cellular damage (Matthews et al., 2022). The blue-black color reaction confirming tannins is consistent with antimicrobial and astringent qualities, which could support the plant's use in traditional remedies (Ubhenin *et al.,* 2023). Saponins, indicated by persistent froth, and terpenoids, detected by the blue-green ring, both contribute to immune support and anti-inflammatory effects. Overall, these compounds highlight *S. aqueum*'s

potential as a therapeutic agent with antioxidant, antimicrobial, and anti-inflammatory properties, providing a foundation for further exploration of its medicinal use.

Table 2: Brine shrimp lethality Test of dried methanolic leaves of S. aqueum.

Concentration	% Mortality	LC_{50}
500	80	
1000	70	
1500	50	794.33
2000	40	
2500	20	

The brine shrimp lethality test conducted at concentrations of 500, 1000, 1500, 2000, and 2500 µg/mL showed a progressive increase in mortality, with the LC50 calculated at 794.33µg/mL. According to Meyer's toxicity scale, substances with LC50 values below 250µg/mL are considered highly toxic, while values above 500µg/mL suggest lower toxicity (Meyer et al., 1982). The relatively high LC50 of 794.33µg/mL for *Syzygium aqueum* indicates a low level of cytotoxicity, aligning with the traditional use of this plant in folk medicine as a relatively safe natural remedy. The low cytotoxicity and presence of bioactive compounds support the potential therapeutic applications of *S. aqueum*. These results suggest that the extract is not highly toxic at the tested concentrations and could be explored further for use in pharmaceuticals, with appropriate dosing considerations. However, additional studies, including in vivo testing and trials on human cell lines are essential to confirm safe dosage limits and therapeutic efficacy.

CONCLUSION

The presence of bioactive compounds in Syzygium *aqueum* indicates the potential use for pharmaceutical properties. Furthermore, the brine shrimp lethality test suggests the presence of toxic compounds highlighted potential therapeutic applications. However, further research is necessary to isolate and identify the specific bioactive compounds and to conduct in-depth toxicological evaluations to assess the safety and efficacy of *S. aqueum* extracts for medical use.

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