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# PYTOCHEMICAL SCREENING AND LC<sub>50</sub> OF METHANOLIC EXTRACT OF PHILODENRON ERUBESCENE OF PANGLIMA SUGALA, TAWI-TAWI

John Mark S. Jacoba<sup>1</sup>\*, Efren Tangon<sup>2</sup>, Xandra S. De Guzman<sup>3</sup>, Fatimah Raiza I. Abubakar<sup>4</sup>, Ian Patrick S. Jaudinez<sup>5</sup> and Indal Fitra S. Daham<sup>6</sup>

<sup>1,3,4</sup>Batu-Batu National High School, Panglima Sugala, Tawi-Tawi, Philippines.

<sup>2,5,6</sup>Graduate School, Mindanao State University-TawiTawi College of Technology and Oceanography, Bongao, Tawi-Tawi, Philippines.

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\*Corresponding Author John Mark S. Jacoba Batu-Batu National High School, Panglima Sugala, Tawi-Tawi, Philippines.

#### ABSTRACT

Phytochemical screening is a rigorous scientific endeavor that involves the isolation and characterization of plant-derived bioactive compounds. Through a series of analytical techniques, researchers can identify and quantify these phytochemicals, paving the way for their potential application in various fields, including medicine, food science, and cosmetics. Plant toxicity results from the consumption of plantderived substances that possess toxic properties. This study aimed to determine the phytochemicals effect and toxicity effects of Philodendron erubescene. Leaflets were collected from Batu-Batu, Panglima Sugala, Tawi-Tawi, and subjected to methanolic process. The results extract was screened for phytochemicals using various tests including Wagner's test for alkaloids, ferric chloride test for phenolics and tannins test, and Salkowski test for triterpenoids. The extract was found to contain alkaloids, flavonoids, saponins phenolics, and tannins steroids. The toxicity of the extract was evaluated using the brine shrimp lethality assay. The results showed that the extract exhibited notable cytotoxic effects, with an LC50 value of 1.901 ppm. This finding underscores the potential toxicity of the extract and its implications for aquatic organisms. The study demonstrates the potential of Philodendron erubescens leaflets contains various phytochemicals and exhibits cytotoxic effects. These findings suggest that the tested plants exhibit potent cytotoxic activity, indicating their potential as natural sources of cytotoxic agents for local applications the findings are consistent with previous studies on the phytochemical composition and biological activities of Philodendron species.

**KEYWORDS:** Philodenron erubescene, Phytochemical screening, Brine Shrimp lethality test.

# INTRODUCTION

Philodendron is a large genus of flowering plants in the family Araceae. It is one of the largest genera in the family; with over 900 species hey are popular houseplants due to their attractive foliage, which can vary widely in shape, size, and color. Croat, (2013) Philodendron, a prominent member of the Araceae family, is renowned for its diverse morphological adaptations to various Neotropical environments. This genus has been the subject of numerous botanical studies, contributing significantly to our understanding of plant evolution and diversity (Loss-Oliveira et al., 2016).

Philodendrons exhibit a remarkable diversity in form and function. These plants can be found as vining, selfheading, or epiphytic species (Croat, 2013). Their leaves display a wide range of shapes, sizes, and textures, adapting to different light conditions and growth environments. Some species develop aerial roots to aid in climbing and nutrient absorption. Their unique inflorescence, consisting of a spadix and spathe, is

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characteristic of the Araceae family. Physiologically, philodendrons are C3 plants, and their growth and development are influenced by factors such as light intensity, temperature, and humidity (Barabé et al., 1997). Philodendron is a member of the Araceae (Arum) family and originates from the Caribbean, Colombia and Venezuela, but also grows in Asia nowadays. Hundreds of species are known, of which around ten have been promoted to houseplant status. The plant was first described in 1644, and was given its name in 1829. That name derives from Greek: 'philo' meanbarts 'love' or 'affection' and 'dendron' is 'tree'. Freely translated it means 'tea hugger', because Philodendron is a real climber that loves to 'embrace' trees. The plant symbolises health and abundance. However the plant is used as an ornamental plant. (Nainwal 2019).

Phytochemical screening is a preliminary method used to identify and categorize the various types of secondary metabolites present in plant extracts. These metabolites, such as alkaloids, flavonoids, tannins, and terpenoids,

often possess significant biological activities. By conducting phytochemical screening, researchers can gain insights into the potential therapeutic applications of plants (Harborne, 1998). The Brine Shrimp Lethality Test (BSLT) is a simple yet effective bioassay used to assess the toxicity of substances, including plant extracts. In this test, brine shrimp larvae (Artemia salina) are exposed to different concentrations of the extract, and their mortality is monitored. The LC50 value, which represents the concentration of the extract required to kill 50% of the brine shrimp, is used to evaluate the toxicity of the sample (Meyer et al., 1982).

## MATERIALS AND METHODS

Sample Collection and Preparation: Leaflets of Philodenron Erubescene were obtained and that were used in this study were collected last September 15, 2024 in the barangay Batu-Batu in the municipality of Panglima Sugala in the province of Tawi-Tawi (BARMM) of the Philippines. Geographically, at approximately 5° 4' North, 119° 53' East. It has a land area of 416.66 square kilometers or 160.87 square miles which constitutes 11.49% of Tawi-Tawi's total area, according to the Philatlas.com Fresh Philodenron Erubescene leaves were harvested during the morning hours to minimize the loss of volatile compounds. Each leaf was individually selected by hand with sterilized tools to avoid contamination and guarantee the highest quality sample. The leaflets were gathered and placed in a sterile plastic bag with essential information, including date, location, and size. The collected leaves were thoroughly rinsed with distilled water to remove dust and other contaminants. The leaves were air-dried under shade for 14-21 days until they reached 30% of their initial mass before being pulverized. After air drying the leaf lets were carefully peel the midrib away from the leaf blade using tweezers, then the leaves were pulverized into a fine powder using a laboratory grinder to standardize the sample for subsequent extraction and analysis. A phytochemical analysis was performed adhering to the standard protocols outlined by Harborne in 1973. The general purpose of this study is to test the toxicity effect of biochemical found in Philodendron erubescens and other chemical constituents based on their ethnopharmaceutical information.

Methanol Extraction: Finely powdered dried sample was weighed. 50.00 grams were submerged in 200 ml of methanol for 48 hours. After this duration, the samples underwent filtration using Whatman filter paper. In the subsequent soaking phase, 100 ml of methanol was utilized. After one hour, filtration was repeated. The resulting filtrate was securely stored in reagent bottle and refrigerated for future use. Phytochemical Screening The study obtained all its chemicals from the Integrated Science Laboratory at Mindanao State University -Tawi-Tawi College of Technology and Oceanography Sanga-Sanga, Bongao, Tawi-Tawi, located at Philippines. The assessment of phytochemicals in Philodendron Erubescene leaflets were carried out in

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accordance with the methods outlined by Goyal et al. (2012) with slight modification. The result were categorized qualitative as either negative (-) or positive (+), using the methanolic extract.

**Test for Alkaloids:** Alkaloid presence was assessed qualitatively through the utilization of Wagner's Test. A sample weighing 0.05 was mixed with 2ml of hydrochloric acid (2N) and filtered. A few drops of Wagner's reagent were added to 2ml of the filtrate, and the formation of a brown or reddish brown precipitate signaled the presence of alkaloids. According to (Chen & Morlock, 2016) the optimized bioassay was applied for profiling of alkaloid-rich herbal drugs such as Philodendron, Coptis, Tinospora, and Ephedra, leading to information-rich biofingerprints.

Test for Flavonoids: Two to three drops of 10% sodium hydroxide were introduced to 2ml of the extract. Initially a deep yellow hue manifested, which upon the addition of a few drops of a dilute hydrochloric acid, gradually. In the studies of (Scapinello et al., 2019) The extract of Philodendron. bipinnatifidum displays antinociceptive anti-inflammatory activities, and causing no toxicological effects. The pharmacological activity of this vegetal species may be related to the presence of flavonoids and phytosterols. Another studies results the antioxidant responses and the accumulation of rutin were evaluated in treated Philodendron erubescens (P. erubescens) shoots using 5.0 mg/L of Ti-Mo-Ni-O NTs. The total phenolic content and total flavonoid content were estimated. The resultant blue complex hue was measured at 630 nm at the conclusion of the incubation period. Data are displayed as means and SDs.( Ebrahim, et al., 2024).

Test for Phenols and Tannins: The existence of phenolics and tannins was ascertained through the application of ferric chloride tests. In this test 2 mL of the methanolic extract was mixed with 5% FeCl<sub>3</sub>. There is formation of a red-brown precipitate suggests that existence of phenols, while a blue-black or greenishblack precipitate serves as confirmation for the presence of Phenolics and tannins compounds. This study results also in the Philodendron meridionale extracts contained saponins, calcium oxalate crystals, and flavonoids, including phenolic compounds, which are known allelochemicals with herbicidal activities. (Swiech et al., 2021) and also in the study in the qualitative analysis of Philodendron. the aqueous extracts of megalophyllum using the TLC method, the presence of coumarins, fatty acids and hydrolysable tannins was found.(Noranathan et al., 2020).

**Test for Saponins:** To determine the saponin presence, the Froth test was conducted. In this test, 2 mL of the methanolic extract was vigorously agitated with 5 mL of distilled water in a test tube for approximately 5 minutes. The formation of a persistent, stable foam, lasting for at least 10 minutes, indicated the presence of saponins.

These compounds are known to possess surface-active properties, reducing surface tension and forming stable bubbles. Saponins, derived from plants, have diverse applications, including use in soaps and medicines, and are associated with various health benefits. According to Dikkala et al., (2023), saponins are secondary metabolites that are naturally found in plants parts (leaves, flowers, and fruits). In the study of Phytotoxic and Enzymatic Study of *Philodendron meridionale* on seeds of Lactuca sativa L. The presence of saponins and the concentration of phenolic compounds were determined (Swiech et al., 2021).

**Test for Triterpenoids:** The Salkowski test was employed to identify the presence of triterpenoids. Salkowski test. To 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. A reddish-brown ring formed at the interface between the chloroform and sulfuric acid layers, confirming the presence of triterpenoids. About ten of the hundreds of species have been designated as ornamental plants, including Philodendron erubescens "Imperial Red". Furthermore, research shows that the plant contains triterpenoids and flavonoids, which have medicinal effects against a variety of diseases (Santiago, et al., 2014).

**Test for Steroids:** To determine the presence of steroids, Liebermann-Burchard test was utilized. In this procedure, 1 mL of the methanolic extract was combined with 2 mL of acetic anhydride. Following gentle agitation, 2 drops of concentrated sulfuric acid were added. A color transition from pink to blue-green indicated the presence of steroidal compounds, which undergo oxidation to produce this characteristic color change. Also, some common plant steroids, fatty acid ethyl esters and a polyprenoid (hexaprenol) were reported from the ethanol extract of *Philodendron imbe* leaves and roots (Ponchet et al., 2017). As far as we know, there are only three reports on the chemical composition of the essential oils of *Philodendron species: Philodendron acutatum Schott.*, *P. imbe Schott.*, *and Philodendron scabrum K. Krause*, all obtained from their roots (Feitosa & Bezerra 2007).

**Test for Cardiac Glycosides:** To detect the presence of cardiac glycosides, the Keller-Kiliani test was conducted. In this procedure, 2 mL of the methanolic extract was combined 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 distinct layer. The absence of a brown ring at the interface and a violet ring below indicated the absence of glycosides.

Brine Shrimp Lethality Assav: A stock solution containing methanolic extract from dried and pulverized sample was created using distilled water as the solvent. Methanolic extract was evaluated using the BSLT, as described by Meyer et al. (1982). Through a serial dilution technique, five different concentrations (2,500 ppm, 2,000 ppm, 1,500 ppm, 1,000 ppm, 100 ppm,) were derived from this stock solution. Subsequently, 1 mL of each concentration was transferred to clean vial. The solutions of dried-methanolic extract, with varying concentrations, were partially supplemented with a small quantity of seawater containing ten (10) nauplii. All test vials were left uncovered under constant lighting for 24 hours. After this period, the number of surviving nauplii was recorded. The LC50 value for the Philodendron erubescens methanolic extract was calculated as 1.901 ppm, indicating high toxicity. According to Gupta et al in 1996 plant extract  $LC_{50}$  values below 1000 ppm were classified as cytotoxic.

#### **RESULTS AND DISCUSSION**

The result of the phytochemical composition of the *Philodenron Erubescene* is summarized in table 1. The results of the phytochemical screening of *Philodenron Erubescene* methanolic extract show the absence of Cardiac Glycosides, while Triterpenoids, Alkaloids, Flavonoids, Saponins, Phenolics, Tannins and Steroids were present.

 Table 1: Results of Phytochemicals of Philodendron erubescene.

Biochemicals	Test	Dried Sample
Alkaloids	Wagner test	+
Tannins	Ferric Chloride test	+
Phenols	Ferric Chloride test	+
Flavonoids	(modified)	+
Saponins	Distilled Water test	+
Steroids	Salkowski test	+
Triterpenoids	Salkowski test	+
Cardiac Glycosides	Keller-Killiani Test	-

Where; + present, - absent

As shown in table, the dried-methanolic extract of *P*. *Erubescene* leaflets contained variety of biochemicals, including alkaloids, flavonoids, phenolics, saponins, triterpenoids, and steroids. These compounds are

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secondary metabolites that the plant produces, which have various biological activities and potential health benefits.

Alkaloids: The methanol extracts the leaflets contains alkaloids. Alkaloids are mainly biosynthetically derived from amino acids resulting in variety of chemical structures, mostly isolated from plants.<sup>[7]</sup> Alkaloids can be found in about 20% of plant species in small qualities.<sup>[8]</sup> and their production (including in biotechnology), extraction and processing remain major areas of research and development (Heinrich et al., 2021)

Flavonoids: Flavonoids are also present in the methanol extract of the leaflets. Flavonoids are natural substances synthesized in several parts of plants that exhibit a high antioxidant capacity. They are a large family, presenting several classes based on their basic structure. Flavonoids have the ability to control the accumulation of reactive oxygen species (ROS) via scavenger ROS when they are formed. Therefore, these antioxidant compounds have an important role in plant stress tolerance and a high relevance in human health, mainly due to their antiinflammatory and antimicrobial properties. (Dias et al., 2021).

Phenolics: Phenolic compounds and extracts with bioactive properties can be obtained from many kinds of plant materials. Various studies have shown that plant phenolics can substitute or support the activity of synthetic food preservatives and disinfectants. (Takó, et al., 2020).

Saponins: Bioactive compounds found in the methanol extracts of the leaflets. Saponins belong to a class of plant metabolites with surfactant properties that are widely distributed in nature. They are eco-friendly because of their natural origin and biodegradable. To date, many plant-based saponins have been investigated for their surface activity.

Triterpenoids: Triterpenoids possess versatile biological activities including antiviral, anticancer, and hepatoprotective activities. They are widely used in medicine and other health-related fields. However, current production of such compounds relies on plant culture and extraction, which brings about concerns for environmental, ecological, and infield problems. (Sun et al., 2019).

**Brine Shrimp Lethality Assay:** This research employed the brine shrimp lethality assay to assess the cytotoxic effects of dried-methanolic leaflet extract derived from the terrestrial plant species *P. Erubescene*. The outcomes of the brine shrimp lethality assay are detailed in table 1.

 Table 1: Brine Shrimp lethality of dried-mthanolic leaflet extract of P. Erubescene.

Concentrations	% Mortality after 24 hrs.	LC <sub>50</sub>
2,500 ppm	60	
2,000 ppm	60	
1,500 ppm	80	1.901
1,000 ppm	80	
500 ppm	90	

 $LC_{50}$  <1000 ppm, classified as cytotoxic as classified by Gupta et. al. (1996).

The data presented in Table 1 highlights the lethality of dried-methanolic leaf extract of *P. erubescens* on brine shrimp (nauplii) after a 24-hour exposure period. The results indicate a dose-dependent increase in mortality rates as the concentration of the extract decreases.

At the highest concentrations of 2,500 ppm and 2,000 ppm, the mortality rate remains at 60%, suggesting that these concentrations may not be sufficient to induce higher lethality in the nauplii. However, as the concentration decreases to 1,500 ppm and 1,000 ppm, the mortality rate increases significantly to 80%. This trend continues at the lowest concentration tested, 500 ppm, where the mortality rate reaches 90%.

The calculated LC50 value of 1.901 ppm indicates that at this concentration, 50% of the nauplii are expected to die, confirming the cytotoxic nature of the extract. Furthermore, the classification of the extract as cytotoxic at concentrations below 1,000 ppm, as referenced from Gupta et al. (1996), reinforces the potential harmful effects of *P. erubescens* on aquatic organisms,

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particularly at lower concentrations. Comparing results with other published one, Ghareeb M. 2015, in his study Cytotoxic Screening of Three Egyptian Plants Using Brine Shrimp Lethality Test. The chloroform extract of Philodendron selloum showed the most potent cytotoxic activity at sub-lethal concentration (LC50=16  $\mu$ g/ml) followed by its methanol extract (LC50=26  $\mu$ g/ml).

Another study show that within the Aracae, genera such as Alocasia, Arisaema, Caladium, Colocasia, Dieffenbachia, and Philodendron contain calcium oxalate crystals in the form of raphides. When consumed, these may cause edema, vesicle formation and dysphagia accompanied by painful stinging and burning to the mouth and throat, with the symptoms occurring for up to two weeks (Nicolson, 1969).

The aqueous extract of Philodendron megalophyllum did not present cytotoxic effects on human fibroblast cells of the MRC-5 line at CI50 (Noranathan et al., 2020).

#### CONCLUSIONS

The study of phytochemical screening of *P. Erubescene* leaflet extract revealed a diverse array of bioactive compounds, including alkaloids, flavonoids, saponins,

phenolics, tannins, steroids, and triterpenoids, while cardiac glycosides were absent. Additionally, the brine shrimp lethality assay indicated significant toxicity effects of the methanolic extract, with an LC50 value of 1.901 ppm, suggesting potential toxicity of the extract and its implications for aquatic organisms.

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