

Cytotoxicity test for Philippine cherry (*Syzygium lineatum* (D.C. Merr & L.M. Perry) leaf extract using brine shrimps

Malana J*

Department of Chemistry, College of Arts and Sciences, Isabela State University, Echague, Isabela, Philippines

Abstract

The Philippine cherry (*Syzygium lineatum* D.C. Merr & L.M. Perry) belongs to the family Myrtaceae. This study investigated the phytochemical composition and cytotoxicity of the plant's methanolic extract. Solvent fractionation and gravity column chromatography revealed the presence of essential oils, phenolic compounds, alkaloids, steroids, flavonoids, tannins and higher alcohols. Cytotoxicity testing using a brine shrimp lethality assay at 500ppm showed the highest mortality rate between 12 and 48 hours after treatment. The results suggest that the bioactive constituents in *S. lineatum* may be responsible for the observed cytotoxic effects, potentially exerting physiological action on the test organisms.

Keywords: Phytochemical study, *S. lineatum*, chemical characterization, cytotoxicity test, brine shrimps

Introduction

Medicinal plants are those that possess therapeutic properties or exert beneficial pharmacological effects on the animal body. They play a vital role in traditional healing systems, where they are used in various forms—fresh or dried, whole, chopped, powdered, or as extracts obtained through different solvents. These botanical medicines, also known as phytomedicines, include plant parts such as seeds, berries, leaves, bark, roots, and flowers, which are valued for their medicinal potential. Medicinal plants naturally synthesize and accumulate secondary metabolites, including alkaloids, glycosides, tannins, volatile oils, as well as essential minerals and vitamins, all of which contribute to their pharmacological significance (Wink, M., 2015)^[7].

As pharmaceutical costs continue to rise, herbal medicines serve as a more accessible and affordable alternative, particularly for economically disadvantaged populations. This highlights the importance of studying the bioactive constituents of medicinal plants, their biological activities, and their therapeutic potential.

One such plant with reported pharmacological properties is *Syzygium lineatum*, commonly known as the Philippine cherry, a member of the Myrtaceae family. Traditionally, *S. lineatum* has been used to treat gonorrhea, headaches, and intestinal pain and is commonly found in swamps and flooded zones. Previous chemical studies have confirmed the presence of alkaloids in this species (Zarate-Manicad 2016)^[8]. In light of this, the present study aims to analyze the bioactive constituents of *S. lineatum* leaf extract, fractionate these compounds based on polarity, and assess the biological activity of the crude extract using brine shrimp as test organisms.

Materials and methods

Plant Materials

Fresh leaves of *Syzygium lineatum* D.C. Merr. & L.M. Perry were collected from Bagumbayan, Ilagan, Province of Isabela, Philippines. This location was chosen due to the plant's availability in the area and its recognized therapeutic properties (Figure 1).



Fig 1: The Philippine cherry plant

Methanolic Extraction of *Syzygium lineatum* D.C. Merr. & L.M. Perry

The leaves were air-dried at room temperature. A total of 560 grams of air-dried and finely ground *S. lineatum* leaves were completely submerged in methanol. The extract was then filtered and refluxed at a temperature below 50°C, yielding 25.32 grams of concentrate. The physical characteristics and pH of the different fractions were assessed before testing.

Fractionation of Phytochemicals of *Syzygium lineatum* D.C. Merr. & L.M. Perry

The concentrated alcoholic extract was partitioned between hexane and water in 1:1 ratio. The aqueous extract was further extracted with dichloromethane, a semi-polar solvent. Butanol was used to extract the polar constituents from the remaining aqueous solution. Figure 2 shows the schematic diagram for the separation and fractionation of the constituents of *S. lineatum*. The non-polar, semi-polar and polar fractions were concentrated separately and were subjected to a series of chromatographic separations

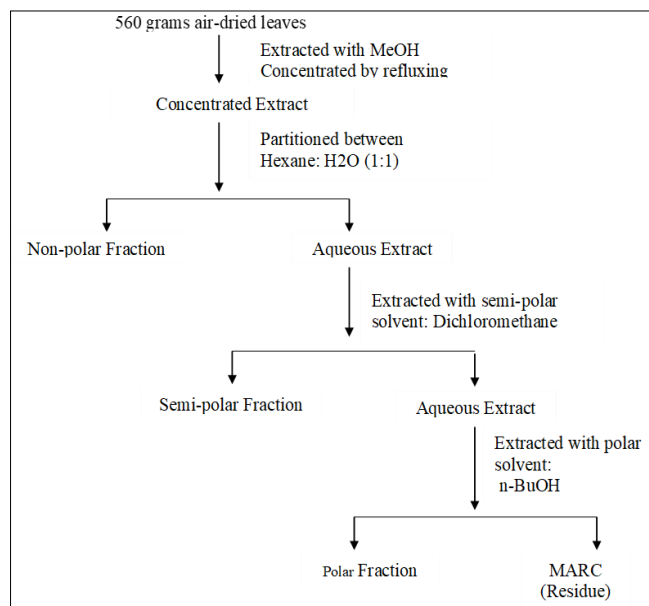


Fig 2: Schematic Diagram for the Separation and fractionation of the Constituents of *S. lineatum*

Gravity Column Chromatographic Profiling

The column was cleaned, dried and filled with adsorbent of Merk's Silica gel G for column chromatography. Packing was achieved by pouring slurry of silica gel. One gram of the concentrated extract was introduced, and the solvent was allowed to flow slowly from the column until all of the constituents of the extract migrate. The solvent was collected as it emerged from the column. Each eluate was spotted on prepared TLC plates. Eluates with similar R_f value were concentrated and further subjected to a series of chromatographic separations.

Characterization of the Bioactive Constituents of the Extract of *S. lineatum*

The concentrated non-polar, semi-polar and polar extracts were subjected to thin layer chromatography. Each sample was spotted on a prepared TLC plates using a capillary tube. The chromatograms were allowed to develop in the equilibrated chamber with filter paper to facilitate equilibration. After the solvent has reached the solvent

front, the plates were removed from the developing chamber and allowed to dry. The chromatograms were sprayed with different spray reagents to visualize the solute spots of each sample and the retention factor (R_f) of every constituent present in the fraction were computed using the formula given by Guevarra (2004).

Brine Shrimp Cytotoxicity Test

Brine Shrimps Culture

A shallow rectangular dish filled with artificial seawater was used to hatch the shrimp eggs. The dish was divided with perforated divider to allow hatched brine shrimps to pass through. One half of the dish was covered with carbon paper and the other half was illuminated with 50 watt lamp. Yeast solution served as food for the newly hatched brine shrimps.

Cytotoxicity Test

Different concentrations of *S. lineatum* were prepared from its crude extract. The prepared concentrations were as follows: 50ppm, 100ppm and 500ppm. Appropriate amounts of the crude extract were used to drench 1.25 centimeter (1/2 inch.) discs and dried using hair dryer to remove excess solvents. Five millimeters of artificial seawater was added to each vial. 10 brine shrimps were transferred into each vial using a 9 inches disposable pipette. They were fed with yeast solution (3mg of yeast/5ml of distilled water). Mortality determinations, average of three replicates were made: 12, 24 and 48 hours after the imposition of treatments.

Experimental Design

The Completely Randomized Design (CRD) was used in this study. Square root transformation was resorted to in the statistical analysis of the data collected having zero values. The formula $\sqrt{x + 0.5}$, where x represents the number of dead brine shrimps and 0.5 as constant. Data were subjected to analysis of variance (ANOVA) and means with significant differences were compared using the Least Significant Difference (LSD) test.

Results

The Plant Extract

The crude extracts from the leaves of *S. lineatum* were sticky and green in color. It possessed a pungent odor and caused dizziness. The concentrated methanolic extract was further extracted using solvents of different polarity. The non-polar fraction gave a dark green sticky concentrate with a pH of 8.0. The dichloromethane fraction yielded a yellow orange concentrate with a pH of 5.0 while the butanol fraction was dark brown in color with a pH of 4.0

Behavioral Observations on the Brine Shrimp Assay

The physiological reactions of the newly hatched brine shrimps were observed after they were confined into the vials containing the prepared concentrations of the crude extract of *S. lineatum*. LC₅₀ of less than 100 ppm was considered as potent (active). As mentioned by Meyer, LC₅₀ value of less than 1000 µg/mL is toxic while LC₅₀ value of greater than 1000 µg/mL is non-toxic. The percentage mortality (%M) was also calculated by dividing the number of dead brine shrimps by the total number, and then multiplied by 100%. This is to ensure that the death (mortality) of the brine shrimps is attributed to the bioactive compounds present in the plant extracts.

Phytochemical Constituents in the Leaf Extract of *S. lineatum*

Table 1 shows the results of the chemical characterization of the constituents of *S. lineatum* using different spray reagents. The non-polar fraction gave a positive result to Vanillin-H₂SO₄ which is indicative of the presence of essential oils. It was also visualized with K₃Fe (CN)-FeCl₃ and the thin layer chromatogram showed blue spots, an indication of the presence of phenols, tannins and flavonoids. The constituents gave negative results to Dragendorff's reagents, Kedde's reagent and Acetic-anhydride- H₂SO₄.

The semi-polar fraction gave positive results to Vanillin-H₂SO₄ and K₃Fe (CN)-FeCl₃. These indicate the presence of phenolic compounds and higher alcohols. Using Kedde's

reagent, Dragendorff's reagent and Acetic-anhydride-H₂SO₄ as spray reagents, the thin layer chromatogram gave negative result for the presence of cardenolides, alkaloids and saponins.

The polar fraction yielded colored spots upon visualization with Vanillin-H₂SO₄ which is indicative for the presence of higher alcohols, steroids and essential oils. The thin layer chromatogram also gave positive result to K₃Fe (CN)-FeCl₃ for the presence of phenolic group, tannins and flavonoids. Using Dragendorff's reagent, the chromatogram gave an orange spot which indicates the presence of alkaloids on the polar fraction of the leaf extract of *S. lineatum*. It was found out that the polar fraction of *S. lineatum* contains various bioactive compounds than non-polar and semi-polar fraction.

Table 1: Phytochemical analysis of the Non-polar, Semi-polar and Polar constituents of the leaf extract from *S. lineatum*

Phytochemicals	Fractions					
	Polar			Semipolar		Nonpolar
	91	57	18.3	73.1	54.2	61
Steroids and saponins	---	---	---	---	---	---
Alkaloids	+	+	+	---	---	---
Cardenolides	---	---	---	---	---	---
Phenols, tannins and flavonoids	+	+	+	+	+	+
Higher alcohol, phenols and essential oils	+	+	+	+	+	+

+ = means presence of the constituents --- = means absence of the constituents

Cytotoxicity Test

Table 2 shows the summary of the average mortality of the Brine Shrimps. On the 12 HAT, the 500ppm (T₄) gave the highest mortality, followed by T₃ and T₂. T₁ (control) gave a zero mortality. On the 24 HAT, 500ppm (T₄) had the

highest mortality followed by T₃ and T₂. T₁ (control) did not show any mortality. On the 48 HAT, the highest mortality was obtained by the brine shrimp treated with 500ppm (T₄) followed by T₃ and T₂. While T₁ (control) did not show any mortality during the duration of observation.

Table 2: Summary of Average Mortality of Brine Shrimps

Treatment	Average Mortality			Total Number of Survivors	% Mortality
	12 HAT	24 HAT	48 HAT		
T ₁ - Control	0	0	0	90	0%
T ₂ - 50ppm	6	15	20	49	54%
T ₃ - 100ppm	9	20	23	38	42%
T ₄ - 500ppm	14	24	28	24	27%



Figure 3. The brine shrimps.

Discussion

The present study demonstrated a clear dose-dependent increase in brine shrimp (*Artemia salina*) mortality with increasing concentrations of *Syzygium lineatum* crude leaf extract. This suggests that the extract contains bioactive compounds capable of exerting toxic physiological effects on aquatic invertebrates. Such a pattern of increasing lethality with concentration is characteristic of plant-derived compounds with cytotoxic or insecticidal properties.

The observed activity may be attributed to the phytochemicals naturally occurring in *S. lineatum*. Previous chemical analyses by Santos (1981)^[5] revealed that various parts of the plant—leaves, stems, fruits, and whole plant extracts—contain alkaloids. Alkaloids are nitrogen-containing secondary metabolites known for their broad spectrum of biological activity, including antimicrobial, insecticidal, and cytotoxic properties. As noted by Guevarra *et al.* (1985)^[2], alkaloids often exhibit strong physiological

actions in both humans and animals, and many have been exploited in pharmacology for their medicinal value.

This dose-dependent mortality pattern is consistent with the findings of Castro *et al.* (2014) ^[1], who tested bean extract preparations against the golden apple snail (*Pomacea canaliculata*). Their study reported 100% mortality at the highest concentrations, while lower concentrations resulted in reduced mortality, establishing a linear relationship between extract concentration, exposure duration, and toxic effect.

Recent studies reinforce these observations. For example, Pal *et al.* (2023) ^[4] found that methanolic extracts of *Clitoria ternatea* leaves and stems displayed LC₅₀ values of approximately 276 µg/ml and 323 µg/ml, respectively, in brine shrimp lethality assays. The presence of alkaloids, flavonoids, and phenolics in these extracts was confirmed through phytochemical screening. Similarly, Ulfa *et al.* (2024) ^[6] reported potent cytotoxic activity in turmeric (*Curcuma longa*) and red ginger (*Zingiber officinale*) extracts, with LC₅₀ values of ~16 ppm and ~3.5 ppm, respectively. Another study by Irawan *et al.* (2024) ^[3] reported that stem extracts of *Piper cf. arcuatum* exhibited very strong toxicity in brine shrimp bioassay, with LC₅₀ values below 35 ppm.

Collectively, these findings provide strong evidence that the bioactivity observed in *S. lineatum* extract is likely due to its alkaloid content, which contributes significantly to its toxicity profile. The consistency of these results with other plant-based studies highlights the reliability of brine shrimp lethality assay as a preliminary screening tool for identifying cytotoxic and pharmacologically active compounds. Further research, including phytochemical isolation and mechanistic toxicity assays, is warranted to identify the specific active compounds and evaluate their potential therapeutic or pesticidal applications.

Acknowledgements

The author would like to express her sincere gratitude to Dr. Oliva C. Ruma, her mentor, for generously sharing her knowledge and expertise throughout the conduct of this study. Special thanks are also extended to the faculty and staff of the Chemistry Department, College of Arts and Sciences, Isabela State University, Echague, Isabela, Philippines. Without the support of these individuals and the institution, the completion of this undertaking would not have been possible.

References

1. Castro JR, Lopez CC, Ramos LJ. Molluscicidal activity of bean extract preparations against golden apple snail (*Pomacea canaliculata*). *J Appl Trop Agric*,2014;21(2):115–21.
2. Guevarra BQ, Baltazar JL, Relos RJ. A Guidebook to Plant Screening: Phytochemical and Biological. UST Publishing House, 1985.
3. Irawan D, Gunawan H, Puspitasari R. Toxicity test of *Piper cf. arcuatum* stem extracts using brine shrimp lethality assay. *Trop J Nat Prod Res*,2024;8(3):45–52.
4. Pal MB, Sultana T, Rahman MM. Cytotoxicity evaluation of *Clitoria ternatea* extracts using brine shrimp lethality assay. *J Pharm Res Int*,2023;35(12):42–51.

5. Santos CR. Phytochemical screening and chemical analysis of *Syzygium lineatum*. *Philipp J Plant Sci*,1981;5(1):23–9.
6. Ulfa M, Rahman MI, Sari DN. Comparative cytotoxic activity of turmeric and red ginger using *Artemia salina* assay. *Afr J Nat Prod Res*,2024;6(1):67–74.
7. Wink M. Modes of action of herbal medicines and plant secondary metabolites. *Medicines*,2015;2(3):251–86. Available from: <https://doi.org/10.3390/medicines2030251>
8. Zarate Manicad MC. Phytochemical analysis of Lubeg (*Syzygium lineatum* (DC.) Merr. & L.M. Perry) species in Apayao. *Int J Novel Res Life Sci*,2016;3(6):1–5. ISSN 2394 966X.