

**PHYTOCHEMICAL EVALUATION AND CYTOTOXIC ACTIVITY
OF “MAKATAYNUK” (*Streblus* sp; Family Moraceae) LEAF
EXTRACTS USING BRINE SHRIMP (*Artemia salina*) LETHALITY
ASSAY**

A RESEARCH PAPER

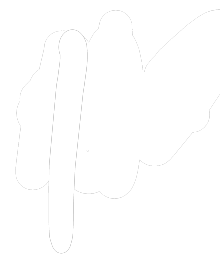
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the Faculty of the College of Medical Technology
Misamis University
Ozamiz City



In Partial Fulfillment
of the Requirements for the Degree
BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY

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CERTIFICATE OF PANEL APPROVAL

The thesis/research paper attached hereto, entitled **“PHYTOCHEMICAL EVALUATION AND CYTOTOXIC ACTIVITY OF *Streblus* sp. (“Makataynuk”) (Family Moraceae) LEAF EXTRACTS USING BRINE SHRIMP (*Artemia salina*) LETHALITY ASSAY”**, prepared and submitted by **HELENA MARGRETHE D. DOLLOSO, WINONA RENZ F. DURAN, and NIEJEN ELFIE L. HATAGUE**, in partial fulfillment of the requirements for the degree **BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY**, is hereby recommended for approval.


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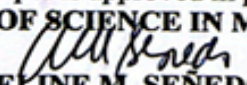
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DEDICATION

This research is sincerely dedicated to our parents and families, whose unwavering love, prayers, sacrifices, and support motivated us throughout this journey. Their encouragement gave us strength during times of difficulties and inspired us to continue striving toward the completion of this study.

We also dedicate this study to our adviser, Dr. Yunalyn L. Villantes, for her patience, guidance, valuable insights, and continuous support in helping us improve and complete this research. Her expertise and encouragement greatly contributed to our learning and growth as student researchers.

This work is likewise dedicated to our professors, panelists, classmates, and friends who shared their knowledge, constructive suggestions, and encouragement throughout this study.

*Most importantly, we dedicate this research to future researchers and individuals interested in medicinal plant studies and scientific exploration. May this study serve as a helpful reference and contribute to further investigations regarding the phytochemical properties and cytotoxic potential of *Streblus* sp. (“Makataynuk”).*

Above all, we offer this achievement to God Almighty for granting us wisdom, guidance, strength, and perseverance throughout the completion of this research.

Researchers

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ABSTRACT

The increasing prevalence of chronic diseases, antimicrobial resistance, and adverse effects associated with synthetic drugs has intensified the global search for safer and more effective bioactive compounds from medicinal plants. *Streblus* sp., locally known as “Makataynuk,” is a medicinal plant with limited scientific information regarding its phytochemical composition and cytotoxic properties. This study evaluated the phytochemical constituents and cytotoxic activity of the leaf extracts of *Streblus* sp. using the Brine Shrimp Lethality Assay with *Artemia salina* nauplii. Fresh leaves were collected, air-dried, pulverized, and subjected to methanolic, aqueous, ethyl acetate, and hexane extraction procedures. Phytochemical screening was conducted to determine the presence of secondary metabolites, and cytotoxic activity was assessed by evaluating mortality responses across varying extract concentrations, including positive and negative controls. The median lethal concentration (LC₅₀) was determined using probit regression analysis. Results revealed the presence of flavonoids, phenolics, saponins, tannins, alkaloids, steroids, and terpenoids in the extracts. A concentration-dependent increase in mortality was observed across all treatments. The positive control (DMSO) exhibited very high mortality, whereas the negative control (artificial seawater) showed no mortality, validating the assay conditions. Among the solvent extracts, the methanolic extract exhibited the highest cytotoxic activity with the lowest LC₅₀ value of 25.11 µg/mL, followed by ethyl acetate (104.72 µg/mL), aqueous (312.45 µg/mL), and hexane extracts (447.83 µg/mL). One-way ANOVA revealed significant differences in mortality rates among concentration levels and controls ($p < 0.0001$), which was further confirmed through Tukey HSD analysis and graphical grouping comparisons. The findings indicate that *Streblus* sp. contains biologically active secondary metabolites with significant cytotoxic potential, particularly in the methanolic extract, suggesting its potential as a source of pharmacologically important compounds for future pharmacological and toxicological investigations.

Keywords: *Artemia salina*, brine shrimp lethality assay, cytotoxic activity, Makataynuk, phytochemical evaluation.

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INTRODUCTION

Background of the study

The increasing prevalence of chronic diseases, antimicrobial resistance, and adverse effects associated with synthetic drugs has become a major global public health concern. Diseases such as cancer, diabetes, cardiovascular disorders, and infectious diseases continue to cause significant morbidity and mortality worldwide despite advances in modern medicine. In addition, the overuse and misuse of synthetic drugs have contributed to the rapid emergence of antimicrobial-resistant microorganisms, reducing the effectiveness of commonly used treatments. These global health challenges have intensified the search for safer, more effective, and naturally derived therapeutic alternatives (Ekor, 2021; Altemimi et al., 2020)

Medicinal plants have consistently played a significant role in the discovery and development of therapeutic agents due to their diverse pharmacological properties and naturally occurring bioactive compounds. Since ancient times, plants have been used in traditional medicine to treat various diseases and health conditions. In recent years, scientific interest in medicinal plants has increased due to the rising incidence of chronic diseases, antimicrobial resistance, adverse effects of synthetic drugs, and the demand for safer, more affordable alternative medicines (Khan et al., 2022; Kavya et al., 2024). According to the World Health Organization, a large percentage of the global population

still depends on herbal medicine for primary healthcare, especially in developing countries where medicinal plants remain accessible and culturally accepted.

The medicinal value of plants is primarily attributed to phytochemicals or secondary metabolites that are naturally synthesized during plant metabolism. These compounds function as defense mechanisms against environmental stress, pathogens, and herbivores while also contributing to plants' biological activities. This concept is supported by the Plant Secondary Metabolite Concept, which explains that secondary metabolites are not only essential for plant survival but also serve as bioactive compounds with pharmacological potential in humans (Mahamat Ousman et al., 2025). Common phytochemicals include flavonoids, alkaloids, tannins, saponins, steroids, glycosides, terpenoids, and phenolic compounds, many of which possess antimicrobial, antioxidant, anti-inflammatory, anticancer, antidiabetic, and cytotoxic activities (Do et al., 2020; Salmerón-Manzano et al., 2020).

Among the various phytochemicals, flavonoids and phenolic compounds are widely recognized for their antioxidant and cytotoxic properties, owing to their ability to neutralize free radicals and mitigate oxidative stress in cells. This mechanism is explained by the Oxidative Stress Concept, which posits that excessive production of free radicals causes cellular damage and contributes to the development of chronic diseases, including cancer and degenerative disorders (Ramakrishna et al., 2021; Liguori et al., 2020). Alkaloids are also known for their diverse pharmacological activities, including antimicrobial, analgesic, and cytotoxic effects (Ramos et al., 2022), while tannins possess protein-binding properties associated with antimicrobial and enzyme inhibitory functions (Khan et al., 2022). Saponins have likewise attracted scientific interest for their

membrane-disrupting properties and their ability to induce cell death in abnormal cells (Osbourn et al., 2011). These biological activities demonstrate the importance of identifying phytochemical constituents in medicinal plants as potential sources of therapeutic compounds.

Cytotoxicity screening is an important step in evaluating the biological activity and safety of medicinal plant extracts. Cytotoxicity refers to the ability of certain substances to inhibit or destroy living cells, which may indicate the presence of biologically active compounds with potential pharmacological applications, particularly in anticancer drug discovery (Singh & Sharma, 2024). This study is also anchored in the Dose–Response Concept, which holds that biological responses vary with the concentration of a substance, with higher concentrations often producing stronger biological effects. Several studies have demonstrated that plant extracts exhibiting cytotoxic effects may contain compounds with significant medicinal value, making preliminary toxicity screening an important component of natural product research (Abdullahi et al., 2021; Apu et al., 2022).

One of the most commonly used methods for preliminary cytotoxicity evaluation is the Brine Shrimp Lethality Assay (BSLT) using *Artemia salina* nauplii. The assay is widely accepted because it is simple, rapid, economical, and reliable for detecting biologically active compounds from natural products (Meyer et al., 1982). In this assay, *Artemia salina* larvae are exposed to different concentrations of plant extracts, and mortality rates are used to determine toxicity through the median lethal concentration (LC₅₀). Previous studies have reported that the Brine Shrimp Lethality Assay shows strong correlation with various biological activities, including cytotoxic, pesticidal,

antimicrobial, and pharmacological effects (Hamidi et al., 2020; Ahmed et al., 2021). Because of its efficiency and cost-effectiveness, BSLT is frequently used as a preliminary screening tool before conducting more advanced pharmacological investigations.

The effectiveness of phytochemical extraction is influenced by the solvent used. Solvent polarity affects the quantity and type of bioactive compounds extracted from plant materials. This is explained by the Solubility (“Like Dissolves Like”) Principle, which states that polar solvents dissolve polar compounds while nonpolar solvents dissolve nonpolar compounds (Mekonnen et al., 2022). Polar solvents such as methanol and water are more effective in extracting phenolic compounds, flavonoids, tannins, and glycosides, whereas nonpolar solvents such as hexane are more suitable for extracting lipophilic compounds such as certain steroids and terpenoids (Do et al., 2020; Sultana et al., 2023). Previous studies have shown that methanolic extracts often exhibit stronger biological activity because methanol can dissolve a broader range of phytochemicals compared with less polar solvents (Azwanida, 2022). Comparative solvent extraction, therefore, provides valuable information regarding the phytochemical composition and cytotoxic activity of medicinal plant extracts.

The Philippines is recognized as one of the world's biodiversity hotspots and is home to a wide variety of medicinal plants traditionally used by local communities. However, many indigenous and locally known plants remain scientifically underexplored despite their possible therapeutic value. One such plant is *Streblus* sp., locally known as “Makataynuk,” for which there is limited scientific literature on its phytochemical constituents and cytotoxic properties. Existing studies involving *Streblus* species mainly focus on antimicrobial and antioxidant activities, while limited information is available

regarding their cytotoxic potential and bioactive compounds (Nguta et al., 2021). This lack of scientific evidence highlights the need for further investigation to validate its medicinal potential through phytochemical and cytotoxicity studies.

Therefore, this study aims to evaluate the phytochemical composition and cytotoxic activity of Makataynuk (*Streblus* sp.) leaf extracts using the Brine Shrimp Lethality Assay. Specifically, it seeks to identify the secondary metabolites present in the extracts and to determine their cytotoxic effects on *Artemia salina* nauplii via LC₅₀ analysis. The findings of this study may contribute to the growing body of scientific knowledge on medicinal plants and provide preliminary evidence supporting the pharmacological potential of *Streblus* sp. as a source of biologically active compounds.

Conceptual Framework

The conceptual framework (Figure 1) of this study outlines the systematic pathway for evaluating the phytochemical composition and cytotoxic activity of Makataynuk (*Streblus* sp.) leaf extracts. It is anchored on the integration of the Plant Secondary Metabolite Concept, the Solubility (“Like Dissolves Like”) Principle, the Dose–Response Concept, and the Bioassay-Guided Screening Concept, which collectively explain how plant-derived compounds are extracted, identified, and evaluated for biological activity.

The framework begins with the collection, authentication, and preparation of plant materials, ensuring accurate identification and sample quality. The leaves are then subjected to standardized extraction procedures using solvents of varying polarity (methanol, aqueous, ethyl acetate, and hexane). This step is guided by the Solubility

Principle, which states that solvent polarity determines the type and efficiency of phytochemical extraction, enabling the isolation of both polar and nonpolar secondary metabolites.

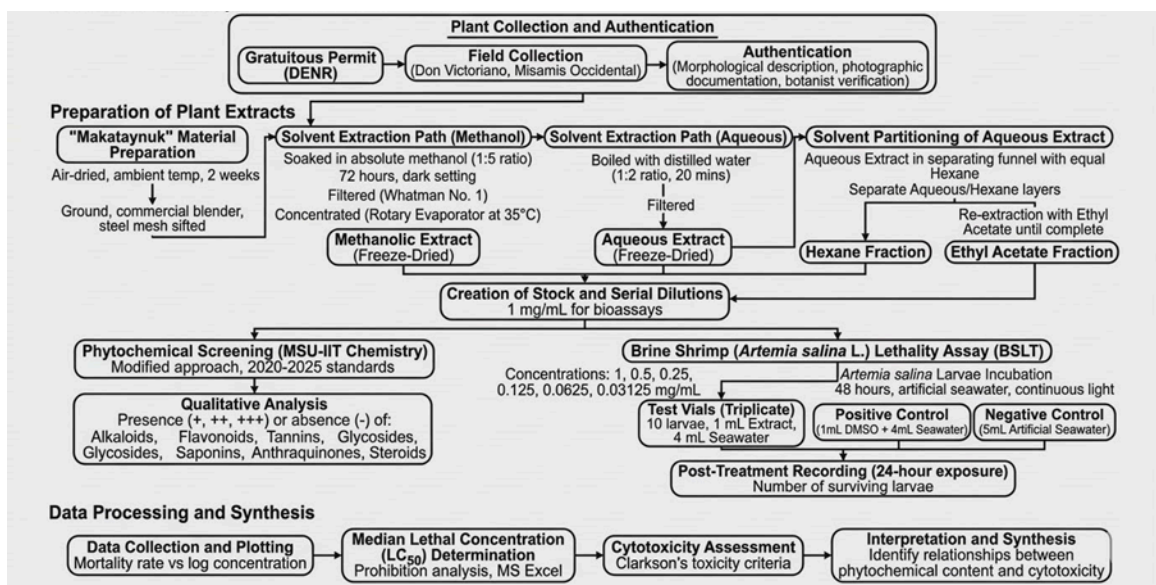


Figure 1. Conceptual Framework of the Study

The extracted crude samples are then analyzed by phytochemical screening, which identifies key secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, terpenoids, and phenolic compounds. These compounds are considered biologically active constituents that contribute to potential therapeutic and toxicological effects, as explained by the Plant Secondary Metabolite Theory.

Following phytochemical identification, the extracts undergo cytotoxicity testing using the Brine Shrimp Lethality Assay (BSLT) involving *Artemia salina* nauplii. This assay serves as a preliminary bioassay model within the Bioassay-Guided Screening Concept, in which simple biological systems are used to detect potential pharmacologically active compounds. Mortality responses are recorded at varying extract concentrations to assess toxicity.

The resulting data are then subjected to statistical analysis, particularly LC_{50} determination using probit regression, along with comparative tests such as ANOVA and post hoc analysis. This phase is supported by the Dose–Response Theory, which posits that biological effects vary with the concentration of bioactive compounds.

Overall, the framework demonstrates a logical progression from plant material acquisition → solvent-based extraction → phytochemical identification → bioassay testing → statistical toxicity evaluation, establishing the relationship between phytochemical content and cytotoxic activity. This systematic approach enables assessment of the pharmacological potential of Makataynuk (*Streblus* sp.) as a source of bioactive compounds.

Objectives of the study

This study aimed to evaluate the phytochemical composition and cytotoxic activity of Makataynuk (*Streblus* sp.) leaf extracts using the Brine Shrimp Lethality Assay (BSLT). Specifically, it sought to:

1. Identify the secondary metabolites present in the different solvent extracts of Makataynuk (*Streblus* sp.) leaves;
2. Analyze the dose–response relationship of the different solvent extracts based on the mortality rate of test organisms;
3. Determine the cytotoxic activity of the plant extracts using *Artemia salina* nauplii through the Brine Shrimp Lethality Assay; and
4. Compute the median lethal concentration (LC_{50}) of each extract using probit analysis.

Significance of the study

This study contributes to the growing body of knowledge on medicinal plants as potential sources of bioactive compounds with pharmacological importance. The findings may provide baseline information on the phytochemical composition and cytotoxic activity of Makataynuk (*Streblus* sp.), which can be useful for future pharmacological, toxicological, and natural product research. For researchers and students, this study serves as a reference for understanding phytochemical screening, solvent extraction, and cytotoxicity testing using the Brine Shrimp Lethality Assay involving *Artemia salina*. In the field of pharmaceutical and biomedical sciences, the results may provide preliminary evidence for the potential development of plant-derived compounds for therapeutic applications, particularly regarding cytotoxic or anticancer activities. For local communities, the study helps validate the scientific use of traditionally used medicinal plants, promoting awareness of their potential health benefits and encouraging evidence-based use. In addition, academic institutions may benefit from this research as it strengthens laboratory-based learning and supports the integration of natural product studies in biology and medical technology curricula. Lastly, by highlighting the potential value of indigenous plant species, this study emphasizes the importance of biodiversity conservation as a source of future medicinal resources.

Scope and Delimitations

This study focused on the phytochemical screening and cytotoxic evaluation of Makataynuk (*Streblus* sp.) leaf extracts using the Brine Shrimp Lethality Assay (BSLT). The scope of the study included the collection and authentication of plant samples, the preparation of crude extracts using methanol, water, ethyl acetate, and hexane, and the qualitative identification of secondary metabolites such as flavonoids, alkaloids, tannins, saponins, steroids, terpenoids, and phenolic compounds. It also covered the assessment of cytotoxic activity using *Artemia salina* nauplii, the determination of LC₅₀ values, and the analysis of dose–response relationships using statistical methods. However, the study was limited to in vitro experimentation. It did not include in vivo testing, clinical trials, or molecular-level investigations such as gene expression analysis or isolation and structural characterization of individual bioactive compounds. Furthermore, only the leaves of *Streblus* sp. were used in the study, and other plant parts, such as roots, stems, and bark, were not included in the analysis. The study was also limited to preliminary toxicity screening and did not extend to pharmacological validation or drug development.

MATERIALS AND METHODS

Research Design

This study employed an experimental quantitative research design to evaluate the phytochemical composition and cytotoxic activity of Makataynuk (*Streblus* sp.) leaf extracts (Creswell & Creswell, 2021). The methodology involved controlled laboratory experiments to determine the presence of bioactive compounds through qualitative phytochemical screening and to assess cytotoxic potential using the Brine Shrimp Lethality Assay (BSLA). Data were collected, statistically analyzed, and interpreted to establish the relationship between phytochemical composition and cytotoxic activity.

Research Setting

This study was conducted in two main laboratory settings to ensure proper execution of all experimental procedures. The extraction and preparation of plant samples, and the Brine Shrimp Lethality Assay (BSLA) using *Artemia salina* nauplii, were carried out at the Natural Sciences Laboratory of Misamis University, which provided controlled environmental conditions suitable for laboratory experimentation and bioassay procedures.

Meanwhile, the qualitative phytochemical screening of the plant extracts was performed at the Department of Chemistry, Mindanao State University–Iligan Institute of

Technology (MSU-IIT), under the supervision of a licensed chemist. This setting provided the necessary facilities and expertise for standardized phytochemical analysis. All procedures were conducted in accordance with institutional laboratory safety protocols and research guidelines to ensure accuracy, reliability, and safety throughout the study. The location of the plant collection site is shown in Figure 2. The geographic map identifies Lake Duminagat in Don Victoriano, Misamis Occidental, where the Makataynuk (*Streblus* sp.) sample was collected.



Figure 2. Geographic Map of Lake Duminagat Indicating the Collection Sites of Plant Samples

Plant Collection and Authentication

The collection of plant samples was conducted in accordance with the regulations of the Mt. Malindang Range Natural Park Protected Area Management Board (PAMB). Prior to sample collection, approval was obtained through PAMB Resolution No. 15,



which authorized the conduct of the research study within the protected area and required the researchers to comply with the necessary permitting requirements, including the application for a Gratuitous Permit (GP) from the Department of Environment and Natural Resources (DENR). The plant samples were collected with the assistance of local farmers and community members in Don Victoriano, Misamis Occidental. As shown in Figure 3, the plant was identified as Makataynuk (*Streblus* sp.) based on its morphological characteristics. The collection process was documented through morphological descriptions and photographs (Cook & Lee, 2021; Mahamat Ousman et al., 2025).

Figure 3. Morphological Features of the Makataynuk Tree

Preparation of Plant Extracts

The gathered Makataynuk plant materials were air-dried at ambient temperature for about two weeks. As shown in Figure 4, the dried Makataynuk leaves were pulverized by blending to obtain fine-powdered samples for extraction. The resulting powder was soaked in absolute methanol at a 1:5 (g: mL) ratio for 72 hours in the dark to prevent compound degradation. The methanolic extract was filtered using Whatman No. 1 filter paper, concentrated via rotary evaporation at 35 °C, and subsequently freeze-dried. As shown in Figure 5, the filtration process was performed to separate the liquid extract from the plant residues.

For the aqueous extraction, the plant powder was boiled with distilled water at a 1:2 ratio for 20 minutes, then filtered and freeze-dried. Solvent partitioning was carried out using a separating funnel containing equal volumes (100 mL each) of the aqueous extract and hexane. The lower aqueous layer is re-extracted with ethyl acetate until the

separation is complete, as shown in Figure 6. Each of these fractions was concentrated and freeze-dried.

A stock solution of 1 mg/mL was created by dissolving 40 mg of each extract in 40 mL of distilled water, followed by serial dilution for later bioassays (Kavya et al., 2024; Sultana et al., 2023).



Figure 4. Pulverization of Dried Makataynuk Leaves Through Blending



Figure 5. Filtration Process of the Soaked Makataynuk Leaf Extract



Figure 6. Solvent Partitioning and Extraction of Makataynuk Leaf Extracts.

Phytochemical Testing

Qualitative phytochemical screening was conducted by a licensed chemist from the MSU-IIT Department of Chemistry using standardized analytical procedures and a modified protocol based on Claustra et al. (2005). The modifications employed in the procedure included the use of semi-quantitative intensity scoring to classify the level of

phytochemical detection as low (+), moderate (++), and high (+++), as well as slight adjustments in reagent volume and extract concentration to suit the available laboratory conditions and crude plant extracts used in the study. The analysis aimed to detect secondary metabolites, including alkaloids, flavonoids, tannins, glycosides, saponins, anthraquinones, and steroids. The intensity of phytochemical presence was recorded using a semi-quantitative scale: (+) low, (++) moderate, (+++) high, and (–) absent (Mahamat Ousman et al., 2025; Khan et al., 2022).

Brine Shrimp (*Artemia salina* L.) Lethality Assay

The cytotoxic activity of the plant extracts was evaluated using the Brine Shrimp Lethality Assay (BSLA) with *Artemia salina* nauplii. The brine shrimp eggs were obtained from Mindanao State University–Naawan and first hatched by incubating them in artificial seawater under continuous illumination for 48 hours, during which they developed into nauplii (Meyer et al., 1982; Hamidi et al., 2020).

The experiment was conducted in the Natural Sciences Laboratory of Misamis University under controlled laboratory conditions. Ten nauplii were transferred into each test vial following standard brine shrimp lethality assay procedures (Syahmi et al., 2021) containing 4 mL of artificial seawater and 1 mL of plant extract at varying concentrations (1 mg/mL, 0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL, 0.0625 mg/mL, and 0.03125 mg/mL). Each concentration was tested in triplicate to ensure the reliability of results (Shabir et al., 2021). As shown in Figure 7, *Artemia salina* nauplii were exposed to different concentrations of Makataynuk leaf extracts during the assay.

The positive control consisted of 1 mL dimethyl sulfoxide (DMSO) mixed with 4 mL of seawater, while the negative control contained only 5 mL of artificial seawater. After 24 hours of continuous light exposure, the number of surviving larvae was counted and recorded. The mortality data obtained were used to determine cytotoxic activity and to compute the median lethal concentration (LC_{50}) of the plant extracts (Pimenta et al.,



2020).

Figure 7. Exposure of Brine Shrimp Nauplii to Different Concentrations of Makataynuk Leaf Extracts

Data Analysis

The data obtained from the phytochemical screening and Brine Shrimp Lethality Assay (BSLA) using *Artemia salina* nauplii and Makataynuk (*Streblus* sp.) leaf extracts were organized, tabulated, and analyzed using appropriate statistical tools.

Phytochemical screening results were analyzed descriptively and presented using a semi-quantitative scale indicating the presence of secondary metabolites as follows: (+)

low, (++) moderate, (+++) high, and (–) absent. This allowed for the identification and comparison of bioactive compounds present in the different solvent extracts.

Mortality data from the BSLA were expressed as percentages and used to establish the dose–response relationship by plotting log-transformed extract concentrations against observed mortality rates. This provided a visual representation of the cytotoxic response of the test organisms.

Statistical Analysis

Prior to inferential analysis, the assumption of normality was tested using the Shapiro–Wilk test in SPSS version 29 to assess whether the data were normally distributed (Razali & Wah, 2011). Based on the results, appropriate statistical procedures were applied.

Differences in mortality rates across concentrations and solvent extracts were analyzed using one-way Analysis of Variance (ANOVA) in SPSS version 29.0 (Kim, 2021). When significant differences were identified, a Tukey Honest Significant Difference (HSD) post hoc test was conducted to determine specific group differences (Field, 2020).

The median lethal concentration (LC_{50}) values of the extracts were determined using probit regression analysis in PAST software version 3.4, which estimates the concentration required to cause 50% mortality of the test organisms (Finney, 1971; Shabir et al., 2021). This method is widely used in toxicological studies for quantifying cytotoxicity.

All statistical tests were performed at a 0.05 level of significance. The toxicity of the extracts was interpreted using established toxicity classification criteria, such as Clarkson's toxicity scale.

RESULTS AND DISCUSSION

Phytochemical screening of the crude extract

The phytochemical screening of the methanolic crude extract of Makataynuk (*Streblus* sp.) revealed the presence of several important secondary metabolites, including flavonoids, total phenolics, saponins, tannins, alkaloids, steroids, and terpenoids (Table 1). Among these, flavonoids, total phenolics, saponins, and tannins were highly detected (+++), alkaloids and steroids were moderately detected (++), while terpenoids were only slightly detected (+). Overall, this indicates that the plant contains a wide range of natural compounds that may be responsible for its biological and cytotoxic effects.

Table 1. Phytochemical screening of the methanolic crude extracts of Makataynuk, indicating its phytochemical constituents and their level of detection.

Phytochemicals	Level of Detections	Interpretation
Flavonoids	+++	High Detection
Total Phenolics	+++	High Detection
Saponins	+++	High Detection
Tannins	+++	High Detection
Steroids	++	Moderate Detection
Alkaloids	++	Moderate Detection
Terpenoids	+	Low Detection

Legend: high detection (+++), moderate detection (++), low detection (+), and no detection (-)

The strong presence of flavonoids and phenolic compounds is particularly important. These compounds are well known for their antioxidant and cytotoxic properties because they help neutralize harmful free radicals that can damage cells (Gulcin, 2020). In simpler terms, they help protect cells from stress and may also

interfere with processes that allow abnormal cells to grow and survive. Because of this, flavonoids and phenolics are often linked to biological activities such as antimicrobial, antioxidant, and cytotoxic effects (Do et al., 2020; Ramakrishna et al., 2021). Their abundance in the extract may therefore be one reason for the observed mortality in the Brine Shrimp Lethality Assay with *Artemia salina*. Similar observations were reported in several phytochemical studies showing that medicinal plants rich in flavonoids and phenolic compounds commonly exhibit strong antioxidant and cytotoxic activities because these compounds can interfere with oxidative pathways and cellular metabolism. Recent investigations have also demonstrated that phenolic-rich plant extracts may induce apoptosis and inhibit the proliferation of abnormal cells through modulation of oxidative stress and mitochondrial disruption (Kavya et al., 2024; Mahamat Ousman et al., 2025; Rahman et al., 2021; Santos et al., 2022). Furthermore, flavonoids are recognized for their ability to chelate metal ions, stabilize reactive oxygen species, and regulate inflammatory pathways, contributing to their broad pharmacological significance (Salmerón-Manzano et al., 2020).

Saponins were also detected at high levels in the extract. These compounds are known to interact with and disrupt cell membranes, thereby increasing membrane permeability and eventually causing cell breakdown or death. This membrane-disrupting property explains why saponins are often associated with cytotoxic activity in biological tests (Osbourn et al., 2011). Alongside this, tannins were also present in high amounts. Tannins can bind with proteins and enzymes, interfering with normal cellular functions such as metabolism and growth. This can further contribute to the plant extract's overall biological and cytotoxic effects (Khan et al., 2022). Previous studies have also associated

saponins with increased membrane permeability and leakage of intracellular components, resulting in cellular damage and mortality in various bioassay organisms. Tannins, meanwhile, may interfere with nutrient absorption and enzyme activity by forming complexes with proteins and cellular macromolecules (Fraga-Corral et al., 202; Osbourn et al., 2011; Khan et al., 2022). These mechanisms collectively support the observed cytotoxic response of the plant extract against *Artemia salina* nauplii.

Moderate levels of alkaloids and steroids were also observed. Alkaloids are important bioactive compounds known for their antimicrobial, analgesic, and cytotoxic properties. Some alkaloids can interfere with DNA replication and cell division (Roy, 2021), which is why they are widely studied in pharmaceutical research (Singh & Sharma, 2024). Steroids, on the other hand, may help regulate inflammation and other cellular processes through their interaction with cell signaling pathways (Salmerón-Manzano et al., 2020). Although terpenoids were only weakly detected, they remain biologically important because many exhibit antioxidant, antimicrobial, and cytotoxic activities, even in small amounts. Several pharmacological studies have demonstrated that alkaloid-containing medicinal plants exhibit significant cytotoxic and antimicrobial activities due to their capacity to inhibit nucleic acid synthesis and alter membrane transport processes (Singh & Sharma, 2024; Abdullahi et al., 2021). In addition, plant steroids and terpenoids have been reported to possess anti-inflammatory, antioxidant, and antiproliferative properties that may further contribute to the biological activity of medicinal plant extracts (Ningsih et al., 2021; Kavya et al., 2024).

The high detection of these polar phytochemicals can be explained by the use of methanol as the extraction solvent. Methanol is highly effective for extracting polar

compounds such as flavonoids, phenolics, tannins, and glycosides because these compounds readily dissolve in polar solvents (Azwanida, 2022). Previous studies have also shown that methanolic extracts often yield a wider range of phytochemicals and stronger biological activity than those from less polar solvents (Sultana et al., 2023). This supports the strong presence of phytochemicals observed in this study.

Brine Shrimp Lethality of Plant Extracts Using Different Solvents

The solvent extracts demonstrated a clear concentration-dependent lethality against *Artemia salina* nauplii, with mortality increasing progressively with extract concentration. This dose–response relationship was consistently observed across all solvent fractions, indicating that the toxic effect of the extracts was directly associated with extract concentration and the abundance of bioactive constituents.

The mean percent mortality values presented in Table 2 further reinforced the concentration-dependent toxicity pattern observed in the mortality counts. Mortality increased steadily with increasing concentration in all extracts, while the methanolic extract maintained the highest mean mortality at every exposure level. At 1.0 mg/mL, the methanolic extract produced $100.00 \pm 0.00\%$ mortality, whereas the hexane extract showed the lowest mortality ($80.00 \pm 0.00\%$) at the same concentration. The relatively low mortality values recorded at lower concentrations indicate that toxic effects diminished substantially as exposure decreased.

Notably, the standard deviation values across treatments were generally low (± 0.00 to ± 1.15), indicating minimal variability among replicates and demonstrating strong experimental consistency and reproducibility. Low variability strengthens confidence that the observed mortality differences were primarily attributable to extract

activity rather than random experimental variation or handling error. Similar observations have been reported in reliable brine shrimp lethality studies employing replicated bioassays (Hamidi et al., 2020; Apu et al., 2022). The high mortality observed in the positive control confirms the sensitivity of the nauplii to toxic substances under the assay conditions. In contrast, the absence of mortality in the negative control validated that the artificial seawater itself produced no toxic effect.

Table 2. Mean percent mortality ($\pm$ SD) of *Artemia salina* nauplii exposed to different solvent extracts and controls

Concentration (mg/mL)	Aqueous	Methanolic	Ethyl acetate	Hexane
1.0	86.67 $\pm$ 0.58	100.00 $\pm$ 0.00	92.22 $\pm$ 0.58	80.00 $\pm$ 0.00
0.50	73.33 $\pm$ 0.58	90.00 $\pm$ 0.00	83.33 $\pm$ 0.58	66.67 $\pm$ 0.58
0.25	56.67 $\pm$ 0.58	73.33 $\pm$ 1.15	66.67 $\pm$ 0.58	50.00 $\pm$ 0.00
0.125	40.00 $\pm$ 0.00	53.33 $\pm$ 0.58	46.67 $\pm$ 0.58	33.33 $\pm$ 0.58
0.0625	23.33 $\pm$ 0.58	33.33 $\pm$ 0.58	26.67 $\pm$ 0.58	16.67 $\pm$ 0.58
0.03125	10.00 $\pm$ 0.00	16.67 $\pm$ 0.58	13.33 $\pm$ 0.58	6.67 $\pm$ 0.58
Positive Control (DMSO)	96.67 $\pm$ 0.58	96.67 $\pm$ 0.58	96.67 $\pm$ 0.58	96.67 $\pm$ 0.58
Negative Control (Artificial Sea Water)	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00

Legend: Values are expressed as mean percentage mortality $\pm$ standard deviation (SD) based on triplicate determinations.

The observed concentration-dependent trend is consistent with the general toxicological principle that increasing exposure levels increase organismal susceptibility to phytochemical toxicity. Similar dose-dependent mortality patterns have been widely reported in brine shrimp lethality assays involving medicinal plant extracts and are considered indicative of biologically active secondary metabolites (Meyer et al., 1982; Hamidi et al., 2020; Ahmed et al., 2021). The assay is widely recognized as a rapid preliminary screening tool for detecting cytotoxic, pesticidal, and pharmacologically active compounds because *A. salina*'s sensitivity correlates well with the presence of potent bioactive metabolites. The increasing mortality observed at higher concentrations

may indicate that greater exposure levels allowed larger amounts of phytochemicals to penetrate the nauplii and interfere with essential physiological processes. Bioactive compounds such as flavonoids, alkaloids, tannins, and saponins are known to alter membrane stability, inhibit respiratory enzymes, and induce oxidative stress, ultimately leading to organismal death (Hamidi et al., 2020; Ahmed et al., 2021; Apu et al., 2022). Similar concentration-dependent mortality responses have been consistently reported in brine shrimp lethality assays involving medicinal plant extracts rich in secondary metabolites (Maghanoy et al., 2022; Ismail et al., 2025).

Among the solvent fractions, the methanolic extract consistently exhibited the highest lethality across all concentrations. This finding suggests that methanol extracted a greater quantity of potent cytotoxic constituents compared with the other solvents. Methanol is highly effective for extracting polar and moderately polar phytochemicals, including flavonoids, phenolic compounds, tannins, alkaloids, glycosides, and certain saponins, many of which exhibit cytotoxic and membrane-disrupting activities (Do et al., 2020; Azwanida, 2022). The strong activity of the methanolic fraction, therefore, implies that the major toxic constituents of the plant are predominantly polar in nature. The stronger activity of the methanolic extract further supports the established principle that polar solvents extract greater quantities of biologically active constituents compared with nonpolar solvents. Methanol is particularly effective in dissolving phenolic acids, flavonoids, glycosides, and alkaloids because of its high polarity and penetration efficiency into plant tissues (Azwanida, 2022; Sultana et al., 2023). Previous comparative extraction studies similarly demonstrated that methanolic fractions frequently exhibit

stronger antioxidant and cytotoxic activities than aqueous and hexane extracts (Do et al., 2020; Khan et al., 2022).

The ethyl acetate extract also demonstrated relatively high toxicity, particularly at higher concentrations, suggesting that semi-polar constituents contributed substantially to the observed lethality. Ethyl acetate commonly extracts flavonoid aglycones, terpenoids, and other semi-polar metabolites that have previously been associated with cytotoxic and antioxidant activities (Ningsih et al., 2021; Rahman et al., 2021). In contrast, the comparatively lower toxicity observed in the hexane extract indicates that nonpolar constituents contributed less to nauplii mortality. Nonpolar solvents such as hexane primarily extract lipids, waxes, chlorophylls, and nonpolar terpenes, which may possess weaker cytotoxic potential than polar phytochemicals (Sultana et al., 2022). The overall toxicity ranking observed in the present study, therefore, supports the established influence of solvent polarity on phytochemical extraction efficiency and biological activity.

The one-way ANOVA analysis confirmed that concentration significantly influenced nauplii mortality in all solvent extracts. As shown in Table 3, highly significant differences were observed among concentration groups for aqueous ($F = 185.14$), methanolic ($F = 131.41$), ethyl acetate ($F = 143.35$), and hexane extracts ($F = 181.23$), with all p -values below 0.0001. These findings indicate that the observed mortality responses were statistically dependent on extract concentration. Among the solvent extracts, the aqueous extract exhibited the highest F -value, indicating substantial variation in mortality responses across concentrations. The highly significant ANOVA results indicate that concentration was a major factor influencing nauplii mortality across

all solvent extracts. Statistical validation through ANOVA is important in cytotoxicity studies because it confirms that observed biological responses are unlikely to have occurred by random variation alone (Kim, 2021). Similar highly significant mortality differences among concentration groups have also been reported in other brine shrimp lethality studies involving medicinal plants with potent phytochemical constituents (Arulpriya et al., 2021; Ahmed et al., 2021).

Table 3. Statistical comparison of mortality responses among extract concentrations and controls using one-way ANOVA

Extract	F-value	p-value	Decision
Aqueous	185.14	<0.0001*	Reject H ₀
Methanolic	131.41	<0.0001*	Reject H ₀
Ethyl acetate	143.35	<0.0001*	Reject H ₀
Hexane	181.23	<0.0001*	Reject H ₀

*Note: *Significant at $p < 0.05$.*

The extremely low p-values demonstrate that differences in mortality among concentration treatments were highly significant, thereby rejecting the null hypothesis of no difference between concentration groups. The large between-group variation relative to within-group variation further indicates that the extracts produced measurable and biologically meaningful toxic effects on *Artemia salina*. Similar statistically significant dose-dependent mortality responses have been reported in other phytotoxicity and cytotoxicity studies using brine shrimp assays (Ahmed et al., 2021; Hamidi et al., 2020).

The Tukey HSD grouping analysis presented in Figure 8 provided further clarification regarding the differences in mortality responses among extract concentrations. Most concentration groups showed statistically significant differences ($p < 0.05$), particularly in comparisons involving the highest concentration (1.0 mg/mL) and the lower-concentration treatments. These findings further confirm that mortality increased substantially with increasing extract concentration. The grouping pattern showed that

higher concentrations generally formed distinct statistical groups relative to lower concentrations, indicating stronger cytotoxic effects at elevated exposure levels.

However, several lower concentration treatments, particularly in the methanolic and ethyl acetate extracts, showed overlapping groupings, suggesting that their mortality responses were not significantly different from one another. This may indicate the presence of a threshold or plateau effect at lower concentrations, wherein reductions in concentration no longer produce proportionally detectable decreases in mortality. Similar plateau responses have been reported in previous brine shrimp lethality studies, in which mortality approaches baseline levels at sublethal concentrations (Arulpriya et al., 2021). The observed grouping patterns suggest that below certain exposure thresholds, the concentration of active phytochemicals may become insufficient to induce substantial physiological disruption in *Artemia salina* nauplii. Similar findings were reported in previous toxicological studies involving medicinal plant extracts, in which low extract concentrations produced minimal differences in mortality responses due to reduced availability of bioactive compounds (Shabir et al., 2021; Pimenta et al., 2020). Overall, the Tukey HSD grouping analysis supports the dose-dependent cytotoxic activity of the solvent extracts and confirms the significant influence of extract concentration on nauplii mortality.

Probit regression analysis provided quantitative evidence of the relative toxicity of the different solvent extracts against *Artemia salina* nauplii. As presented in Table 4, the methanolic extract exhibited the lowest LC_{50} value (25.11 $\mu\text{g/mL}$), indicating the highest toxicity among all tested extracts, followed by the ethyl acetate extract (104.72 $\mu\text{g/mL}$), aqueous extract (312.45 $\mu\text{g/mL}$), and hexane extract (447.83 $\mu\text{g/mL}$).

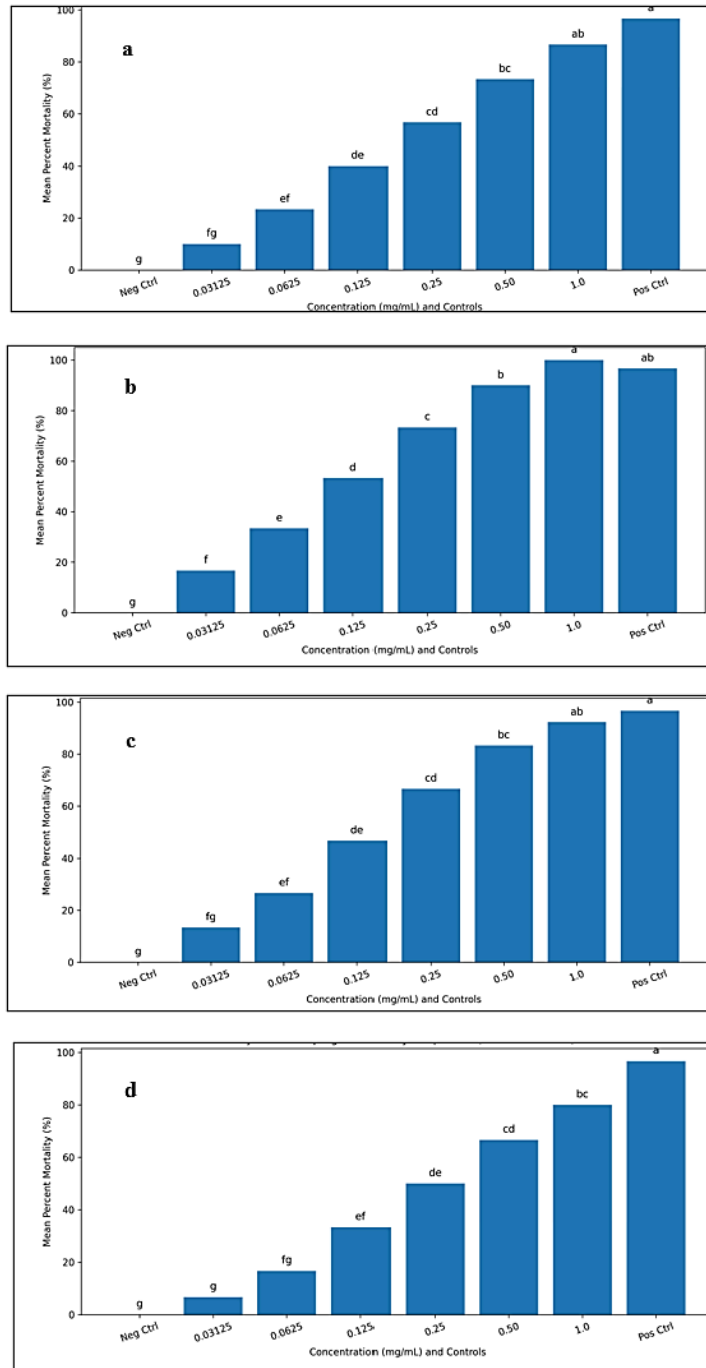


Figure 8. Tukey HSD Grouping of Mortality Responses in Different Extract Concentrations: (a) aqueous, (b) methanolic, (c) ethyl acetate, and (d) hexane extracts. Concentrations followed by the same letter are not significantly different, whereas concentrations followed by different letters indicate significant differences at $p < 0.05$.

Since lower LC₅₀ values correspond to greater biological potency, the results clearly demonstrate that the methanolic fraction possessed substantially stronger cytotoxic activity compared with the semi-polar and nonpolar extracts. This toxicity ranking is consistent with the mortality trends observed in previous assays, in which the methanolic extract consistently produced the highest nauplii mortality across all concentration levels.

Table 4. Probit regression analysis and LC₅₀ values of different solvent extracts against *Artemia salina* nauplii

Extract	LC ₅₀ (µg/mL)	95% Confidence Interval (µg/mL)	Probit Slope (b ± SE)	R ²	Toxicity Classification
Methanolic	25.11	19.42–32.87	2.08 ± 0.21	0.981	Highly toxic
Ethyl acetate	104.72	86.15–129.33	1.74 ± 0.14	0.998	Moderately toxic
Aqueous	312.45	255.60–392.18	1.56 ± 0.12	0.997	Slightly toxic
Hexane	447.83	365.22–559.41	1.55 ± 0.13	0.995	Least toxic

Note: Lower LC₅₀ values indicate greater cytotoxic activity.

Based on the toxicity classification proposed by Meyer et al. (1982), extracts with LC₅₀ values below 100 µg/mL are considered highly toxic, while higher LC₅₀ values indicate moderate to low toxicity. Accordingly, the methanolic extract can be classified as highly toxic. In contrast, the ethyl acetate extract exhibited moderate to high toxicity, whereas the aqueous and hexane extracts showed comparatively lower toxicity. The markedly lower LC₅₀ value of the methanolic extract suggests the presence of highly active cytotoxic phytochemicals with potential pharmacological or pesticidal applications. Brine shrimp lethality has long been recognized as a preliminary indicator of biologically active compounds with possible anticancer, antimicrobial, insecticidal,

and antitumor properties; therefore, extracts exhibiting strong lethality may warrant further phytochemical and bioactivity-guided investigations (Hamidi et al., 2020; Pimenta et al., 2020). According to established toxicity criteria, extracts with LC_{50} values below 100 $\mu\text{g/mL}$ are generally considered highly bioactive and potentially suitable for further pharmacological investigation (Meyer et al., 1982). The high lethality of the methanolic extract, therefore, suggests the presence of potent cytotoxic constituents with anticancer, pesticidal, or antimicrobial activity. Previous studies have shown that plant extracts demonstrating strong brine shrimp lethality often correlate with cytotoxic effects in more advanced biological systems, supporting the usefulness of BSLT as a preliminary screening tool in natural product research (Hamidi et al., 2020; Singh & Sharma, 2024).

The strong activity observed in the methanolic extract may be attributed to methanol's high extraction efficiency for polar and moderately polar secondary metabolites. Methanol is widely regarded as one of the most effective solvents for extracting bioactive phytochemicals such as flavonoids, tannins, alkaloids, phenolic acids, glycosides, and saponins, many of which possess membrane-disrupting, oxidative, or cytotoxic properties (Do et al., 2020; Azwanida, 2022). These compounds may interfere with cellular respiration, membrane permeability, enzyme activity, or ion regulation in the nauplii, ultimately leading to mortality. In contrast, the lower toxicity observed in the hexane extract suggests that nonpolar constituents contributed less to the plant's overall cytotoxic activity. Hexane primarily extracts lipophilic compounds such as fats, waxes, chlorophylls, and nonpolar terpenes, which may possess comparatively lower toxicity in brine shrimp assays (Sultana et al., 2022). The intermediate toxicity of the

ethyl acetate fraction further suggests that semi-polar compounds also contributed significantly to the observed bioactivity.

The probit slopes obtained from the regression analysis further provided insight into the dose–response characteristics of the extracts. The methanolic extract exhibited the highest slope (2.08 ± 0.21), indicating a steeper dose–response relationship, in which small increases in concentration led to rapid increases in mortality. Steeper slopes are generally associated with more uniform biological responses and stronger toxic potency because the active compounds exert their effects within a narrower concentration range. Conversely, the relatively lower slope values observed in the aqueous and hexane extracts suggest more gradual mortality responses, indicating weaker or less concentrated toxic constituents.

Moreover, the narrow 95% confidence intervals and high coefficients of determination ($R^2 = 0.981\text{--}0.998$) demonstrate that the probit regression models fitted the mortality data exceptionally well. High R^2 values indicate that concentration strongly explained the variation in mortality response, while narrow confidence intervals suggest good precision and reliability of the LC_{50} estimates. These findings strengthen the validity of the bioassay results and indicate that the experimental data were highly consistent across replicates. Probit analysis is widely applied in toxicological and pharmacological studies because it effectively models sigmoid dose–response relationships and provides robust estimation of median lethal concentrations (Shabir et al., 2021).

The probit analysis confirms that solvent polarity played a major role in determining the extraction of cytotoxic constituents from the plant material. The significantly greater toxicity of the methanolic extract indicates that polar phytochemicals

were likely the principal contributors to the observed lethality against *A. salina*. Since brine shrimp lethality is commonly used as an inexpensive preliminary screening method for identifying biologically active natural products, the present findings suggest that the methanolic extract may contain promising bioactive compounds with potential pharmaceutical, pesticidal, or anticancer applications. Further studies involving phytochemical profiling, compound isolation, and mechanistic toxicity evaluation are therefore recommended to identify the specific metabolites responsible for the observed cytotoxic effects.

CONCLUSIONS AND RECOMMENDATIONS

This study demonstrated that Makataynuk (*Streblus* sp.) leaf extracts contain several bioactive secondary metabolites, including flavonoids, phenolics, saponins, tannins, alkaloids, steroids, and terpenoids. The extracts exhibited concentration-dependent cytotoxic activity against *Artemia salina* nauplii, with the methanolic extract showing the highest toxicity and lowest LC₅₀ value among all solvent extracts tested. These findings indicate that *Streblus* sp. possesses biologically active compounds with potential pharmacological importance.

Based on the study's findings, it is recommended that future research conduct quantitative phytochemical analysis to determine the concentrations of specific secondary metabolites in the different solvent extracts of Makataynuk (*Streblus* sp.). Further evaluation using advanced cytotoxicity assays, such as cell-line testing and in vivo studies, is also recommended to validate the biological activity and safety of the extracts. In addition, isolation and characterization of the specific bioactive compounds responsible for the observed cytotoxic effects should be performed using chromatographic and spectroscopic methods. Future studies may also evaluate other biological properties of *Streblus* sp., including antimicrobial, antioxidant, and anticancer activities, using standardized laboratory methods.

DECLARATION OF THE USE OF AI TOOLS

We acknowledge the use of artificial intelligence (AI) tools such as ChatGPT, QuillBot, and Grammarly in assisting with the preparation of this research paper. These tools were used mainly to correct grammar, improve sentence construction, and paraphrase selected statements into formal academic language.

Prompts Used:

“Improve the grammar of this paragraph.”

“Paraphrase this sentence using formal academic language.”

“Revise this sentence for clarity.”

“Check the grammar and sentence structure.”

Uses:

We reviewed and modified the generated outputs to improve grammar, sentence structure, clarity, and overall readability of the manuscript. All ideas, interpretations, analyses, and conclusions presented in this study remain the responsibility of the researchers.

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APPENDICES

APPENDIX A: Informed Consent

 **Misamis University**
Ozamiz City

MISAMIS UNIVERSITY RESEARCH CENTER
CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippine Association of Colleges and Universities Commission on Accreditation (PACUCOA)

MU-RC-042/13 November 2024

Thesis/Research Data Gathering Approval Form

Date: January 22, 2024

Name of Authors: Helena Margrethe D. Doloso, Winona Renz F. Duran, Nicjen Elfir L. Malague

College/Department: College of Medical Technology

Research Title: Phytochemical Evaluation and Anti-Angiogenic Activity of "Makataynuk" Leaf Extracts Using the Chorioallantoic Membrane Assay on Duck Embryo

Data Gathering Procedure:

a. Type of Research: (Kindly check)

☐ Quantitative	☐ Qualitative	☒ Experimental
☒ Approved Research Title & Problem/Objectives	☒ Approved Data Gathering Procedure	☒ Approved Data Gathering Instrument/Data Sheets

b. Respondents/Participants/Subject/Samples to be Collected: Makataynuk leaf and Duck Embryo

c. Procedure:

☒ Survey	☐ Interview	☒ Experimentation	☒ Laboratory Testing
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d. Place of Implementation/Study Area: Misamis University Science Building

If to be conducted outside the school campus, indicate the nature of transportation to be used:

☒ Own private vehicle	☒ public utility vehicle	☐ rental	☐ Others, please indicate _____
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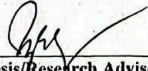
e. Date/s of Implementation/Sampling: February 10, 2024 on wards

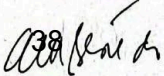
Attachments:

☐ Approved Letter of Consent from the designated authority in the area/place where the study is to be conducted
☒ Signed Parents' Consent

The thesis proposal mentioned above is hereby recommended for the commencement of data gathering implementation.

Recommended by:

 Thesis/Research Adviser	 Thesis/Research Instructor
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Approved by: 
College Dean

CONTROLLED DOCUMENT

APPENDICES

APPENDIX B: Approved Letter of Consent



Misamis University

Ozamiz City



COLLEGE OF MEDICAL TECHNOLOGY

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippines Association of Colleges and Universities Commission on Accreditation (PACUCOA)

January 22, 2026

DR. HAYDEE D. VILLANUEVA
Dean, College of Arts and Sciences
Misamis University

Dear Dr. Villanueva,

Good day!

We, the undersigned third-year Bachelor of Science in Medical Technology students, respectfully request permission from your good office to use the facilities in Natural Sciences Laboratory for the **pulverization (blending) of our plant samples** in connection with our research entitles: **“PHYTOCHEMICAL EVALUATION AND ANTI-ANGIOGENIC ACTIVITY OF ‘MAKATAYNUK’ LEAF EXTRACTS USING THE CHORIOALLANTOIC MEMBRANE ASSAY ON DUCK EMBRYO.”**

We assure you that proper laboratory rules, cleanliness, and safety protocols will be strictly observed during the activity under the supervision of our research adviser.

We hope for your kind approval. Thank you very much.

Respectfully yours,

HELENA MARGRETHE D. DOLLOSO

WINONA RANZ F. DURAN

NIEJEN ELFIE L. HATAGUE
BS Medical Technology – 3rd Year Researchers

Noted by:
YUNALYN L. VILLANTES, PhD
Research Adviser

ARJADE O. ALCABAZA, RChT
Laboratory In-charge

EVANGELINE M. SEÑEDO, RMT, MATMRS
Dean, College of Medical Technology

Recommending Approval:

NELFA D. CANINI, PhD
Chairperson, Department of Natural Sciences

Approved by:

HAYDEE D. VILLANUEVA, PhD
Dean, College of Arts and Sciences

APPENDICES

APPENDIX B Cont'd

	MISAMIS UNIVERSITY Ozamiz City Department of Student Affairs & Services
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MU-DSAS-006/02June2023

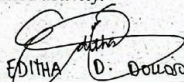
PARENT'S CONSENT

Sponsoring Group/Organization

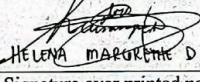
I am allowing my son/daughter/ward, Helena Margrethe D. Dollosro, to join the Research Paper Writing and Presentation (activity) to be held on January - May 2026 at Misamis University under the supervision of Yunallyn L. Villantes (Adviser/Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.


EDITHA D. Dollosro
Signature over printed name of
Parent/Guardian

Jan 18, 2026
Date


HELENA MARGRETHE D. DOLLOSRO
Signature over printed name of
Student

Jan 18, 2026
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this 20 JAN 2026 at OZAMIZ CITY, Philippines, that the herein affiant personally came and appeared with his/her _____ as evidence of his/her personal identity.

Doc. No. 277
Page No. 46
Book No. VII
Series of 20 26

ATTY. JASON ERIC MARAVE
NOTARY PUBLIC
WASHINGTON ST., 5TH DISTRICT, OZAMIZ CITY
NOTARIAL COMMISSION VALID UNTIL 06/30/27
PTR NO. 9409 DATE 06/30/21
IBP NO. 82221 DATE 05/30/23
MCLE COMPLIANCE NO. VIII-0041696 VALID UNTIL 04/14/2028
Roll No. _____

(For DSAS Personnel only)

Attested by:


RODUL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date: _____

APPENDICES

APPENDIX B Cont'd



MISAMIS UNIVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, Winona Renz F. Duran, to join the Research Paper Writing and Presentation (activity) to be held on January - May 2026 at Misamis University under the supervision of Yunolyn L. Villantes (Adviser/Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

ELENA F. DURAN
Signature over printed name of
Parent/Guardian

Jan 18, 2026
Date

WINONA RENZ F. DURAN
Signature over printed name of
Student

Jan 18, 2026
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this 20 JAN 2026 day of JANUARY at OZAMIZ CITY, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 224
Page No. 49
Book No. 111
Series of 20 46

ATTY. JASON CHONG MARAVE
NOTARY PUBLIC
WASHINGTON ST., 50TH DISTRICT, OZAMIZ CITY
NOTARIAL COMMISSION AND UNTIL DECEMBER 31, 2027
PTR NO. 800592 DATE: 01/05/2025
IBF NO. 469 DATE: 05/21/2022
ATTORNEY'S REG. NO.: 82221 DATE: 05/30/2022
MCLE COMPLIANCE NO. 0041696 VALID UNTIL 04/14/2028
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by:

RODEL L. SALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date: _____

APPENDICES

APPENDIX B Cont'd



MISAMIS UNIVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, NIELO ELITE L. VILLANUE, to join the RESEARCH PAPER WRITING & PRESENTATION (activity) to be held on JANUARY 10 MM 2024 at MISAMIS UNIVERSITY under the supervision of YONALYN L. VILLANUE (Adviser/Faculty).

I understand that Misamis University exercises the necessary safety precautions in this activity.

In consideration of the benefits to be derived from the above activity, I expressly waive any and all claims against the administration or any member of the faculty and staff of the Misamis University on account of any unforeseen accident or injury that my son/daughter/ward might incur in connection with the aforementioned activity.

ARVINE L. VILLANUE
Signature over printed name of
Parent/Guardian

01/19/24
Date

NIELO ELITE L. VILLANUE
Signature over printed name of
Student

01/19/24
Date

Important: This form shall be submitted to the DSAS Office at least two (2) days before the conduct of the activity.

SUBSCRIBED AND SWORN to before me, this 20 JAN 2026 day of JANUARY at OZAMIZ CITY, Philippines, that the herein affiant personally came and appeared with his/her [Signature], as evidence of his/her personal identity.

Doc. No. 272
Page No. 48
Book No. VII
Series of 20 26

ATTY. JASON CHONG MARAVE
NOTARY PUBLIC
WASHINGTON ST., SOUTH DISTRICT, OZAMIZ CITY
NOTARIAL COMMISSION VALID UNTIL FEBRUARY 31, 2027
PTR NO. 007 DATE: 01/05/2023
IBP NO. 007 DATE: 05/30/2022
ATTORNEY'S REG. NO. 82221 DATE: 05/30/2022
MCLE COMPLIANCE NO. 0041696 VALID UNTIL 04/14/2028
Roll No. _____

(For DSAS Personnel only)

Attested by:

RODEL L. BALDADO, MHist, MAEd
OIC, Department of Student Affairs & Services

Date: _____

APPENDICES

APPENDIX C: Letter Request for Plant Collection and Gratuitous Permit



Misamis University

Ozamiz City



COLLEGE OF MEDICAL TECHNOLOGY

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippines Association of Colleges and Universities Commission on Accreditation (PACUOCA)

January 22, 2026

FOR. ALSON D. POTUTAN
PARK OPERATION
SUPERINTENDENT IV
DENR-MMRNP

SUBJECT: Request for Permit/Clearance to Collect Leaf Samples of "Makataynuk" in Lake Duminagat

Dear For. Potutan:

Warm greetings!

We, the undersigned 3rd year Medical Technology students, respectfully request permission from your good office to collect leaf samples of the plant locally known as "Makataynuk," a native species found in Lake Duminagat, for academic research purposes.

The leaf samples will be used in our study entitled: "PHYTOCHEMICAL EVALUATION AND ANTI-ANGIOGENIC ACTIVITY OF 'MAKATAYNUK' LEAF EXTRACTS USING THE CHORIOALLANTOIC MEMBRANE ASSAY ON DUCK EMBRYO." This research aims to evaluate the phytochemical constituents of *Makataynuk* leaves and determine their potential anti-angiogenic activity using the chorioallantoic membrane (CAM) assay on duck embryos. The study is purely for educational and scientific purposes and will be conducted in compliance with ethical standards and environmental conservation guidelines. Only a minimal amount of plant material will be collected to avoid any negative impact on the natural population of the species.

We assure your good office that proper documentation, responsible collection, and respect for biodiversity will be strictly observed throughout the conduct of the study.

In this regard, we humbly seek your approval to collect the necessary leaf samples from Lake Duminagat. We are hoping for your favorable response.

Thank you very much for your time and consideration.

Respectfully yours,

NIEJEN ELFIE L. HATAGUE

WINONA RENZ E. DURAN

HELENA MARGRETHE D. DOLLOSO

Noted by:

YUNALYN L. VILLANTES, MSc. Bio, PhD
Research Adviser

EVANGELINE M. SEÑEDO, RMT, MATMRS
Dean, College of Medical Technology

Approved by:

FOR. ALSON D. POTUTAN
PARK OPERATION
MMRNP

APPENDICES

APPENDIX C Cont'd

**Misamis University**
Ozamiz City
COLLEGE OF MEDICAL TECHNOLOGY

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippines Association of Colleges and Universities Commission on Accreditation (PACUCOA)

May 20, 2026

HENRY A. ADORNADO, Ph.D.
Regional Executive Director
DENR-10/PAMB Chair

Subject: Letter of Intent for the Application of Gratuitous Permit

Dear Sir,

We are Third Year Bachelor of Science in Medical Technology students of Misamis University currently conducting our undergraduate research entitled **“PHYTOCHEMICAL EVALUATION AND CYTOTOXIC ACTIVITY OF *Streblus* sp. (“Makataynuk”) (Family Moraceae) LEAF EXTRACTS USING BRINE SHRIMP (*Artemia salina*) LETHALITY ASSAY”**

In line with this, we respectfully submit this letter of intent as part of the requirements for securing a Gratuitous Permit (GP). The said permit will allow us to collect limited plant specimens necessary for our study from selected areas in Don Victoriano, Misamis Occidental for academic and research purposes.

We assure your good office that all research activities and plant collection procedures will strictly comply with the rules, regulations, and guidelines set by the Department of Environment and Natural Resources (DENR). Collection activities will be conducted responsibly, minimally, and with full consideration for environmental conservation and ecological integrity. We are also willing to comply with any additional requirements or conditions that your office may deem necessary.

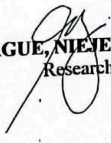
We sincerely hope for your favorable consideration of our request. Should you require further information or supporting documents, we would be willing to provide them anytime.

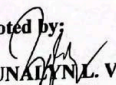
Thank you very much, and we look forward to your kind assistance and support.

Respectfully yours,

DOLLOSO, HELENA MARGRETHE D.
Researcher

DURAN, WINONA RENZ F.
Researcher


HATAGUE, NIJEN ELFIE L.
Researcher


YUNALYN L. VILLANTES, Ph.D.
Research Adviser
Misamis University


NELFA D. CANINI, Msc, Bio, Ph.D.
Research Instructor
Misamis University

APPENDICES

APPENDIX C Cont'd



Misamis University

Ozamiz City



COLLEGE OF MEDICAL TECHNOLOGY

CERTIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippines Association of Colleges and Universities Commission on Accreditation (PACUCOA)

May 20, 2026

HENRY A. ADORNADO, PhD

Regional Executive Director
Department of Environment and Natural Resources – Region X
Cagayan de Oro City

Subject: Endorsement for Gratuitous Permit Application

Dear Regional Director:

Greetings of peace!

This letter serves as a formal endorsement of the undergraduate research study entitled **“PHYTOCHEMICAL EVALUATION AND CYTOTOXIC ACTIVITY OF *Streblus* sp. (“Makataynuk”) (Family Moraceae) LEAF EXTRACTS USING BRINE SHRIMP (*Artemia salina*) LETHALITY ASSAY”** to be conducted by the following Bachelor of Science in Medical Technology students of Misamis University:

- Dolloso, Helena Margrethe D.
- Duran, Winona Renz F.
- Hatague, Niejen Elfie L.

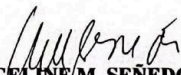
The study aims to evaluate the phytochemical composition and cytotoxic activity of “Makataynuk” leaf extracts for academic and scientific research purposes. In connection with this, the researchers intend to collect limited plant samples from designated areas in Don Victoriano, Misamis Occidental, in compliance with existing environmental laws, rules, and regulations.

The College of Medical Technology fully supports this academic undertaking and assures your good office that all collection activities will be conducted responsibly, ethically, and strictly in accordance with the guidelines and requirements set by the Department of Environment and Natural Resources (DENR). Proper documentation and reporting procedures shall likewise be observed.

In view thereof, we respectfully endorse the application of the above-named students for a Gratuitous Permit (GP) from your good office.

Thank you for your continued support in promoting academic research and environmental conservation.

Respectfully yours,


EVANGELINE M. SENEDO, RMT, MATMRS
Dean, College of Medical Technology

APPENDICES

APPENDIX C Cont'd



Misamis University
Ozamiz City
COLLEGE OF MEDICAL TECHNOLOGY



STIFIED: ISO 21001: 2018 Educational Organizations Management System by DNV
ACCREDITED: Philippines Association of Colleges and Universities Commission on Accreditation (PACUCOA)

April 14, 2026

ORLYN RECOLITO

Gokum
Brgy. Lake Duminagat
Don Victoriano, Misamis Occidental

Dear Gokum Orlyn,

Maayong adlaw!

Kami ang mga Third Year Bachelor of Science in Medical Technology nga mga estudyante sa Misamis University. Sa pagkakaran, nagahimo kami sa among research nga adunay titulo: *"Phytochemical Evaluation and Anti-Angiogenic Activity of 'Makataymuk' Leaf Extracts Using the Chorioallantoic Membrane Assay on Duck Embryo."*

Kabahin sa among pagtuon, nagkinahanglan kami og mga sample sa tanom nga makolekta gikan sa lugar sa Brgy. Lake Duminagat, Don Victoriano, Misamis Occidental.

Busa, kami nagpadala niini nga sulat aron mangayo og pagtugot sa pagkolekta sa gikinahanglan nga mga plant specimens sa maong lugar, alang lamang sa akademikong katuyoan ug panukiduki.

Among ginapasalig nga ang pag-collect sa mga tanom buhaton namo sa responsable, hapsay, ug kontrolado nga paagi aron malikayan ang kadaot sa kalikupan. Andam usab kami mosunod sa tanang guidelines, regulasyon, ug mga kondisyon nga inyong ipatuman.

Nagalaum kami sa inyong maayong kasingkasing ug paborableng konsiderasyon sa among hangyo. Daghang salamat sa inyong suporta ug tabang.

Matinahuron,

NIEJEN ELFIE L. HATAGUE
Researcher

HELENA MARGRETHE D. DOLLOSO
Researcher

WINONA RENZ F. DURAN
Researcher

Noted by

YUNALYN L. VILLANTES, Ph.D.
Research Adviser

NELFA D. CANINI, MSc. Bio, Ph.D.
Research Instructor

EVANGELINE M. SEÑEDO, RMT, MATMRS
Dean, College of Medical Technology

APPENDICES

APPENDIX D: Certification for Plant Collection Issued by the Gokum of Lake Duminagat

SERTIPIKASYON

Kini nga sertipikasyon nagapamatuod nga ako naghatag ug pagtugot sa mga Third Year Bachelor of Science in Medical Technology nga mga estudyante gikan sa Misamis University sa pagpahigayon og pagkolekta sa mga sample sa tanom diri sa Brgy. Lake Duminagat, Don Victoriano Chiongbian, Misamis Occidental, alang sa ilang pagtuon nga adunay titulo: *"Phytochemical Evaluation and Anti-Angiogenic Activity of 'Makataynuk' Leaf Extracts Using the Chorioallantoic Membrane Assay on Duck Embryo."*

Gitugotan ang mga nagtuon sa pagkolekta sa mga samples sa makataynuk na tanom sulod sa maong lugar alang lamang sa akademikong katuyoan ug panukiduki. Ang maong kalihokan pagabuhaton sa responsableng paagi ug sa pamaagi nga maminusan ang kadaot sa kalikupan.

Kini nga sertipikasyon gipagawas aron suportahan ang ilang hangyo ug alang sa bisan unsang legal nga katuyoan nga mahimong gamiton.

Gihatag niadtong ika-____ nga adlaw sa bulan sa _____ 2026, diri sa Brgy. Lake Duminagat, Don Victoriano, Misamis Occidental.


MR. ORLYN RECOLITO

Gokum, Brgy. Lake Duminagat
Don Victoriano Chiongbian, Misamis Occidental

APPENDICES

APPENDIX E: Resolution Issued by the Department of Environment and Natural Resources (DENR)

Republic of the Philippines
Department of Environment and Natural Resources
Mt. Malindang Range Natural Park
29th ASEAN Heritage Park
Protected Area Management Board (PAMB)
Misamis Occidental

EXCERPTS OF THE 78TH PROTECTED AREA MANAGEMENT BOARD 1ST
QUARTER EN BANC MEETING OF MT. MALINDANG RANGE NATURAL PARK
HELD AT LA ELENA FISHYALAN AQUAPARK, SINACABAN, MISAMIS
OCCIDENTAL ON MARCH 26, 2026 AT 10:00 A.M.

PRESENT:

HENRY A. ADORNADO, PhD.	Chairperson
MS. ESTELA O. ESTAÑO	Regional Executive Director, DENR 10
ATTY. CRESTELLE PEDROSA	Ex-Officio Member, Municipal Mayor, Tudela
GOKUM TANNY S. LUMBAY	Representative with authorization to vote, Office of the 2 nd District Representative, Misamis Occidental
MR. CHRISTOPHER D. GARCIA	Ex-Officio Member, IPMR, 2 nd District of Misamis Occidental
MR. ANTONIO S. CAYLAN, JR.	Representative with authorization to vote, Office of the Municipal Mayor, Don Victoriano
MR. FROILAN A. BONDACO JR.	Representative with authorization to vote, Office of the City Mayor, Tangub City
MR. ARBIE M. DOMPALES	Representative with authorization to vote, Office of the City Mayor, Ozamiz City
MS. CHERLIE P. CABAUG	Representative with authorization to vote, Office of the Municipal Mayor, Clarin
MS. LUZ T. MADRIO	Representative with authorization to vote, Office of the Municipal Mayor, Jimenez
MS. BIANELYN B. LOMOSO	Representative with authorization to vote, Office of the Municipal Mayor, Aloran
MS. XENIA C. CEBUA	Representative with authorization to vote, Office of the Municipal Mayor, Calamba
MS. MARIA ESTELLA L. JALANDONI	Representative with authorization to vote, Office of the Municipal Mayor, Bonifacio
PLTCOL MARK LAGUNA	Representative with authorization to vote, Office of the Regional Director, DA
MR. JASPER JACILDO	Representative with authorization to vote, Office of the Provincial Director, PNP Misamis Occidental
CPT. RUFINO N. VELASCO	Representative with authorization to vote, Office of the Regional Director, DEPDev
MR. BUEN ENRI TUMILAP	Representative with authorization to vote, Office of the 10 th Infantry Battalion, Misamis Occidental
MS. MELANIE O. LAUDE	Representative with authorization to vote, Office of the Provincial Director, DILG Misamis Occidental
MR. MILAN C. SAMBILE	Representative with authorization to vote, Office of the Regional Director, DOST
	Representative with authorization to vote,

APPENDICES

APPENDIX E Cont'd

MR. JUAN M. PELARE	Office of the Provincial Director, NCIP Misamis Occidental
HON. WILSON M. TIATE	Non Ex-Officio Member, Private Sector
HON. MAURITA L. BURLAT	Ex-Officio Member, Brgy. Captain, Brgy. Penacio
HON. RUBEN CATHLON	Ex-Officio Member, Brgy. Captain, Brgy. Matugas Alto
HON. PHIL T. APEPE	Representative with authorization to vote, Office of the Brgy. Captain, Brgy. Owayan
HON. ARNOLD S. CASINTO	Ex-Officio Member, Brgy. Captain, Brgy. Manamong
HON. MYRNA M. DUMALTE	Ex-Officio Member, Brgy. Captain, Brgy. Stimson Abordo
HON. MODESTO R. ONTO	Ex-Officio Member, Brgy. Captain, Brgy. New Liboron
HON. LENIE L. ANSAO	Ex-Officio Member, Brgy. Captain, Brgy. Mara-Mara
HON. GINA I. SEROJIHOS	Ex-Officio Member, Brgy. Captain, Brgy. Bagong Clarin
HON. HERMANO G. CABANAG	Ex-Officio Member, Brgy. Captain, Brgy. Lampasan
HON. JUANITO J. TAUTU-AN	Ex-Officio Member, Brgy. Captain, Brgy. Gandawan
HON. ELIAS M. ALAYAAY SR.	Ex-Officio Member, Brgy. Captain, Brgy. Lahud
HON. EMILIANO A. SUKO	Ex-Officio Member, Brgy. Captain, Brgy. Lake Duminagat
HON. MARY ANN S. REPOLDO	Ex-Officio Member, Brgy. Captain, Brgy. Petianan
HON. RUSTICO M. PALARAN	Ex-Officio Member, Brgy. Captain, Brgy. Hoyohoy
HON. FLORENCIO T. BLAYA JR.	Ex-Officio Member, Brgy. Captain, Brgy. Mansawan
HON. FLORENCIA O. TUBAL	Ex-Officio Member, Brgy. Captain, Brgy. Tuno
HON. HENRY D. JUMALON	Representative with authorization to vote, Office of the Brgy. Captain, Brgy. Kanao-Kanao
HON. ROGER C. ABES	Ex-Officio Member, Brgy. Captain, Brgy. Sebucal
HON. ARNOLD PELARCA	Ex-Officio Member, Brgy. Captain, Brgy. Trigos
HON. ELISA L. SUANER	Representative with authorization to vote, Office of the Brgy. Captain, Brgy. Salimpuno
HON. PEDRO L. ATABAN	Representative with authorization to vote, Office of the Brgy. Captain, Brgy. Banglay
HON. ARNEL O. BUAGAS	Representative with authorization to vote, Office of the Brgy. Captain, Brgy. Kimat
HON. NELSON M. ADAZA	Ex-Officio Member, Brgy. Captain, Brgy. Peniel
HON. ARFIL P. PERICO	Ex-Officio Member, Brgy. Captain, Brgy. Dalingap
HON. JOSEPHINE BONGCAWEL	Representative with authorization to vote, Office of the Brgy. Captain, Brgy. Buenavista
HON. EVELYN T. AGUILAR	Representative with authorization to vote, Office of the Brgy. Captain, Brgy. Mamalad
HON. LUCIANO M. TUMANOS	Ex-Officio Member, Brgy. Captain, Brgy. Roxas
HON. JUDY DAGPIN	Ex – Officio Member Brgy. Captain, Brgy. Sinampongan
	Ex-Officio Member, Brgy. Captain, Brgy. Gala

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APPENDIX E Cont'd

HON. HELEN T. MACAS	Ex-Officio Member, <i>Brgy. Captain, Brgy. San Pedro</i>
HON. JOSEPH CABUG	Ex-Officio Member, <i>Brgy. Captain, Brgy. Carmen</i>
HON. JOCELYN S. SARAJENA	Ex-Officio Member, <i>Brgy. Captain, Brgy. Sicot</i>
HON. PERLA B. PACCARO	Ex-Officio Member, <i>Brgy. Captain, Brgy. Clarin Settlement</i>
HON. FLOREFE PALMERO	Ex – Officio Member <i>Brgy. Captain, Brgy. Baluc</i>

ABSENT:

GOV. HENRY S. OAMINAL, SR.	Ex – Officio Member <i>Provincial Governor, Misamis Occidental</i>
HON. JAYSON P. ALMONTE	Ex – Officio Member <i>Congressman, 1st District</i>
GOKUM EUGENIO T. LUMONTOD	Non-Ex – Officio Member <i>Office of the 1st District IPMR</i>
HON. LEMUEL MEYRICK ACOSTA	Ex – Officio Member <i>Mayor, Oroquieta City</i>
HON. JESSE S. VIÑA	Ex – Officio Member <i>Mayor, Concepcion</i>
HON. DAWN M. ANIMAS	Ex – Officio Member <i>Mayor, Sapang Dalaga</i>
HON. GOLDA CATHERINE YAP-RESMA	Ex – Officio Member <i>Mayor, Baliangao</i>
HON. GADWIN E. HANDUMON	Ex – Officio Member <i>Mayor, Plaridel</i>
HON. ANDREA CHERRY PINK L. GUTTIEREZ-CAGAS	Ex – Officio Member <i>Mayor, Lopez Jaena</i>
HON. FRANCISCO L. PAYLAGA JR.	Ex – Officio Member <i>Mayor, Panaon</i>
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HON. CRISTY MAE P. CANÑO	Ex – Officio Member <i>Brgy. Captain, Brgy. Guimad</i>

APPENDICES

APPENDIX E Cont'd

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HON. JESUS S. TOLOYLONGSOD	Ex – Oficio Member <i>Brgy. Captain, Brgy. Upper Salimpuno</i>
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HON. BERNY G. LUMANDA	Ex – Oficio Member <i>Brgy. Captain, Brgy. Small Potongan</i>
HON. CHARITO M. LUMBAY	Ex – Oficio Member <i>Brgy. Captain, Brgy. Poblacion</i>
HON. JUNNIE G. ETOL	Ex – Oficio Member <i>Brgy. Captain, Brgy. Guiban</i>
HON. DANNY T. LUMAYOG	Ex – Oficio Member <i>Brgy. Captain, Brgy. Capule</i>
HON. ANECITA J. JUANCHO	Ex – Oficio Member <i>Brgy. Captain, Brgy. Virayan</i>
HON. JHEV-KLOYD L. RASONABLE	Ex – Oficio Member <i>Brgy. Captain, Brgy. Lingatongan</i>
HON. ALFREDO T. LAMOSTE SR.	Ex – Oficio Member <i>Brgy. Captain, Brgy. Pogan</i>
HON. LEO M. MANDAY	Ex – Oficio Member <i>Brgy. Captain, Brgy. New Casul</i>
HON. JAMES V. TINA-E	Ex – Oficio Member <i>Brgy. Captain, Brgy. Upper Dapitan</i>
HON. RIZALDO A. TUMAGNA	Ex – Oficio Member <i>Brgy. Captain, Brgy. Marugang</i>
HON. ALBERTO L. JUGUILON	Ex – Oficio Member <i>Brgy. Captain, Brgy. Sosoon</i>
HON. DORLITO O. UBAS	Ex – Oficio Member <i>Brgy. Captain, Brgy. Bagong Nayon</i>
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HON. MARIFE BARINAS	Ex – Oficio Member <i>Brgy. Captain, Brgy. Burgos</i>
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HON. NESTOR B. NAPUNE	Ex – Oficio Member <i>Brgy. Captain, Brgy. Napangan</i>

APPENDICES

APPENDIX E Cont'd

Resolution No. 15 Series of 2026

A RESOLUTION GRANTING CLEARANCE TO VARIOUS RESEARCH PROPOSALS TO BE CONDUCTED WITHIN MT. MALINDANG RANGE NATURAL PARK

Page 5 of PAMB Resolution No. 15

WHEREAS, the Protected Area Management Board is mandated by law, pursuant to R.A. 7586 as amended by R.A. 11038 otherwise known as the "Expanded National Integrated Protected Area System," to oversee the management of the Protected Area;

WHEREAS, Ms. Jenesa Dumalay from the Sacred Heart College of Calamba, Incorporated, Mr. Reysan Cristalen from the National Museum of the Philippines, and Ms. Niejen Hatague, et al. from the Misamis University - Ozamiz City, presented the details of their research studies to the Subcommittee on Research and subsequently to the PAMB En Banc, which will be conducted within Mt. Malindang Range Natural Park;

WHEREAS, the study results will provide invaluable data which can be used for the future decision making of the PAMB in relation to PA management, to wit:

- A. Water Quality Assessment of Lake Duminagat and its Relationship to Surrounding Vegetation located at Brgy. Lake Duminagat, Don Victoriano by Ms. Jenesa Dumalay - provide data on the detailed information on water quality of Lake Duminagat;
- B. Mammals of Mindanao: Mammals of Malindang (Brgy. Lake Duminagat, Don Victoriano) by Mr. Reysan Cristales - provide data on mammalian diversity in Mount Malindang, Misamis Occidental through systematic sampling along an elevational gradient from lowland forest to mossy forest;
- C. Phytochemical Evaluation and Anti-angiogenic Activity of "Makataynuk" Leaf Extracts using the Chorioallantoic Membrane Assay on Duck Embryo) located at Brgy. Lake Duminagat, Don Victoriano by Ms. Niejen Hatague, et al. - provide scientific evidence on the cytotoxic and anti-angiogenic properties of "Makataynuk" leaf extracts, which may contribute to the development of plant-based therapeutic agents;

WHEREAS, paragraph (b) of section 10 of RA 11038 stated that the PAMB shall have the power and function to approve proposals and other related documents for the management of the protected areas;

NOW THEREFORE, on motion of Hon. Estela Estaño of LGU Tudela, and duly seconded by Hon. Hermano Cabanag of BLGU Gandawan;

BE IT RESOLVED, as it is hereby resolved, to grant clearance for the research proposals to be conducted within Mt. Malindang Range Natural Park, to wit:

- a. Water Quality Assessment of Lake Duminagat and its Relationship to Surrounding Vegetation located at Brgy. Lake Duminagat, Don Victoriano
- b. Mammals of Mindanao: Mammals of Malindang (Brgy. Lake Duminagat, Don Victoriano)
- c. Phytochemical Evaluation and Anti-angiogenic Activity of "Makataynuk" Leaf Extracts using the Chorioallantoic Membrane Assay on Duck Embryo) located at Brgy. Lake Duminagat, Don Victoriano

APPENDICES

APPENDIX E Cont'd

Mt. Malindang Range Natural Park
PAMB Resolution No. 15

RESOLVED FURTHER, that the researchers must comply to the following conditions set forth by the PAMB En Banc for the said research studies, to wit:

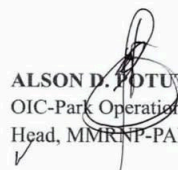
1. For research studies (b) and (c), the proponent must secure Gratuitous Permit (GP) from the DENR Region X;
2. For research studies (a) and (c), the proponent must secure Resolution of Consent from the local ICCs/IPs ; and
3. For research study (b), the proponent must secure Certificate of Precondition (CP) from the NCIP ; and
4. All the proponent must provide copy of the results of the research studies to the MMRNP PAMB;

RESOLVED FINALLY, that copies of this resolution be furnished to all the researchers, Department of Environment and Natural Resources-Biodiversity Management Bureau at Diliman, Quezon City and to the DENR Regional Office at Cagayan de Oro City, for their information and reference.

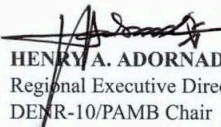
APPROVED UNANIMOUSLY and ADOPTED, this 26th day of March, 2026 at La Elena Fishyalan Aquapark, Sinacaban, Misamis Occidental.

I HEREBY CERTIFY to the correctness of this resolution.

FOR THE SECRETARIAT:


ALSON D. POTUTAN, RPF.
OIC-Park Operation Superintendent V
Head, MMRNP-PAMB Secretariat

AFFIRMED BY:


HENRY A. ADORNADO, Ph.D.
Regional Executive Director,
DENR-10/PAMB Chair

APPENDICES

APPENDIX E Cont'd Turnitin Result



Page 2 of 54 - Integrity Overview

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APPENDICES

APPENDIX E Cont'd Grammarly Report

General metrics

58,246	7,803	545	31 min 12 sec	1 hr 0 min
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6

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Sentence Length

14.3

Measures average sentence length

words per sentence

APPENDICES

APPENDIX F: Documentation



F.1. Presentation to the Department of Environment and Natural Resources (DENR) for Gratuitous Permit Processing

APPENDICES

APPENDIX F Cont'd



F.2. Preparation of Plant Samples Through Blending, Soaking, and Filtration

APPENDICES

APPENDIX F Cont'd



F.3. Solvent Partitioning of Plant Extracts

APPENDICES

APPENDIX F Cont'd



F.5. Exposure and Counting of Dead *Artemia salina* nauplii

APPENDICES

APPENDIX G: Statistical Tables for Cytotoxicity Analysis

Concentration (mg/mL)	Aqueous	Methanolic	Ethyl acetate	Hexane
1.000	26/30 (86.7%)	30/30 (100.0%)	28/30 (93.3%)	24/30 (80.0%)
0.500	22/30 (73.3%)	27/30 (90.0%)	25/30 (83.3%)	20/30 (66.7%)
0.250	17/30 (56.7%)	22/30 (73.3%)	20/30 (66.7%)	15/30 (50.0%)
0.125	12/30 (40.0%)	16/30 (53.3%)	14/30 (46.7%)	10/30 (33.3%)
0.0625	7/30 (23.3%)	10/30 (33.3%)	8/30 (26.7%)	5/30 (16.7%)
0.03125	3/30 (10.0%)	5/30 (16.7%)	4/30 (13.3%)	2/30 (6.7%)
Positive Control (DMSO)	29/30 (96.7%)	29/30 (96.7%)	29/30 (96.7%)	29/30 (96.7%)
Negative Control (Artificial Sea Water)	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)	0/30 (0.0%)

Values represent number dead/total tested (% mortality).

G.1. Dose-dependent mortality of *Artemia salina* nauplii exposed to different solvent extracts and controls after 24 h (N = 30/concentration)

APPENDICES

APPENDIX G Cont'd

Comparison	Aqueous	Methanolic	Ethyl acetate	Hexane
1.000 vs 0.500	0.0402*	0.4423ns	0.3659ns	0.0402*
1.000 vs 0.250	<0.001*	0.0007*	0.0004*	<0.001*
1.000 vs 0.125	<0.001*	<0.001*	<0.001*	<0.001*
1.000 vs 0.0625	<0.001*	<0.001*	<0.001*	<0.001*
1.000 vs 0.03125	<0.001*	<0.001*	<0.001*	<0.001*
1.000 vs Positive Control	0.1971ns	0.9955ns	0.9933ns	0.0071*
1.000 vs Negative Control	<0.001*	<0.001*	<0.001*	<0.001*
0.500 vs 0.250	0.0071*	0.0436*	0.0274*	0.0071*
0.500 vs 0.125	<0.001*	<0.001*	<0.001*	<0.001*
0.500 vs 0.0625	<0.001*	<0.001*	<0.001*	<0.001*
0.500 vs 0.03125	<0.001*	<0.001*	<0.001*	<0.001*
0.500 vs Positive Control	0.0002*	0.8383ns	0.1110ns	<0.001*
0.500 vs Negative Control	<0.001*	<0.001*	<0.001*	<0.001*
0.250 vs 0.125	0.0071*	0.0112*	0.0063*	0.0071*
0.250 vs 0.0625	<0.001*	<0.001*	<0.001*	<0.001*
0.250 vs 0.03125	<0.001*	<0.001*	<0.001*	<0.001*
0.250 vs Positive Control	<0.001*	0.0028*	0.0001*	<0.001*
0.250 vs Negative Control	<0.001*	<0.001*	<0.001*	<0.001*
0.125 vs 0.0625	0.0071*	0.0112*	0.0063*	0.0071*
0.125 vs 0.03125	<0.001*	<0.001*	<0.001*	<0.001*
0.125 vs Positive Control	<0.001*	<0.001*	<0.001*	<0.001*
0.125 vs Negative Control	<0.001*	<0.001*	<0.001*	<0.001*
0.0625 vs 0.03125	0.0402*	0.0436*	0.1110ns	0.1971ns
0.0625 vs Positive Control	<0.001*	<0.001*	<0.001*	<0.001*
0.0625 vs Negative Control	0.0002*	0.0001*	0.0004*	0.0071*
0.03125 vs Positive Control	<0.001*	<0.001*	<0.001*	<0.001*
0.03125 vs Negative Control	0.1971ns	0.0436*	0.1110ns	0.6354ns
Positive Control vs Negative Control	<0.001*	<0.001*	<0.001*	<0.001*

Statistically significant differences at $p < 0.05$.

G.2. Pairwise comparison of concentration-dependent mortality including controls using Tukey HSD test

CURRICULUM VITAE

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PRIMARY : Victorious Christian School
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