TOXICOLOGICAL EVALUATION AND IN VITRO ANTHELMINTIC ACTIVITY OF ETHANOLIC BARK EXTRACT OF KATURAY (*Sesbania grandiflora* (L.) Pers.) AGAINST *Pontoscolex corethrurus*

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CERTIFICATE OF PANEL APPROVAL

The research paper attached hereto, entitled **"TOXICOLOGICAL EVALUATION AND IN VITRO ANTHELMINTIC ACTIVITY OF ETHANOLIC BARK EXTRACT OF KATURAY (*Sesbania grandiflora*) AGAINST *Pontoscolex corethrurus*"**, prepared and submitted by **DARYHL ANGEL E. ABIJAY, CHRISTIAN T. ALVARICO, JANE BARAQUIL, PRINCESS ANNE G. ENGRACIA, ANGEL L. OBAOB, JOSHUA B. TIU, and JOELINA P. VILLEJO**, in partial fulfillment of the requirements for the degree **BACHELOR OF SCIENCE IN MEDICAL TECHNOLOGY**, it is hereby recommended for approval.

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ABSTRACT

The increasing concerns regarding the side effects, limited availability, and possible drug resistance associated with commercial deworming drugs have encouraged researchers to explore safer and more affordable plant-based alternatives. This study first evaluated the toxicity and then investigated the in vitro anthelmintic activity of the ethanolic bark extract of *Sesbania grandiflora* against *Pontoscolex corethrurus*. Bark samples were collected, air-dried, ground into powder, and extracted through maceration using 70% ethanol. Toxicity testing was conducted using the Brine Shrimp Lethality Assay (BSLA) with *Artemia salina* nauplii to determine the median lethal concentration (LC_{50}) of the extract. The results showed an LC_{50} value of 1.79 mg/mL, indicating that the extract has low toxicity and may be relatively safe for biological use. After the toxicity evaluation, the anthelmintic activity of the extract was assessed by measuring the time to paralysis and time to death of *Pontoscolex corethrurus* exposed to different extract concentrations (5, 10, and 15 mg/mL). Albendazole was used as the positive control, while distilled water served as the negative control. The findings showed that the extract exhibited concentration-dependent anthelmintic activity, where higher concentrations produced faster paralysis and death of the worms. Statistical analysis using one-way ANOVA and Tukey's post-hoc test revealed significant differences among treatment groups ($p < 0.05$). Overall, the findings suggest that the ethanolic bark extract of *S. grandiflora* possesses promising anthelmintic properties with relatively low toxicity.

Keywords: *bioassay, cytotoxicity, dose-response relationship, phytochemicals, soil-transmitted parasites*

DEDICATION

To our Almighty God, whose unending grace, fortitude, and direction have seen us through every difficulty and uncertainty. To our families whose sacrifices, love, and support have been the cornerstone of our journey. To our instructors, who have patiently given their effort, time, knowledge, and wisdom, helping shape not only our academic development but also our character. Moreover, to the citizens of Misamis Occidental, whose well-being inspired the very heart of this research. It is through our concern that we found the purpose and motivation to pursue this study, hoping that, in some way, our work may contribute to the betterment of our community.

- The Researchers

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INTRODUCTION

Background of the Study

There is a persistent issue in public health surrounding the infections and diseases caused by pathological helminths, especially in countries that lack proper sanitation practices, inadequate safety measure of water supplies, high population density, and health disparities. Research by the World Health Organization indicates that children and economically disadvantaged populations are the most susceptible to soil-transmitted helminths among the 1.5 billion people measured worldwide. Ascaris lumbricoides, Trichuris trichuria, and hookworms are primary causative parasites contracted through contaminated soil and poor hygiene practices (Al Amin & Wadhwa, 2023). According to a study conducted by Fissiha & Kinde (2021), mass drug administration programs were found to be ineffective; this is evident in the high persistence of reinfection rates in many countries, suggesting that addressing the issue fully goes beyond control measures alone.

Synthetic anthelmintic drugs, namely albendazole and mebendazole, are the primary source of management for parasitic helminth infections. Anthelmintic agents are substances that eliminate or destroy parasitic worms by disrupting their metabolic and neuromuscular activities. However, prolonged and repeated use of these drugs has raised concerns regarding reduced effectiveness, side effects, and the possible development of drug resistance (Bekele et al., 2024). The concept of drug resistance explains how parasites gradually adapt to medications through repeated exposure, ultimately reducing the drugs' therapeutic effectiveness. In addition, some communities, especially those in remote areas,

have limited access to commercial medications due to economic and geographical barriers (Fenta et al., 2024). Because of these concerns, researchers have become increasingly interested in exploring medicinal plants as alternative or complementary sources of anthelmintic agents.

Ethnopharmacology, a field that focuses on understanding indigenous health traditions and the medicinal use of plants, provides a framework for using plants as an alternative approach to medicine. Ethnopharmacology emphasizes the importance of validating traditional knowledge through scientific inquiry to identify bioactive compounds that may contribute to modern medicine (Tan et al., 2025). In the Philippines, traditional medicinal plants remain important in community healthcare, especially in rural areas where access to medical services is limited (Flores et al., 2025). This growing interest in plant-based medicine is connected to phytotherapy, which uses plant extracts and natural compounds to prevent and treat diseases.

One medicinal plant commonly found in the Philippines is *Sesbania grandiflora*, locally known as Katuray. *Sesbania grandiflora* is a fast-growing tree in the family Leguminosae and is widely distributed throughout Southeast Asia (Taculao, 2021). Katuray (*S. grandiflora*) is acknowledged for its nutritional value and its ethnopharmacological properties. Its flowers, leaves, and pods are commonly consumed as vegetables in Filipino cuisine (Guillasper, 2020), while different parts of the plant have traditionally been used to manage fever, inflammation, wounds, diabetes, and other illnesses. Previous research has highlighted that *S. grandiflora* is abundant in various phytochemicals, including flavonoids, tannins, saponins, and phenolic compounds. These bioactive constituents are associated with a broad spectrum of beneficial biological

activities, including antioxidant, antimicrobial, anti-inflammatory, antiviral, and antiparasitic activities (Mokhtar et al., 2025).

The therapeutic efficacy of medicinal plants can be understood through the lens of the phytochemical theory, which states that biologically active compounds naturally present in plants are responsible for their pharmacological properties. For instance, flavonoids are recognized for their antioxidant activity and ability to combat parasites, while tannins may interfere with the structural proteins and energy metabolism of parasites (Zahrah, Abrahamase, & George, 2024). Saponins are also believed to affect parasite membrane integrity, leading to paralysis and death. According to Ullah et al. (2020), flavonoids possess various biological functions, including antioxidant, antimicrobial, antimalarial, anti-inflammatory, and antiparasitic activities. Similarly, Abdullah (2024) emphasized that phytochemicals from medicinal plants may exhibit significant antimicrobial and antiparasitic properties. These findings suggest that *S. grandiflora* may contain compounds with anthelmintic activity.

In addition, the dose-response relationship is a pertinent concept in this study, illustrating how the biological effects of a substance vary with concentration or dosage. In pharmacological studies, higher concentrations of biologically active compounds often produce stronger therapeutic effects. This concept is important for evaluating the effectiveness of medicinal plant extracts because it helps determine whether increasing concentrations yield greater anthelmintic activity. In the present study, different concentrations of the ethanolic bark extract were evaluated to determine whether it exhibits concentration-dependent effects against parasitic worms.

In medicinal plant research, evaluating safety is equally important as determining therapeutic effectiveness. This principle is supported by the toxicological concept that "the dose makes the poison," which explains that any substance may become harmful depending on the amount administered. Toxicological screening is vital for assessing whether plant extracts may cause harmful biological effects before their therapeutic use. A widely utilized preliminary toxicity test is the Brine Shrimp Lethality Assay (BSLA), which employs Artemia salina nauplii as a straightforward, rapid, and cost-effective bioassay for evaluating cytotoxicity (Torno et al., 2024). Cytotoxicity refers to the capability of a substance to harm or eliminate living cells. Extracts showing low toxicity in BSLA are generally considered safer for further pharmacological investigation.

For evaluating anthelmintic activity, *Pontoscolex corethrurus* is widely used as an experimental model because of its physiological and anatomical similarities to human intestinal helminths (None Shilpashree, 2022). As stated in the same study, the effectiveness of anthelmintic agents is commonly assessed by measuring the time to paralysis and time to death of worms after exposure to different concentrations of plant extracts. Paralysis occurs when the worms lose their ability to move, while death is confirmed when they no longer respond to external stimuli. These parameters provide measurable evidence of the extract's biological activity.

Although *S. grandiflora* is widely recognized for its medicinal value, limited scientific studies have specifically investigated the toxicological profile and anthelmintic activity of its ethanolic bark extract. Most existing studies focus on the plant's flowers and leaves, leaving a gap in the scientific literature regarding the bark. This study was conducted to assess the toxicological profile and in vitro anthelmintic activity of the ethanolic bark

extract of *Sesbania grandiflora*. The specific objectives included determining the safety of the extract through toxicity testing; evaluating its efficacy against *Pontoscolex corethrurus* by measuring time to paralysis and time to death; comparing its activity with that of albendazole as the standard reference drug; and investigating its concentration-dependent effects. The results of this study have the potential to enhance the existing body of knowledge on medicinal plants. They may help develop cost-effective, locally accessible plant-based alternatives for treating parasitic worm infections.

Objectives of the Study

The present study aimed to evaluate the toxicological profile and in vitro anthelmintic activity of the ethanolic bark extract of *Sesbania grandiflora* against *Pontoscolex corethrurus*. Specifically, the study sought to:

1. Determine the toxicological profile of the ethanolic bark extract of *Sesbania grandiflora* using the Brine Shrimp Lethality Assay (BSLA) and estimate its median lethal concentration (LC_{50});
2. Evaluate the in vitro anthelmintic activity of the extract against *Pontoscolex corethrurus* by measuring the time to paralysis and time to death at varying concentrations;
3. Determine the concentration-dependent or dose-response effects of the ethanolic bark extract on the paralysis and death of *Pontoscolex corethrurus*; and
4. Compare the anthelmintic activity of the ethanolic bark extract with the standard anthelmintic drug, albendazole.

Significance of the Study

This study aims to deliver significant scientific insights into the toxicological safety and anthelmintic potential of the ethanolic bark extract of *Sesbania grandiflora*. The findings have the potential to advance the fields of pharmacology and drug development by providing preliminary evidence for the efficacy of this plant-based extract as a potential source of natural anthelmintic compounds. The observed dose-dependent effects and biological activity of the extract may support future studies involving the development and production of plant-based therapeutic agents.

The study may also benefit toxicology researchers by providing baseline information on the safety profile of the katuray bark sample extract through the Brine Shrimp Lethality Assay. Since medicinal plants are increasingly being explored for pharmaceutical applications, determining their toxicity is essential before further biological and clinical investigations can be conducted. In public health, the findings may support ongoing efforts to manage soil-transmitted helminth infections, especially in areas that have limited access to commercial medications. The identification of affordable and locally available plant-based alternatives may help strengthen existing deworming programs and community healthcare initiatives.

In addition, the study may benefit local communities by scientifically validating the traditional medicinal use of *S. grandiflora* and encouraging the proper utilization of indigenous medicinal plants. The use of plant-based products offers an alternative pathway to enhancing environmental sustainability by reducing reliance on synthetic chemical agents. Furthermore, results may serve as a reference for future academes conducting

studies related to medicinal plants, antiparasitic activity, phytochemical analysis, and toxicity evaluation.

Scope and Delimitations

This research study emphasizes the toxicological evaluation and in vitro anthelmintic activity of the ethanolic bark extract of *Sesbania grandiflora*. The bark samples were collected from selected areas in Ozamiz City and processed through dehydration, pulverization, and maceration using 70% ethanol. To assess the toxicity profile, the Brine Shrimp Lethality Assay (BSLA) was employed to evaluate the response of Artemia salina nauplii to varying concentrations of the ethanolic bark extract. The median lethal concentration (LC_{50}) was determined based on the mortality observed within the nauplii population. The anthelmintic activity was evaluated through an in vitro assay using adult *Pontoscolex corethrurus*, with the primary measures of efficacy being the time to paralysis and time to death of the worms. Following the experimental procedure of Das, Dey, & Ghosh (2011), a five-interval series of extract concentrations (5, 10, and 15 mg/mL) was prepared, with albendazole at 25 mg/mL as the positive control and distilled water as the negative control. Furthermore, 100-mL beakers were used instead of petri dishes, and the length of the worms was measured but not their weight.

All experiments were conducted under laboratory conditions, and to establish the reliability of the results, triplicates were performed. Statistical tools used in the study included the Shapiro-Wilk normality test, one-way analysis of variance (ANOVA), and Tukey's post hoc test ($p < 0.05$) as the primary descriptive tools. It is important to note that the study used only an in vitro laboratory setup, and no in vivo animal or human clinical trials were

conducted. Furthermore, the phytochemical composition responsible for the observed biological reactions in the study was not investigated. Therefore, the conclusions drawn from this study only adhere to preliminary toxicity assessments and anthelmintic evaluation and cannot yet be directly generalized to humans without further pharmacological and clinical investigations.

MATERIALS AND METHODS

Research Design

A true experimental research design was employed to determine the toxicological profile and anthelmintic activity of *Sesbania grandiflora* bark extract. Systematic collection, preparation, extraction, and assessment were conducted in a controlled laboratory setting. In assessing the toxicological profile of the extract, a recognized preliminary bioassay used to evaluate the cytotoxicity and biological activity of various plant extracts, namely the Brine Shrimp Lethality Assay (BSLA), was employed. The identification of the bioactive potential of crude plant extracts and naturally occurring substances is done efficiently, as BSLA provides simplicity, reliability, and cost-effectiveness.

To evaluate anthelmintic activity, an in vitro assay using *Pontoscolex corethrurus* as the experimental model was conducted. Human intestinal nematodes share anatomical and physiological traits with earthworms; thus, the study uses an earthworm model to substitute for gastrointestinal worms and evaluate the biological response to the extract. To evaluate the effectiveness of the ethanolic bark extract, the study measured the time until the worms became paralyzed and died after exposure to various concentrations of the extract. The positive control agent in the study was albendazole, and distilled water was the negative control. The research also explored how the extract's effectiveness varied with concentration to understand its dose-response relationship..

Research Setting

The fresh bark of *Sesbania grandiflora* was collected from Doña Consuelo, Ozamiz City. The area was selected because *S. grandiflora* naturally grows abundantly in the locality, ensuring the availability and authenticity of the plant specimens used in the study. The collection site is situated within a tropical rainforest climate zone (Type IV climate), characterized by warm temperatures and evenly distributed rainfall throughout the year, conditions that support the healthy growth of various plant species (PAGASA, 2021). The area also contains fertile loam and mountain soils, predominantly classified as Adtuyon clay, which provide favorable environmental conditions for the growth of *S. grandiflora* (MPDO- Ozamiz City, 2017).

For authenticating the collected plant specimen. Bobby Alaman from the Misamis University Community Extension Program Office confirmed that the plant used in the study was genuinely *Sesbania grandiflora*.

In coordination with the Naawan Mindanao State University Institute of Fisheries Research and Development, Artemia salina eggs were obtained. The institution is recognized for its research programs in fisheries and aquaculture and serves as a reliable source of biological materials required for the toxicity assay.

Meanwhile, the earthworms (*Pontoscolex corethrurus*) used in the anthelmintic assay were collected from Barangay Dela Paz, Clarin. The earthworm model was identified by Dr. Nonillon M. Aspe, a highly distinguished scientist and academic leader from Mindanao State University – Naawan, through an image shared via the messaging app Messenger by Mrs. Yunalyn Villantes. Barangay Dela Paz, Clarin, was selected because of the dense number of earthworms present in the locality. The moist soil condition, rich

organic matter, and minimal soil disturbance in the area provide a suitable environment for earthworm growth and reproduction. Previous studies have shown that earthworm populations thrive in areas with high soil fertility, adequate moisture, and active microbial activity, all of which contribute to their survival and reproduction (Zhang et al., 2024). In addition, agricultural activities in the area contribute organic plant debris that serves as a natural food source for earthworms, making the location appropriate for specimen collection.

Figure 1 showcases the areas that were covered in sourcing the Katuray (*S. grandiflora*) bark, Artemia salina Nauplii, and the earthworm model, *Pontoscolex corethrurus*, that were essential in conducting the experimental study.

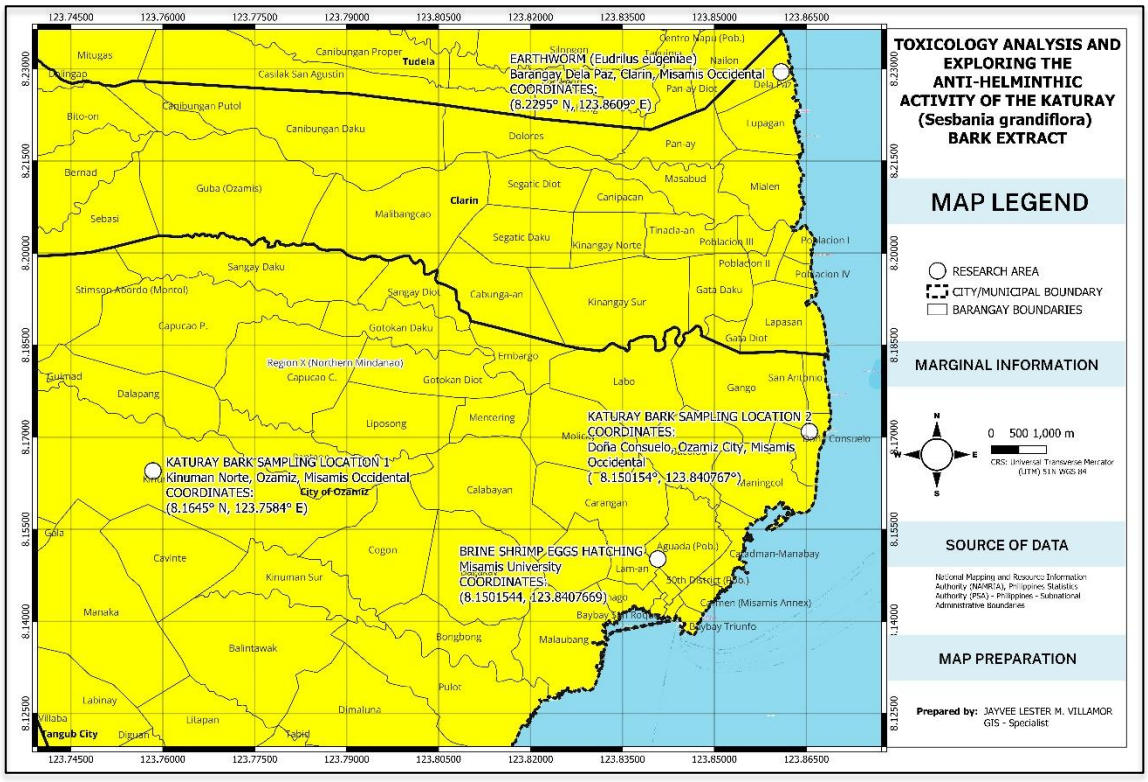

Figure 1. Areas Involved in the Sourcing of *Sesbania grandiflora, Artemia salina* Nauplii, and *Pontoscolex corethrurus*

Plant Materials and Extract Preparation

A modified extraction technique, based on the approach outlined by Terrazola et al. (2025), was used to prepare the *S. grandiflora* bark sample using 70% ethanol. Fresh bark samples were collected, thoroughly cleaned with running tap water to remove soil, dust, and other impurities, and then rinsed with distilled water. This is followed by further producing smaller pieces of the bark; instead of air-drying, the samples were dried in a laboratory oven at 43°C for 12 hours or until a consistent dry weight was reached.

A laboratory-grade blender was used to grind the dried bark samples. Subsequently, 500 g of the powdered bark was placed in a sterile glass container and immersed in 70% ethanol at a weight-to-volume ratio of 1:10, rather than the more concentrated 1:5 ratio, which corresponds to 500 g of bark in 5 liters of solvent. The container was sealed securely, and the solution was allowed to macerate at ambient temperature (25–28°C) for 7 days. During this period, the mixture was gently stirred twice daily to enhance the extraction and penetration of bioactive compounds from the bark.

After the initial maceration period, the mixture was allowed to stand for an additional seven days to maximize extraction efficiency. The extract was then filtered using Whatman™ No. 1 filter paper to separate the liquid filtrate from the plant residues. The collected filtrate was concentrated under reduced pressure at 45°C using a rotary evaporator at the Department of Chemistry laboratory of Mindanao State University – Iligan Institute of Technology until a semi-solid crude ethanolic extract was obtained. The concentrated extract was transferred into sterile amber glass containers and stored at 4°C until use for the toxicological and in vitro anthelmintic assays.

Figure 2 delineates the preparation of the 70% ethanolic bark extract of the katuray (*S. grandiflora*) tree.

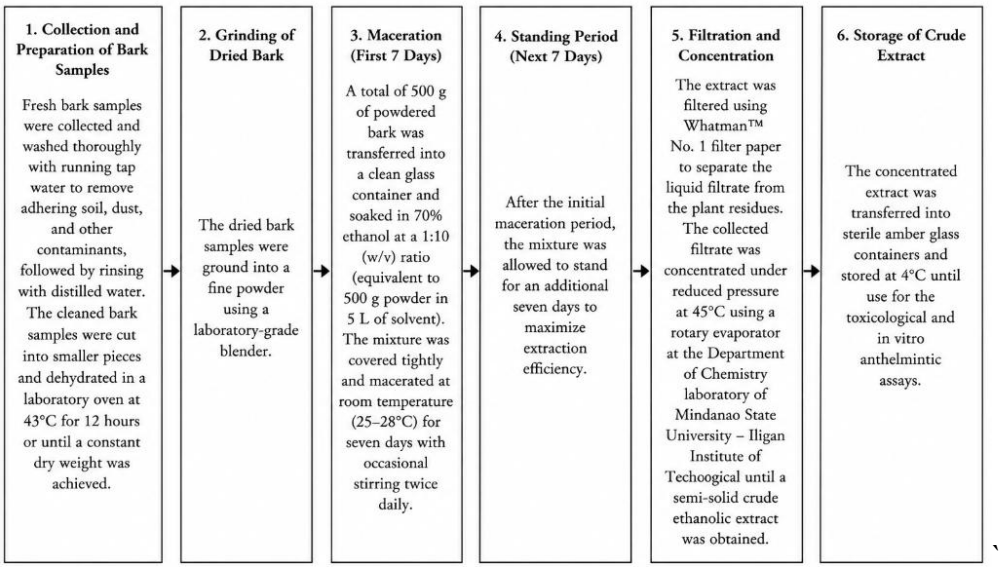

Figure 2. Schematic diagram of the preparation of the plant ethanolic extract

Toxicological Evaluation Using the Brine Shrimp Lethality Assay (BSLA)

The toxicological profile of the ethanolic bark extract of *Sesbania grandiflora* was evaluated using the Brine Shrimp Lethality Assay (BSLA), a widely used preliminary bioassay for determining the cytotoxicity and biological activity of plant extracts and natural compounds (Clemen-Pascual et al., 2022). The BSLA is recognized as a simple, rapid, reliable, and cost-effective screening method that requires only a small amount of sample and is commonly used for the initial assessment of the safety and bioactive potential of medicinal plants (Meyer et al., 1982). The assay is based on the plant extract's ability to cause mortality in laboratory-cultured brine shrimp nauplii (*Artemia salina*), which serves as an indicator of toxicity.

Following the procedure for conducting BSLA by Clemen-Pascual et al. (2022), 38 g of sea salt was dissolved in 1 L of distilled water to produce artificial seawater, which

was then filtered to remove suspended particles. Brine shrimp eggs (*A. salina*) were incubated in a glass hatching chamber containing the prepared artificial seawater. The chamber was continuously aerated and illuminated to facilitate hatching. With a 48-hour incubation period at 32°C, the hatched nauplii were attracted to the illuminated side of the chamber and collected with a glass pipette.

Increasing concentrations of the ethanolic bark extract were prepared in previously labeled sample vials containing filter paper discs loaded with the extract. Vials containing four milliliters of artificial seawater were prepared, and ten live swimming nauplii were transferred into the treatment vials using a clear glass pipette. To obtain a final volume of 5 mL per vial, additional artificial seawater was added, and each vial was provided with a food source using a single drop of dry yeast suspension (3 mg of yeast dissolved in 5 mL of artificial seawater). Continuous illumination at 32°C throughout the assay period was provided to maintain a stable environment.

The number of surviving nauplii in each vial was counted after 24 hours, and the percent mortality was calculated. The median lethal concentration (LC_{50}) of the extract was subsequently determined through probit analysis. The BSLA was selected for this study because it provides a practical and ethical preliminary approach to toxicity screening, as *Artemia* species are not subject to the same regulatory requirements as vertebrates. (Clemen-Pascual et al., 2022). Furthermore, the assay is an initial screening tool in pharmacological and toxicological studies to identify plant extracts with potential biological activity before more advanced in vivo investigations.

Figure 3 displays the process in conducting the Brine Shrimp Lethality Assay using the 70% ethanolic bark extract of *S. grandiflora*.

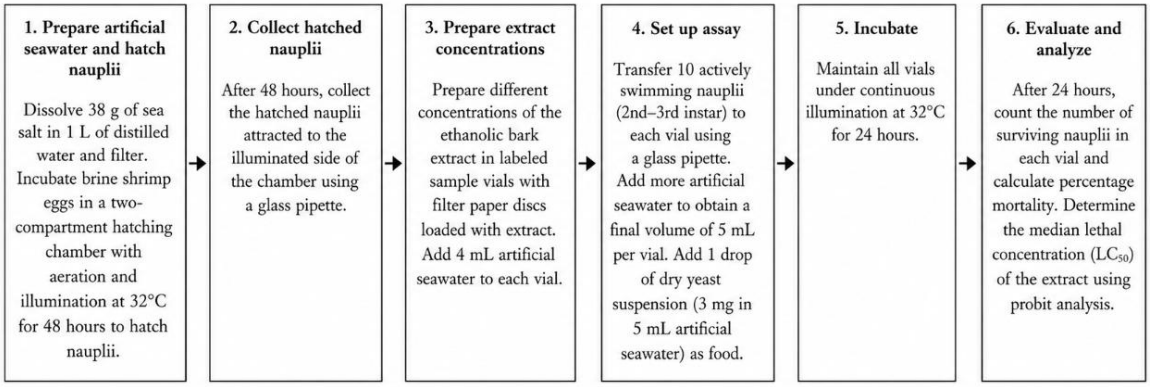

Figure 3. Schematic diagram of conducting the Brine Shrimp Lethality Assay

Anthelmintic Activity of *Sesbania grandiflora* Ethanolic Bark Extract Against *Pontoscolex corethrurus*

The in vitro anthelmintic activity of the ethanolic bark extract of *Sesbania grandiflora* was evaluated using adult *Pontoscolex corethrurus* as the experimental model following a modified method adapted from Das et al. (2011). Earthworms were selected because of their anatomical and physiological similarities to human intestinal helminths, making them suitable organisms for preliminary anthelmintic screening.

Adult earthworms measuring approximately 5–7 cm in length and of similar body size were collected from moist soil in Barangay Dela Paz, Clarin, Misamis Occidental. The collected worms were washed thoroughly with tap water, then with distilled water, to remove adhering soil particles and fecal matter. Healthy and active worms were selected for the assay.

The ethanolic bark extract was prepared at concentrations of 5 mg/mL, 10 mg/mL, and 15 mg/mL using distilled water as the diluent. Twenty milliliters of each concentration were transferred into separate, properly labeled beakers. Six earthworms were introduced into each treatment dish. Albendazole (25 mg/mL) was used as the positive control, while

distilled water served as the negative control. Each treatment and control group was prepared in triplicate.

The worms were continuously observed after exposure to the different treatments. The time to paralysis (P) and time to death (D) were recorded in minutes using a digital timer. As stated by Asiimwe et al. (2023), paralysis was confirmed when the worms showed no visible movement except upon vigorous shaking of the beaker, and death was confirmed when the worms exhibited complete loss of motility and failed to respond even after immersion in warm water at 50°. Observations were conducted for a maximum period of 240 minutes. The recorded times of paralysis and death were used as indicators of the extract's anthelmintic activity.

Figure 4 shows the systematic flow of conducting the Anthelmintic Assay on the earthworm model, *Pontoscolex corethrurus*.

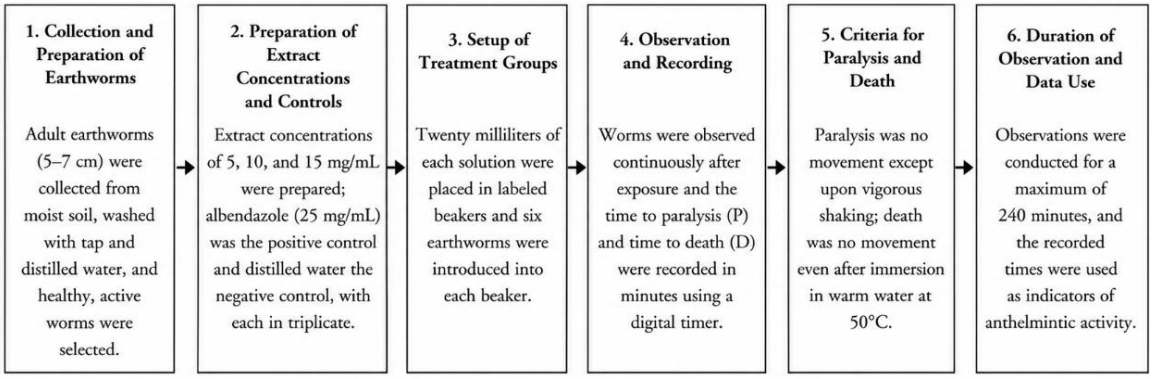

Figure 4. Schematic diagram of conducting the Anthelminthic Assay

Data Analysis

The data generated from the toxicological and in vitro anthelmintic assays of the ethanolic bark extract of *Sesbania grandiflora* were analyzed using both descriptive and

inferential statistical methods. Data processing and statistical computations were performed using the Statistical Package for the Social Sciences (SPSS) version 29 and PAST Statistics software.

For the Brine Shrimp Lethality Assay (BSLA), the number of surviving and dead *Artemia* nauplii was recorded after 24 hours of exposure to the different extract concentrations (Maurel et al., 2024). Percentage mortality was calculated using the formula:

$$\% \text{ Mortality} = \left(\frac{Number\ of\ Dead\ Nauplii}{Initial\ Number\ of\ Nauplii}\right) \times 100 \quad (1)$$

The mortality data obtained from the different concentrations were subjected to probit analysis to determine the median lethal concentration (LC_{50}) of the extract. Linear regression analysis of the probit-transformed mortality values against the logarithm of concentration was used to estimate the LC_{50} value, which the calculated LC_{50} served as the basis for interpreting the toxicity level of the extract according to established toxicity classifications (Bajatovska et al., 2023)

For the in vitro anthelmintic assay using *Pontoscolex corethrurus*, the time to paralysis and time to death were recorded in minutes for each treatment group. Descriptive statistics, including mean and standard error of the mean (SEM), were calculated for all experimental groups and controls.

Statistical Analysis

Before inferential analysis, the data were subjected to the Shapiro–Wilk test to assess normality of distribution. A one-way analysis of variance (ANOVA) was then

performed to determine whether significant differences existed among the treatment groups at varying extract concentrations. When significant differences were observed, Tukey's post hoc test was applied to pairwise comparisons of means among groups. Statistical significance was set at $p < 0.05$.

Concentration-dependent effects of the extract were evaluated by assessing changes in mortality, paralysis time, and death time across the concentrations teste.

RESULTS AND DISCUSSION

Toxicological Evaluation of *Sesbania grandiflora* Ethanolic Bark Extract Using Brine Shrimp Lethality Assay (BSLA)

The results showed that the extract exhibited generally low toxicity across all tested concentrations, while mortality increased progressively with concentration. The data presented in Table 1 highlight the toxicological effects of *the* ethanolic bark extract of Sesbania grandiflora, as determined by the Brine Shrimp Lethality Assay (BSLA).

After 24 hours, the highest mortality was observed at the 1.0 mg/mL concentration with a mean mortality rate of 40%, exhibiting toxicological activity, followed by 30.0% at 0.5 mg/mL and 16.7% at 0.25 mg/mL, demonstrating moderate toxicity. Lower concentrations produced minimal mortality, particularly at 0.0625 mg/mL and 0.03125 mg/mL, where near-complete survival of the nauplii was observed. This finding is consistent with the results reported by Agha et al. (2022), which showed that Fenugreek (*Trigonella foenum-graecum*), a member of the same *Fabaceae* family, has a low toxicity profile.

Table 1. Mortality of *Artemia salina* Nauplii Exposed to Varying Concentrations of *S. grandiflora* Ethanolic Bark Extract

Concentration (mg/mL)	Dead (T1)	Dead (T2)	Dead (T3)	Mean Dead	% Mortality
1.0	4	5	3	4.00	40%
0.5	3	2	4	3.00	30%
0.25	2	1	2	1.67	16.7%
0.125	1	1	0	0.67	6.7%
0.0625	0	1	0	0.33	3.3%
0.03125	0	0	0	0.00	0%
Negative Control (Artificial Seawater)	0	0	0		0%
Positive Control (DMSO)	9	10	10		96.7%

To estimate the median lethal concentration (LC_{50}), probit analysis was employed to linearize the dose-response relationship by converting mortality percentages into probit units and plotting them against the logarithm of concentration. Adjusted mortality values were applied to account for zero responses and allow valid probit transformation. Linear regression analysis generated the following equation:

$$\text{Probit} = 0.99\ (Log - Concentration\ Index) + 4.75 \quad (2)$$

Using this model, the LC50 (median lethal concentration) was estimated at:

$$\text{LC}_{50} \approx 1.79\ \text{mg/mL}$$

The obtained LC_{50} value indicates that the ethanolic bark extract exhibits low toxicity across the tested concentration range. Based on a study conducted by Torno et. al. (2024), which followed Clarkson's Toxicity Criteria, it was found that extracts are considered non-toxic if their LC50 value is above 1000 ppm, low toxic if the LC50 value falls between 500-1000 ppm, medium toxic if it ranges from 100-500 ppm, and highly toxic if the value is between 0-100 ppm.

The findings revealed that the extract exhibited generally low toxicity across all tested concentrations, although mortality increased progressively with concentration. Low toxicity can be attributed to the extraction process used. As stated by Duarte et al. (2022), extraction methods can reduce the co-extraction of unwanted compounds that may interfere with the desired biological yields. For example, deep eutectic solvents have been shown to extract phenolic compounds while minimizing undesirable substances selectively (Nisca & Tanase, 2025)—the phenolics of the *S. grandiflora* bark. Among the extract-treated groups, the highest mortality was observed at 1.0 mg/mL with a mean mortality of 40.0%, followed by 30.0% at 0.5 mg/mL and 16.7% at 0.25 mg/mL. Lower concentrations

produced minimal mortality, particularly at 0.0625 mg/mL and 0.03125 mg/mL, where near-complete survival of the nauplii was observed. This finding is consistent with the results reported by Agha et al. (2022), which showed that Fenugreek (*Trigonella foenum-graecum*), a member of the same *Fabaceae* family, has a low toxicity profile. Fenugreek (*Trigonella foenum-graecum*) also contains active compounds similar to those of *S. grandiflora*, which may explain the low toxicity at high doses, including flavonoids, alkaloids, coumarins, saponins, and other antioxidants (Amini et al., 2023).

Furthermore, the negative control containing artificial seawater produced no mortality, confirming that the experimental conditions did not contribute to organism death. Meanwhile, the positive control (DMSO) exhibited 96.7% mortality, validating the assay's sensitivity and reliability.

Overall, the results indicate that the ethanolic bark extract of *S. grandiflora* did not exhibit strong cytotoxic properties and may therefore be considered non-toxic to slightly toxic within the tested concentration range. Although a concentration-dependent increase in mortality was observed, the extract remained within the low-toxicity category. This suggests that the bioactive compounds in the bark extract may exert biological activity without causing substantial toxicity under laboratory conditions.

The relatively low toxicity observed in this study may be associated with the phytochemical composition of *S. grandiflora*. Previous phytochemical investigations have reported the presence of flavonoids, tannins, saponins, and phenolic compounds in the plant (Singh & Srivastava, 2024). The amount present at a given concentration is determined by the extraction process used. A study conducted by Lohvina, Sándor, and Wink (2022) revealed that the use of 70% ethanol as the extraction solvent maximizes the extraction of

the total phenolic compound. These compounds are known for their pharmacological properties while generally exhibiting low cytotoxicity at moderate concentrations. Flavonoids and phenolic compounds, in particular, possess antioxidant and protective activities that may contribute to the reduced lethality observed in the BSLA (Mutha, Tatiya, & Surana, 2021).

The findings of the present study are consistent with previous toxicity screenings using the brine shrimp assay, in which extracts with low mortality rates are generally interpreted as having low cytotoxic potential. Similarly, Lin et al. (2024), in their toxicological study of Meliaceae extracts, reported that several extracts exhibited minimal to no toxicity in brine shrimp assays, supporting their potential safety for further biological and pharmacological investigations. Likewise, Hamidi et al. (2014) emphasized that plant extracts that produce minimal lethality in BSLA are generally considered relatively safe during preliminary toxicity screening and may serve as promising candidates for future therapeutic studies.

Anthelmintic Activity of *Sesbania grandiflora* Ethanolic Bark Extract Against *Pontoscolex corethrurus*

The anthelmintic activity of the ethanolic bark extract of *Sesbania grandiflora* was evaluated using *Pontoscolex corethrurus* as the experimental model. Paralysis time and death time were used as the primary indicators of anthelmintic efficacy. Earthworms are commonly utilized in preliminary anthelmintic studies because of their physiological and anatomical similarities to human intestinal helminths (Nath & Das, 2026).

Table 2 presents the anthelmintic activity of the ethanolic bark extract of *Sesbania grandiflora* against *Pontoscolex corethrurus* in terms of paralysis time and death time

across different concentrations. The findings revealed that the extract exhibited a clear concentration-dependent anthelmintic effect, with higher concentrations resulting in faster paralysis and death of the worms.

Among all treatments, the positive control, Albendazole (25 mg/mL), demonstrated the strongest anthelmintic activity, causing paralysis at 9.40 ± 0.10 minutes and death at 14.44 ± 0.10 minutes. This confirms the potent activity of the standard drug and validates its use as a reference treatment for comparison.

Among the extract-treated groups, the 15 mg/mL concentration produced the most pronounced effect, with paralysis occurring at 24.32 ± 0.59 minutes and death at 45.20 ± 0.42 minutes, indicating strong bioactivity at higher concentrations. The 10 mg/mL concentration exhibited moderate activity, producing paralysis at 40.50 ± 0.65 minutes and death at 66.43 ± 0.59 minutes. Meanwhile, the 5 mg/mL concentration showed the weakest activity, with paralysis occurring at 66.70 ± 0.15 minutes and death at 86.58 ± 0.05 minutes, indicating reduced efficacy at lower concentrations.

Table 2. Anthelmintic Activity of *Sesbania grandiflora* Ethanolic Bark Extract Against *Pontoscolex corethrurus*

Treatment	Concentration (mg/mL)	Time of Paralysis (min) (Mean ± SEM)	Time of Death (min) (Mean ± SEM)
Negative Control (Artificial Seawater)	—	—	—
S. grandiflora Bark Extract	5	66.70 ± 0.15	86.58 ± 0.05
S. grandiflora Bark Extract	10	40.50 ± 0.65	66.43 ± 0.59
S. grandiflora Bark Extract	15	24.32 ± 0.59	45.20 ± 0.42
Positive Control (Albendazole)	25	9.40 ± 0.10	14.44 ± 0.10

In contrast, the negative control (artificial seawater) showed no observable anthelmintic activity, as the worms remained active throughout the observation period.

This confirms that the paralysis and death observed in the treated groups were caused by the extract and not by the experimental conditions.

The results clearly demonstrate a dose-dependent biological response, wherein increasing extract concentration enhanced the anthelmintic activity of the bark extract. This observation suggests that higher concentrations contain more active phytochemical constituents that interfere with the worms' physiological and neuromuscular activities, ultimately resulting in paralysis and death.

The anthelmintic activity observed in this study may be attributed to bioactive phytochemicals, including flavonoids, tannins, saponins, and phenolic compounds, that have been previously reported in *S. grandiflora*. According to Mokhtar et al. (2025), *Sesbania grandiflora* possesses a rich and diverse phytochemical profile across different plant parts, depending on the extraction solvent used. Similarly, Deepthi et al. (2021) reported the presence of tannins and flavonoids in the ethanolic bark extract of *S. grandiflora*.

These phytochemicals are known to exert anthelmintic effects through different mechanisms. Tannins may bind to proteins in worms' cuticles, leading to structural damage and disruption of metabolic processes. At the same time, flavonoids and saponins may interfere with neuromuscular coordination and membrane integrity. Bhattacharya (2024) explained that plant-derived phytochemicals can disrupt worm neuromuscular activity, eventually causing paralysis followed by death.

The findings of the present study are consistent with those of Eswar et al. (2025), who reported that plant-derived extracts exhibited significant dose-dependent anthelmintic activity against *Pheretima posthuma*. Their study further emphasized that tannins and

flavonoids contribute substantially to anthelmintic activity by impairing worm motility and physiological functioning.

Dose-Response Relationship and Toxicity Classification of *Sesbania grandiflora* Ethanolic Bark Extract

Table 3 presents the dose-response relationship and toxicity classification of the ethanolic bark extract of *Sesbania grandiflora* based on the Brine Shrimp Lethality Assay (BSLA). The results demonstrated a clear concentration-dependent pattern, with higher extract concentrations leading to higher mortality rates among *Artemia salina* nauplii.

Table 3. Dose-Response Relationship and Toxicity Classification of *Sesbania grandiflora* Ethanolic Bark Extract (BSLA)

Concentration (mg/mL)	Mean Mortality (%)	Dose-Response Pattern	Toxicity Interpretation
1.000	40.0%	Highest mortality observed	Low toxicity
0.500	30.0%	Decreased with lower concentration	Low toxicity
0.250	16.7%	Progressive decline in mortality	Low toxicity
0.125	6.7%	Progressive decline in mortality	No observed toxicity
0.0625	3.3%	Minimal mortality observed	No observed toxicity
0.03125	0.0%	No mortality observed; lowest dose	No observed toxicity

Despite the increasing mortality with increasing concentration, the extract did not exhibit strong cytotoxic effects within the tested concentration range. The negative control (artificial seawater) showed 0.0% mortality, indicating normal survival conditions and confirming that the test environment did not contribute to organism death. Meanwhile, the positive control (DMSO) exhibited 96.7% mortality and was classified as highly toxic, thereby validating the assay's sensitivity and reliability.

Overall, the results suggest that the ethanolic bark extract of *S. grandiflora* possesses relatively low cytotoxic potential and may therefore be considered relatively safe

at the tested concentrations. Most treatment groups produced only minimal mortality, particularly at lower concentrations.

These findings are consistent with the study of Hamidi et al. (2014), which reported that plant extracts exhibiting low lethality responses in BSLA are generally interpreted as having low or negligible cytotoxic activity, thereby supporting their potential safety and suitability for further pharmacological investigation.

Table 4 presents the results of the one-way analysis of variance (ANOVA) for paralysis time among the different concentrations of the ethanolic bark extract of *S. grandiflora*. The between-group sum of squares (SS = 2656.41) was substantially greater than the within-group sum of squares (SS = 6.70), indicating that most of the variation in paralysis time was due to differences in extract concentration rather than random variation within groups.

Table 4. One-way ANOVA Time of Paralysis

Source of Variation	SS	df	MS	F	p-value	Decision	Interpretation
Between Groups	2656.41	2	1328.21	**1190.00**	**<0.001**	Reject H_0	Significant difference
Within Groups	6.70	6	1.12	—	—	—	—
Total	2663.11	8	—	—	—	—	—

The computed F-value of 1190.00 was extremely high, indicating a strong treatment effect relative to experimental error. Furthermore, the obtained p-value (<0.001) was significantly lower than the 0.05 level of significance, leading to the rejection of the null hypothesis. Therefore, the findings confirm that significant differences existed in mean paralysis times across the extract concentrations.

These results indicate that increasing concentrations of the ethanolic bark extract significantly enhanced anthelmintic activity by reducing the time required to paralyze the worms.

Table 5 presents the pairwise comparisons of paralysis times among the three extract concentrations. All comparisons showed statistically significant differences ($p < 0.001$), indicating that each increase in concentration significantly reduced the paralysis time of *Pontoscolex corethrurus.*

The findings further support the dose-dependent anthelmintic activity of the ethanolic bark extract, wherein higher concentrations produced more rapid paralysis of the earthworms.

Table 5. Tukey Post-hoc Test for Paralysis Time

Comparison	Mean Difference	p-value	Decision	Interpretation
5 vs 10	26.20	<0.001	Reject H_0	Significant
5 vs 15	42.38	<0.001	Reject H_0	Significant
10 vs 15	16.18	<0.001	Reject H_0	Significant

Table 6 shows the one-way ANOVA results for death time among the different concentrations of the ethanolic bark extract. The between-group variation was considerably greater than the within-group variation, indicating that the differences in death time were primarily due to extract concentration.

The computed F-value of 1098.00 and the p-value of less than 0.001 indicate a highly significant effect of extract concentration on worm death time. Therefore, the null hypothesis was rejected, confirming that increasing extract concentration significantly reduced the time required to kill the worms.

Table 6. One-way Anova for Time of Death

Source of Variation	SS	df	MS	F	p-value	Decision	Interpretation
Between Groups	2574.75	2	1287.38	**1098.00**	**<0.001**	Reject H_0	Significant difference
Within Groups	7.04	6	1.17	—	—	—	—
Total	2581.79	8	—	—	—	—	—

Table 7 presents the pairwise comparisons of death time among the different extract concentrations. All comparisons revealed statistically significant differences ($p < 0.001$), indicating that increasing extract concentration significantly accelerated worm mortality.

Table 7. Tukey Post-hoc Test for Death Time

Comparison	Mean Difference	p-value	Decision	Interpretation
5 vs 10	20.15	<0.001	Reject H_0	Significant
5 vs 15	41.38	<0.001	Reject H_0	Significant
10 vs 15	21.23	<0.001	Reject H_0	Significant

Overall, the findings indicate that the ethanolic bark extract of *Sesbania grandiflora* possesses significant anthelmintic activity, although its efficacy remained lower than that of Albendazole. Nevertheless, the extract demonstrated strong dose-dependent biological activity, suggesting that increasing concentrations enhanced the effectiveness of the bioactive compounds present in the bark extract.

The observed activity may be associated with the phytochemical constituents of *S. grandiflora*, particularly tannins, flavonoids, and saponins, which are known to interfere with worm neuromuscular coordination and metabolic processes. These compounds likely contributed to the paralysis and eventual death of the earthworms observed during the assay.

CONCLUSION AND RECOMMENDATIONS

The study supports the plant's potential as a naturally derived medicinal agent, demonstrating that the bark extract of *S. grandiflora* has a low toxicity profile and an effective ability to eliminate helminths. The findings indicate that the bark extract did not produce severe lethal effects on *Artemia salina* nauplii under laboratory conditions, thereby supporting its suitability for further biological and pharmacological investigation. Furthermore, the anthelmintic assay confirmed that the *S. grandiflora* ethanolic bark extract is effective against *Pontoscolex corethrurus*, demonstrating a concentration-dependent response. Higher extract concentrations led to faster paralysis and death of the earthworms, indicating greater efficacy at higher dosages.

Further investigations are recommended to strengthen and expand the present results. To establish the safety and therapeutic potential of the extract in complex biological systems, toxicity assessment with an in vivo setup and efficacy studies in mammalian models are recommended. Moreover, future researchers are encouraged to test the extract against medically important intestinal parasites to validate its applicability for parasite control and treatment. Additional studies involving different extraction methods, concentration ranges, and formulation techniques may also help improve the therapeutic potential of the plant extract. Furthermore, the findings may serve as reference material for future researchers, educators, and health-related institutions to support the development of plant-based therapeutic agents and alternative health interventions.

DECLARATION OF THE USE OF AI TOOLS

The researchers acknowledge the use of ChatGPT, developed by OpenAI, to assist with refining grammar, sentence structure, clarity, coherence, and academic phrasing during the preparation of this research paper/thesis.

Prompt/s Used:

The following prompts were entered during the writing and revision process:

- "Revise this paragraph for academic tone and clarity."
- "Help me understand what this statistical result means."
- "Help improve the transition between paragraphs."
- "Check the flow and redundancy of this discussion section."
- "Improve the grammar and coherence of this statement."
- "Generate preliminary research-related suggestions."

Use of the Output:

The generated outputs were used as writing assistance for language refinement, clarification of statistical concepts, improvement of paragraph flow, and enhancement of academic readability. All content was reviewed, modified, and validated by the researchers before inclusion in the final manuscript.

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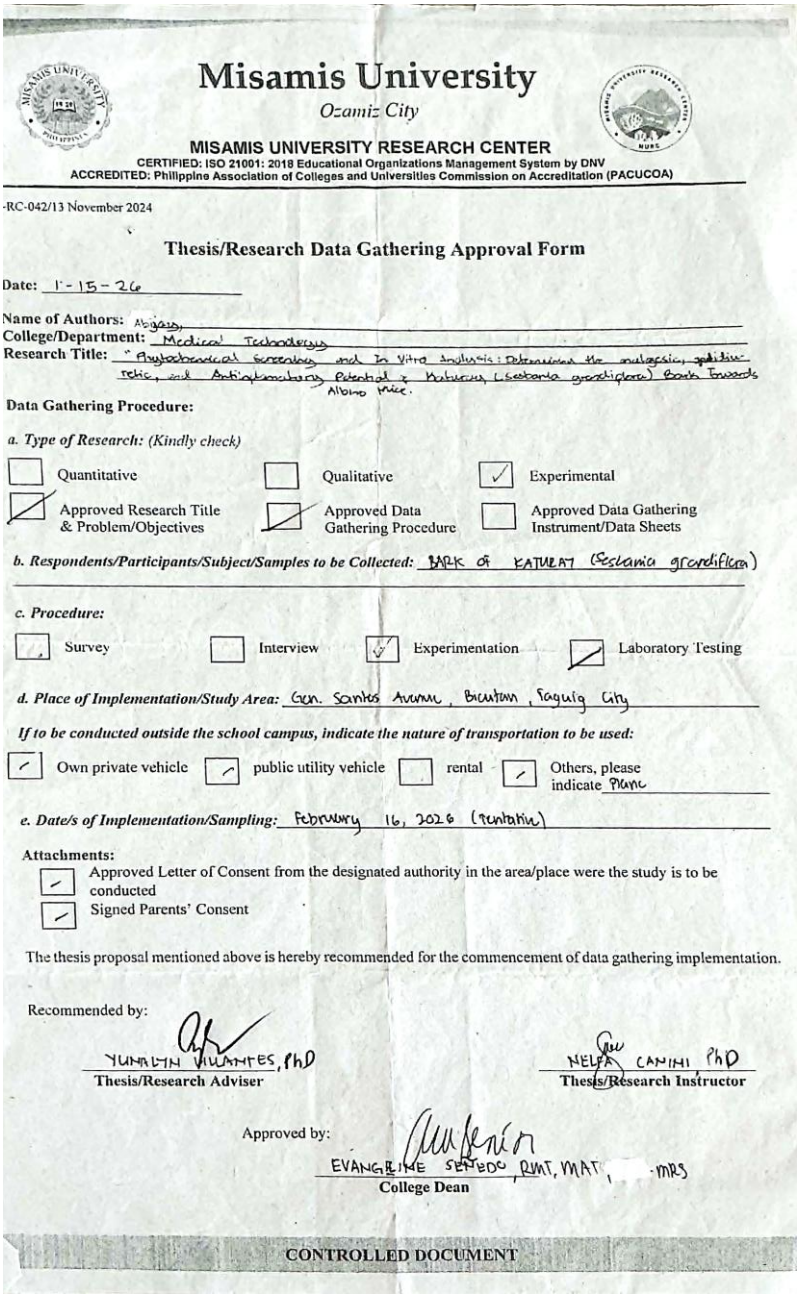
APPENDICES

APPENDIX A

Approved Letter of Consent



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Thesis/Research Data Gathering Approval Form

Date: 1-15-26

Name of Authors: Abigay,
College/Department: Medical Technology
Research Title: "Phytochemical screening and In Vitro analysis: Determining the analgesic, spasmolytic, and Anticholinesterase Potential of Kalanchoe Laciniata grandiflora) Bark Towards Albino mice.

Data Gathering Procedure:

a. Type of Research: (Kindly check)

- [ ] Quantitative
- [ ] Qualitative
- [x] Experimental
- [x] Approved Research Title & Problem/Objectives
- [x] Approved Data Gathering Procedure
- [ ] Approved Data Gathering Instrument/Data Sheets

b. Respondents/Participants/Subject/Samples to be Collected: BARK OF KATUBAT (Kalanchoe grandiflora)

c. Procedure:

- [ ] Survey
- [ ] Interview
- [x] Experimentation
- [x] Laboratory Testing

d. Place of Implementation/Study Area: Gen. Santos Avenue, Bicentan, Zaguig City

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

- [x] Own private vehicle
- [x] public utility vehicle
- [ ] rental
- [x] Others, please indicate Plane

e. Date/s of Implementation/Sampling: February 16, 2026 (tentative)

Attachments:

- [x] Approved Letter of Consent from the designated authority in the area/place were the study is to be conducted
- [x] Signed Parents' Consent

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

Recommended by:

YUMALYN VILLANTES, PhD
Thesis/Research Adviser

NELFA CANINI, PhD
Thesis/Research Instructor

Approved by:

EVANGELINE SEVEDO, RMT, MAT, MRS
College Dean

CONTROLLED DOCUMENT

APPENDICES A Cont'd

MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, Daryhl Angel E. Abijay, to join the Research Activity "Phytochemical Screening and In Vivo Analysis of Kalawat (*activity*) to be held on _____ at Heneral Santos Avenue, Bitan-an, Tagoloan, Misamis Oriental under the supervision of Yuna 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.

Vedamia E. Abijay — January 16, 2026
Signature over printed name of **Parent/Guardian** — Date

Daryhl Angel E. Abijay — January 16, 2026
Signature over printed name of **Student** — 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 ____ day of _____ at _____, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 264
Page No. 95
Book No. LX
Series of 2026

ATTY. REVEL S. BALDADO, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
PTR No. 6007751; 01/05/2026; Ozamiz City
IBP O.R. No. 576185; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCLE Compliance No. VIII-0014127, Valid until 04-14-2028

Notary Public
Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(*For DSAS Personnel only*)

Attested by:

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

Date: _____

APPENDICES A Cont'd

MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, Christian T. Alvarico, to join the Research Activity "Phytochemical Screening and In-vitro Analysis" *(activity)* to be held on _____ at General Santos Avenue, Bicutan Taguig City Metro Manila under the supervision of Yuna 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.

Mary Ellaine I. Alvarico — 01-16-2026
Signature over printed name of **Parent/Guardian** — Date

Christian T. Alvarico — 01-16-2026
Signature over printed name of **Student** — 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 ____ day of _____ at _____, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 226
Page No. 46
Book No. IX
Series of 2026

ATTY REY P. PUNAY, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
PTR No. 6007751; 01/05/2026; Ozamiz City
IBP O.R. No. 576165; 12/29/2025; Pasig City
Attorney's Roll No. 70649
MCLE Compliance No. VIII-0014727; Valid until 04/14/2028

Notary Public
Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(*For DSAS Personnel only*)

Attested by:

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

Date: _____

APPENDICES A Cont'd

MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, Princess Ainne G. Engracia, to join the phytochemical screening & in vitro analysis of S. grandiflorum (Research) (activity) to be held on _____ at DOST complex, Brgy Santos Ave, Bacayan, Tagum City under the supervision of Mrs. Yumuth 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.

Aclyn G. Engracia — 1-21-26
Signature over printed name of **Parent/Guardian** — Date

PRINCESS ANNE G. ENGRACIA — 1-21-26
Signature over printed name of **Student** — 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 ____ day of _____ at _____, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 262
Page No. 45
Book No. 14
Series of 20 26

ATTY. REY B. BINAG, JR., RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
PTR No. 8007751; 01/05/2026; Ozamiz City
IBP O.R. No. 576165; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCLE Compliance No. VIII-0014127; Valid until 04/14/2028

Notary Public
Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(***For DSAS Personnel only***)

Attested by:

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

Date: _____

MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, Angel L. Obaob, to join the "Phytochemical Screening and In vitro Analysis of 5 granulopan" *(activity)* to be held on _____ at DST Complex, Gen. Santos Ave., Bitan under the supervision of Ms. Jennalyn Villamor *(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.

ADELFA L. OBAOB
Signature over printed name of **Parent/Guardian**

01-21-2026
Date

ANGEL L. OBAOB
Signature over printed name of **Student**

01-21-2026
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 ____ day of _____ at _____, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 241
Page No. 45
Book No. 17
Series of 2026

ATTY REY D. BINAG, JR.
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Demad Subdivision, Aguada, Ozamiz City
PTR No. 6007751; 01/05/2026; Ozamiz City
IBP O.R. No. 576165; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCLE Compliance No. VIII-0014127; Valid until 04/14/2028

Notary Public
Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by:

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

Date: _____

APPENDICES A Cont'd

MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, Joshua B Tila, to join the Research Activity on "Phytochemical Screening and In vitro Analysis of Katmon (activity) to be held on _____ at General Santos Ave. - Bicutan, Taguig City, Metro Manila, Philippines under the supervision of Yuna Villagates (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.

Daniel Mala — Signature over printed name of **Parent/Guardian** — January 16, 2026 — Date

Joshua Tila — Signature over printed name of **Student** — January 16, 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 ____ day of _____ at _____, Philippines, that the herein affiant personally came and appeared with his/her _____, as evidence of his/her personal identity.

Doc. No. 260
Page No. 55
Book No. 12
Series of 2026

ATTY. REY B. BINAG, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
PTR No. 6007751; 01/05/2026; Ozamiz City
IBP O.R. No. 576165; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCLE Compliance No. VIII-0014127; Valid until 04/14/2028

Notary Public
Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

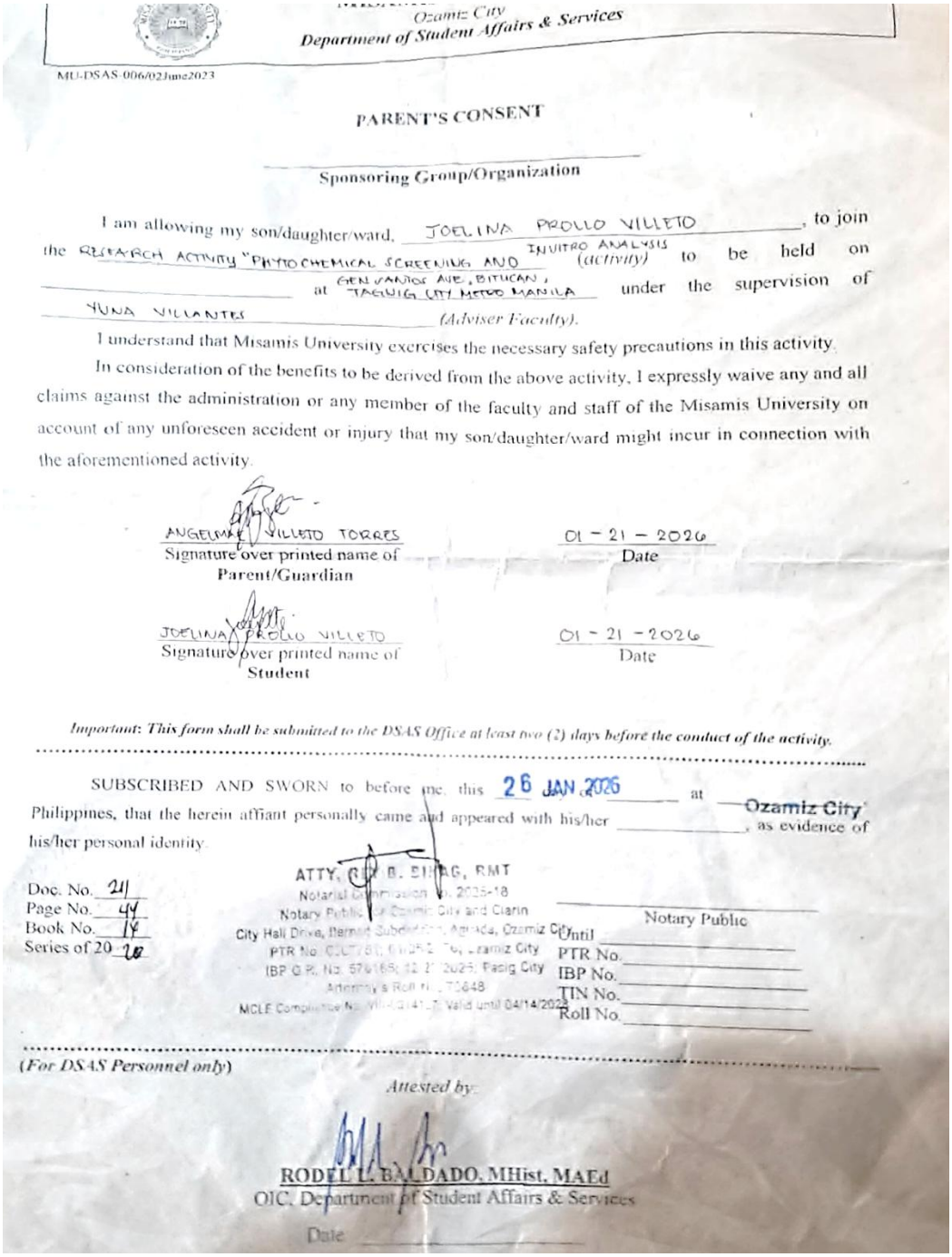
(For DSAS Personnel only)

Attested by:

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

Date: _____

APPENDICES A Cont'd



Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, JOELINA PROLLO VILLETO, to join the RESEARCH ACTIVITY "PHYTOCHEMICAL SCREENING AND IN VITRO ANALYSIS (activity) to be held on at GEN. VARIOS AVE., BITUCAN, TAGUIG CITY, METRO MANILA under the supervision of YUNA 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.

ANGELINE VILLETO TORRES — Signature over printed name of Parent/Guardian — 01 - 21 - 2026 — Date

JOELINA PROLLO VILLETO — Signature over printed name of Student — 01 - 21 - 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 26 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. 21
Page No. 44
Book No. 14
Series of 20 26

ATTY. [illegible] B. ELIAG, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Barrio Subdivision, Aguada, Ozamiz City
PTR No. [illegible] Ozamiz City
IBP O.R. No. 576169; 12.1.2025; Pasig City
Attorney's Roll No. 73648
MCLE Compliance No. VII-0041[illegible] Valid until 04/14/2028

Notary Public
Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(For DSAS Personnel only)

Attested by:

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

APPENDICES A Cont'd

MISAMIS UNVERSITY
Ozamiz City
Department of Student Affairs & Services

MU-DSAS-006/02June2023

PARENT'S CONSENT

Sponsoring Group/Organization

I am allowing my son/daughter/ward, JANE B. BARAQUIL, to join the RESEARCH ACTIVITY "PHYTOCHEMICAL SCREENING AND INVITRO ANALYSIS" (activity) to be held on _____ at GEN. SANTOS AVE., BITUKANG, TAGUIG CITY, METRO MANILA under the supervision of YUNA 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.

MERLINDA BARAQUIL
Signature over printed name of **Parent/Guardian**

01-16-2026
Date

JANE BARAQUIL
Signature over printed name of **Student**

01-14-2026
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 ____ day of 26 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. 22
Page No. 44
Book No. IV
Series of 2026

ATTY. [illegible] B. BINAG, RMT
Notarial Commission No. 2025-18
Notary Public for Ozamiz City and Clarin
City Hall Drive, Bernad Subdivision, Aguada, Ozamiz City
PTR No. 6007731; 01/05/2026; Ozamiz City
IBP O.R. No. 576165; 12/29/2025; Pasig City
Attorney's Roll No. 70648
MCLE Compliance No. VIII 0014127; Valid until 04/14/2028

Notary Public
Until _____
PTR No. _____
IBP No. _____
TIN No. _____
Roll No. _____

(*For DSAS Personnel only*)

Attested by:

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

Date: _____

APPENDICES A Cont'd

Misamis University

Ozamiz City

COLLEGE OF MEDICAL TECHNOLOGY

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

February 21, 2026

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

Subject: *Request for Permission to Use Laboratory Equipment, Rooms, and Reagents for Research Data Gathering*

Dear Dean Villanueva,

We, the undersigned undergraduate researchers from the College of Medical Technology, respectfully seek permission from your good office to **use selected laboratory equipment, laboratory rooms, and reagents located at the Natural Sciences Building** under the College of Arts and Sciences. This request is in line with our ongoing research data gathering for our research paper entitled: **"In Vitro Analysis: Determining the Analgesic, Diuretic, and Anti-inflammatory Potential of Katuray (*Sesbania grandiflora*) Bark Towards Albino Mice."**

The experimental procedures of our study are scheduled to be conducted from **February to April 2026**. The facilities and resources available at the National Science Building are essential for the successful completion of specific laboratory components of our research.

We give our full assurance that all laboratory work will be carried out **in accordance with university rules and regulations**, under proper supervision, and with utmost care for laboratory safety, cleanliness, and responsible use of equipment and reagents. We likewise commit to restoring all facilities to their proper condition after use.

Furthermore, all information and data obtained will be utilized **exclusively for academic research purposes**, and due acknowledgment will be given to the College of Arts and Sciences and the National Science Building in all research outputs.

We humbly request your approval and support for this academic endeavor. Your favorable response will greatly aid us in completing our research requirements.

Thank you very much for your time and kind consideration.

APPENDICES B

Statistical Computations

Step 1: Compute % Mortality

Each replicate has 10 nauplii, so:

\text{% Mortality} = \frac{\text{Mean Dead}}{10} \times 100

Concentration (mg/mL)	Dead (T1)	Dead (T2)	Dead (T3)	Mean Dead	% Mortality
1.000	4	5	3	4.00	40%
0.500	3	2	4	3.00	30%
0.250	2	1	2	1.67	16.7%
0.125	1	1	0	0.67	6.7%
0.0625	0	1	0	0.33	3.3%
0.03125	0	0	0	0.00	0%

Step 2: Apply Correction (for Probit)

Probit **cannot use 0% or 100%**, so we apply a correction:

\text{Adjusted %} = \frac{x + 0.5}{n + 1} \times 100

Concentration	Adjusted % Mortality
1.000	40.9%
0.500	31.8%
0.250	18.2%
0.125	9.1%
0.0625	4.5%
0.03125	4.5%

Step 3: Convert to Probit Values

Concentration	Log_{10} Conc	Adjusted %	Probit
1.000	0.000	40.9	4.77
0.500	-0.301	31.8	4.52
0.250	-0.602	18.2	4.08
0.125	-0.903	9.1	3.66
0.0625	-1.204	4.5	3.33
0.03125	-1.505	4.5	3.33

Step 4: Regression Equation

Linear regression (Probit vs Log Concentration):

$$\text{Probit} = 0.99(\log\ C) + 4.75$$

Step 5: LC_{50} Calculation
For LC_{50}:

$$\text{Probit} = 5$$
$$5 = 0.99(\log C) + 4.75$$
$$\log C = 0.253$$
$$LC_{50} = 10^{0.253} \approx 1.79 \text{ mg/mL}$$

 $LC_{50} \approx 1.79$ mg/mL
 Indicates **low toxicity** (since LC_{50} is higher than your tested range)

RESULTS: Normality Test

Normality of the data was assessed using the **Shapiro–Wilk Test** at $\alpha = 0.05$.

A. Death Time

Concentration (mg/mL)	Data	W-value	p-value	Interpretation
5	86.66, 86.50, 86.59	0.99	>0.05	Normal
10	66.35, 65.45, 67.48	0.96	>0.05	Normal
15	45.00, 46.02, 44.58	0.97	>0.05	Normal

Result: All groups show **no significant deviation from normality**.

B. Paralysis Time

Concentration (mg/mL)	Data	W-value	p-value	Interpretation
5	66.59, 66.50, 67.01	0.99	>0.05	Normal
10	39.34, 40.57, 41.59	0.97	>0.05	Normal
15	23.30, 24.32, 25.33	0.98	>0.05	Normal

Table 1. One-way ANOVA for Death Time

Source of Variation	SS	df	MS	F	p-value	Decision	Interpretation
Between Groups	2574.75	2	1287.38	**1098.00**	**<0.001**	Reject H_0	Significant difference
Within Groups	7.04	6	1.17	—	—	—	—
Total	2581.79	8	—	—	—	—	—

Table 2. Tukey Post-hoc Test for Death Time

Comparison	Mean Difference	p-value	Decision	Interpretation
5 vs 10	20.15	**<0.001**	Reject H_0	Significant
5 vs 15	41.38	**<0.001**	Reject H_0	Significant
10 vs 15	21.23	**<0.001**	Reject H_0	Significant

Table 3. One-way ANOVA for Paralysis Time

Source of Variation	SS	df	MS	F	p-value	Decision	Interpretation
Between Groups	2656.41	2	1328.21	**1190.00**	**<0.001**	Reject H_0	Significant difference
Within Groups	6.70	6	1.12	—	—	—	—
Total	2663.11	8	—	—	—	—	—

Table 4. Tukey Post-hoc Test for Paralysis Time

Comparison	Mean Difference	p-value	Decision	Interpretation
5 vs 10	26.20	**<0.001**	Reject H_0	Significant
5 vs 15	42.38	**<0.001**	Reject H_0	Significant
10 vs 15	16.18	**<0.001**	Reject H_0	Significant

Probit Analysis and LC_{50} Determination

To estimate the median lethal concentration, mortality data were subjected to Probit Analysis, which linearizes the dose-response relationship by converting percentage mortality into probit units and plotting them against the logarithm of concentration.

Adjusted mortality values were used to account for zero responses, enabling valid probit transformation. Linear regression analysis yielded the following equation:

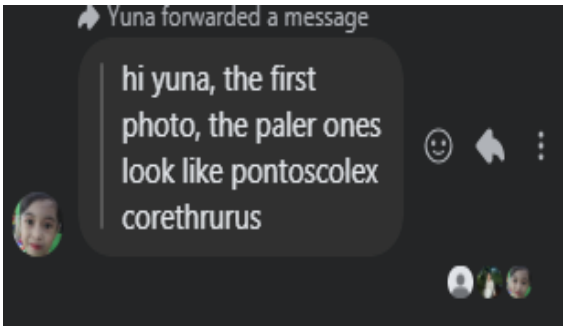
$$\text{Probit} = 0.99(\log\ C) + 4.75$$

Using this model, the **LC50** (median lethal concentration) was estimated at:

$$LC_{50} \approx 1.79\ \text{mg/mL}$$

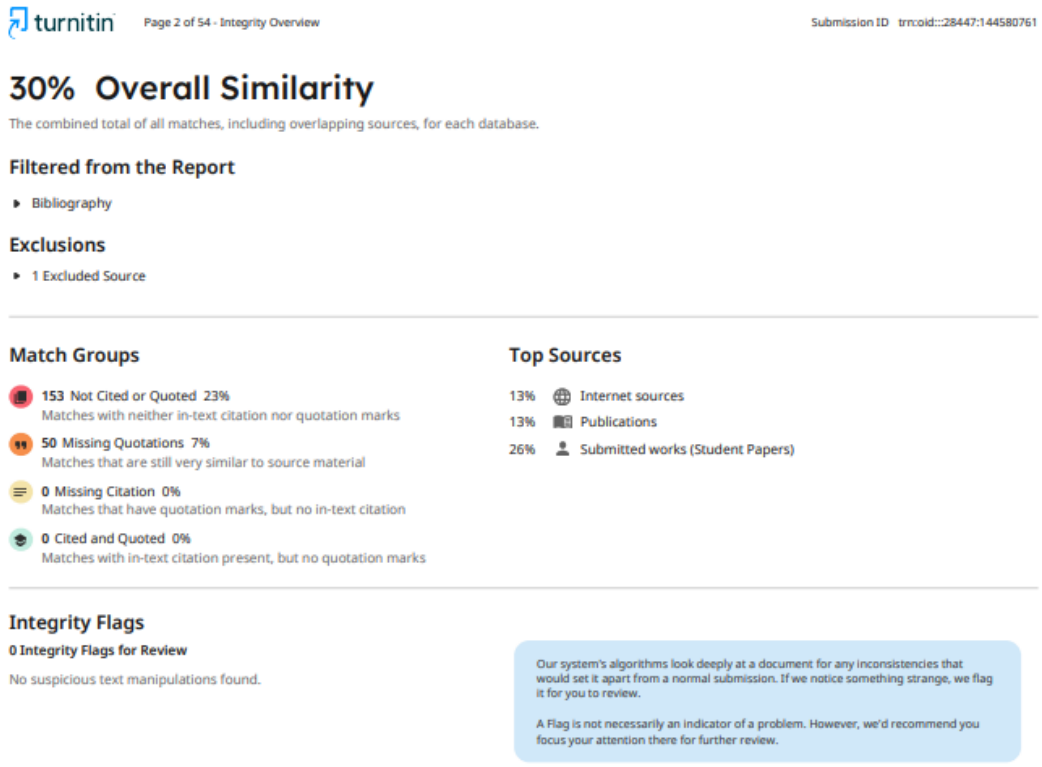
APPENDICES C

Document



APPENDICES D

Turnitin Results



turnitin Page 2 of 54 - Integrity Overview Submission ID trn:oid:::28447:144580761

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APPENDICES D Cont'd

Grammarly Results

TOXICOLOGICAL EVALUATION AND IN VITRO ANTHELMINTIC ACTIVITY OF ETHANOLIC BARK EXTRACT OF KATURAY (Sesbania grandiflora (L.) Pers.) AGAINST Pontoscolex corethrurus

by Coify

General metrics

57,742	7,562	462	30 min 14 sec	58 min 10 sec
characters	words	sentences	reading time	speaking time

Score

This text scores better than 99% of all texts checked by Grammarly

Writing Issues

17	3	14
Issues left	Critical	Advanced

Plagiarism

This text hasn't been checked for plagiarism

CURRICULUM VITAE

PERSONAL INFORMATION

Name: Joshua B. Tiu

Birthdate: April 18, 2004

Home Address: Gata, Jimenez, Misamis Occidental

Contact Number: 0927-228-2292

E-mail Address: joshuabtiu7@gmail.com

EDUCATIONAL BACKGROUND

Tertiary School: Bachelor of Science in Medical Technology Misamis University H.T. Feliciano Street Aguada, Ozamiz City, Philippines 7200 (2022-2026)

Secondary School: Misamis University - H.T. Feliciano Street, Ozamiz City, Misamis Occidental, 7200 Philippines (2021-2023); FMC MA, Dapdap, Maningcol, Ozamiz City, Philippines 7200 (2016-2020)

Primary School: Jimenez Central School, Nacional, Jimenez, Misamis Occidental, Philippines 7204 (2011-2017)

PERSONAL INFORMATION

Name: Princess Anne G, Engracia

Birthdate: May 28, 2005

Home Address: Bañadero, Ozamiz, Misamis Occidental

Contact Number: 09663557185

E-mail Address: princessengracia2005@gmail.com

EDUCATIONAL BACKGROUND

Tertiary School: Bachelor of Science in Medical Technology Misamis University H.T. Feliciano Street Aguada, Ozamiz City, Philippines 7200 (2023 - 2025)

Secondary School: Misamis University - H.T. Feliciano Street, Ozamiz City, Misamis Occidental, 7200 Philippines (2021-2023); Ozamiz City National High School, Lam-an, Ozamiz, Misamis Occidental Philippines 7200 (2018 - 2021)

Primary School: Ozamiz City Central School Special Education Center (OCCS-SPED), Tinago, Ozamiz City, Misamis Occidental, Philippines 7200 (2011 - 2017)

PERSONAL INFORMATION

Name: Joelina P. Villejo

Birthdate: June 28, 2001

Home Address: Zone 3, Purok Bariles Brgy. Himo-aw Hilongos, Leyte

Contact number: 096385238274

Email: joelinavillejo60@gmail.com

EDUCATIONAL BACKGROUND

Tertiary School: Bachelor of Science in Medical Technology (2023-2026)

Misamis University H.T. Feliciano St. Ozamis City

Secondary School: Greliña Osmeña Christian College (Senior High School)

(2017-2019); R.V. Fulache St. Eastern Brgy. Hilongos Leyte (2016-2012) Hilongos National Vocational School (Junior High School)

R.V. Fulache St. Eastern Brgy. Hilongos Leyte

Primary School: Himo-aw Elementary School

(2012-2006) Himo-aw Hilongos Leyte

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EDUCATIONAL BACKGROUND

Tertiary School: Bachelor of Science in Medical Technology Misamis University H.T. Feliciano Street Aguada, Ozamiz City, Philippines 7200 (2022-2026)

Secondary School: Clarin National Highschool Poblacion III, Clarin, Misamis Occidental, 7201, Philippines (2018-2022)

Primary School: Fourth Estate Elementary School Brgy. San Antonio, Fourth Estate, Parañaque City (2011-2017)

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EDUCATIONAL BACKGROUND

Tertiary School: Bachelor of Science in Medical Technology Misamis University
H.T. Feliciano Street Aguada, Ozamiz City, Philippines 7200
(2022-2026)

Secondary School: Magsaysay National High School
Magsaysay, Lanao Del Norte, Philippines, 9221 (2016-2022)

Primary School: Magsaysay Central Elementary School Magsaysay, lanao Del Norte
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EDUCATIONAL BACKGROUND

Tertiary School: Bachelor of Science in Medical Technology

Misamis University H.T. Feliciano Strreet Aguada, Ozamiz City, Philippines, 7200 (2023-2027)

Secondary School: Ozamiz City National High School

Lam-an, Ozamiz City, Misamis Occidental Philippines, 9221

(2021-2023)

Primary School: Felipe Carreon Central School Banadero, Ozamiz City, Misamis Occidental Philippines, 7200 (2012-2021)

PERSONAL INFORMATION

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EDUCATIONAL BACKGROUND

Tertiary School: Bachelor of Science in Medical Technology Misamis University
H.T. Feliciano Street Aguada, Ozamiz City, Philippines 7200
(2023 - 2025)

Secondary School: La Salle University Ozamiz (2018 - 2021)

Primary School: Ozamiz City Central School (OCCS), Tinago, Ozamiz City, Misamis Occidental, Philippines 7200 (2011 - 2017)