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In Vivo Investigation of the Molluscicidal Effect of Sambong (*Blumea balsamifera)* on Golden Apple Snail (*Pomacea canaliculata*)

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Abstract
One of the top 100 worst invasive species in the world is the golden apple snail (*Pomacea canaliculata*). Golden apple snails damage rice crops in the Philippines annually for $1.2 billion. GAS also presents a major health danger to people by carrying *Angiostrongylus cantonensis*, *Echinostoma ilocanum*, and *Schistosoma spp*. Applying artificial "instant kill" molluscicides is the simplest way to reduce GAS, however, it poses negative impacts on the environment, non-target species, and human health. The study aims to extract phytochemicals from *Blumea balsamifera* that are effective in killing invasive snails. The extract was obtained through maceration and was then evaporated through open-dish evaporation, it was then allowed to stand for 3 days. Five treatments were made; 2 control groups: the negative control and positive control; 3 experimental groups: 50000 PPM, 150000 PPM, and 300000 PPM concentration of *B. balsamifera* extract. The treatments were then distributed through water treatment to the five aquariums with labels containing 10 snails each. The snails were observed for 48 hours; a 24-hour exposure period and a 24-hour recovery period. The commercial molluscicide (Niclosamide), the 150000 PPM, and the 300000 PPM concentration of *B. balsamifera* extract killed all 10 snails. The 50000 PPM concentration killed 3 snails, and 1 moribund which eventually died, and the other 6 were still alive. All snails in negative control were alive. The 150000 PPM and 300000 PPM concentration was found effective according to the LC50 and LC90 test. Thus, the *B. balsamifera* extract can be used as a molluscicide.

Keywords: Invasive Snails, Sambong, Golden Apple Snail, Plant-Based Molluscicide, Molluscicidal Activity

Introduction
The golden apple snail (*Pomacea canaliculata*) is a freshwater snail that is one of the world's top 100 worst invasive species and a well-known problem in agriculture and a quarantine pest that results in significant financial losses (Liu *et al*., 2018) [9]. It is distinguished by rapid growth, great stress tolerance, rapid reproduction, and adaptation to a wide variety of environments. Species of Pomacea that have been imported from South America are commonly referred to as "golden apple snails" in Southeast Asia. This is either because of the occasionally bright orange-yellow color of their shells or because their initial introduction was seen as a potential source of significant financial success. The term "golden apple snail" does not apply to only one species. *Pomacea canaliculata* appears to be the Pomacea species that has been introduced the most frequently in Southeast Asia (Lamarck, 1822), however at least one other species has also been present and is sometimes confused with *P. canaliculata*. In the 1980s, they were brought from South America to Asia as possible human food, however, they turned out to be a significant rice pest. Young and developing rice plants are eaten by golden apple snails. The entire plant is destroyed when they chop the rice stem at the base. Without any preventative measures, they can completely destroy 1 m^2 of a field overnight. A loss in yield of over 50% could result from this damage. Out of the 24 invaded countries, Nghiem *et al*. projected that 3 out of 24 infested countries (Vietnam, the Philippines, and Thailand) had annual GAS destruction costs between 806 and 2138 million USD. Infestations of snails were most common in the Philippines when rice was first planted on newly prepared soil.
This problem was widespread throughout the nation's major areas, particularly in Northern Luzon and Western Visayas, both of which were regarded as the Philippines' rice granaries. Farmers in several places noted the infestation and losses caused by the snails every year. Out of the approximately 3 million hectares of rice-planting land in the Philippines, it was estimated that

about 800,000 hectares (ha) of rice farms were infested with the snails (Mendoza, 2014). Aside from being an agricultural pest, *Pomacea canaliculata* was discovered to be a significant intermediate host of *Angiostrongylus cantonensis*, the rat lungworm. The intermediate hosts are mollusks, such as freshwater and terrestrial snails. By consuming foods like snails that are undercooked or uncooked that contain live third-stage larvae of *A. cantonensis*, the parasite can cause angiostrongyliasis, an infection that manifests as eosinophilic meningitis in humans (Yang *et al*., 2013) [19]. In mainland China, the potentially fatal disease *Angiostrongylus cantonensis*-caused eosinophilic meningitis is regarded as an emerging infectious disease. A total of 32 species of mollusks in China have been tested for *A. cantonensis*, 22 of them containing the parasite. *Pomacea canaliculata* had an infection rate of 69.4%, according to the data. Yang *et al*. (2013) [19] noted that an increasing amount of epidemiological data suggests that P. canaliculata is becoming the most significant natural intermediate host of *A. cantonensis* in mainland China due to its strong parasite susceptibility and broad environmental tolerance. Furthermore, they are known to transmit the intestinal fluke (*Echinostoma ilocanum*) as well as the blood fluke (*Schistosoma spp.*). Humans' gastrointestinal tracts are affected by the food-borne disease echinostomiasis, which is brought on by intestinal trematodes of the Echinostomatidae family. Patients frequently experience nonspecific symptoms, but severe infections can result in catarrhal inflammation, stomach pain, anorexia, nausea, vomiting, diarrhea, and weight loss. Sah *et al*. (2018) noted that eating fish and mollusks uncooked or undercooked is associated with this infection. Several hundred thousand people or possibly more are infected in Southeast Asian nations like the Philippines, Japan, and India, although symptoms are typically mild (Mehlhorn, 2016). On the other hand, blood flukes (trematode worms) of the genus Schistosoma are the parasites that cause the acute and chronic parasitic disease schistosomiasis. According to projections, at least 251.4 million individuals will need preventative care in 2021 (World Health Organization [WHO], 2023). Abdominal discomfort, diarrhea, and blood in the stool can be symptoms of intestinal schistosomiasis. In severe cases, liver enlargement is typical and frequently accompanied by fluid buildup in the peritoneal cavity and abdominal blood vessel hypertension. Additionally, the spleen may enlarge in such circumstances.

Pomacea canaliculata are among the phylum mollusca. Mollusks are a group of animals that includes tens of thousands of invertebrate organisms. Some mollusks like the Zebra Mussel (*Dreissena polymorpha*), Brown Garden Snail (*Cantareus aspersa*), Giant African Snail (*Lissachatina fulica*), Golden Apple Snail (*Pomacea canaliculata*), and many others are considered invasive. To eliminate them commercial molluscicides are often used. Molluscicides often target snails and slugs as well as other mollusks like octopi and squid. They are agents created expressly to kill mollusks, such as chemicals or biocides (Claudi & Mackie, 1994). They can be used as a means of limiting the growth and spread of snails. The use of molluscicides is currently the most successful intervention technique for the management of snails in endemic areas (King *et al*., 2015). Molluscicides are nonselective for mollusk species, but their effectiveness depends on the right dose, administration technique, timing, and contact time. Numerous of these chemicals work by stressing mollusk species' water balance systems. According to McCullough *et al*. (1980), mollusks can die from stress on the water balance system alone. In addition, the restriction of normal water flow in the mollusk body causes various changes in metabolic or physiological function, which frequently result in organism death. The gill membranes experience harmful effects as a result of other compounds (Sprecher & Getsinger, 2000). Typically, molluscicides can be either oxidizing or non-oxidizing chemicals. Non-oxidizing molluscicides are more expensive per volume than oxidizing agents, but they are still less expensive overall because of their low use rates, short exposure times, and quick toxicity. However, molluscicides have numerous disadvantages, including that they are not cost-effective, have deadly consequences for other organisms, and need to be applied frequently. Additionally, uniform dispersal and area coverage are difficult to achieve, well-informed technical capacity is needed, and there is a risk of collateral molluscicide effect on other aquatic organisms that are not the intended target, environmental impact, and time demands for implementation and evaluation of control are greater than for mass drug administration.

Various plants were investigated as possible sources of phytochemical molluscicides. Due to their accessibility and ease of use, molluscicidal plant products are becoming more and more appealing to employ. Phytochemical-based molluscicides are highly effective, rapidly biodegradable, less expensive, readily available, and probably easily applicable with simple techniques than synthetic molluscicides.

Phytochemicals are organic compounds found in plants. These substances aid in defending plants against pathogens, fungi, pests, and other dangers. Phytochemicals are widely categorized into six groups based on their chemical characteristics and structures: carbohydrates, lipids, phenolics, terpenoids, alkaloids, and other nitrogen-containing compounds (Iyer *et al*., 2023) [8]. According to the Iranian Journal of Pharmaceutical Research [IJPR] (2014), there have been reports of many plants acting as molluscicides. However, before a plant with demonstrated molluscicidal activity in lab experiments can be applied widely, a number of requirements must be met. Therefore, the plant material must be readily available in the area where it is needed and, if necessary, be able to be easily propagated; the active ingredients must be easily extractable from the plant source and water-soluble; the molluscicidal activity must be high; and the toxicity toward other organisms, including humans, must be minimal (Evans and Evans, 2009) [5]. In a 2009 study, Evans and Evans found that the berries of the Ethiopian plant *Phytolacca dodecandra* were successful in getting rid of snails from long stretches of rivers. Triterpenoid saponins made of oleanolic acid with a branched sugar side-chain at C-3 are the plant's most potent constituents. Isobutylamides of the *Asteraceae*, *Rutaceae*, and *Piperaceae*, steroidal glycoalkaloids of *Solanum mammosum*, anthraquinones of *Morinda lucida*, and flavonoids of diverse families are further phytochemical classes of chemicals with documented molluscicidal activity. Moreover, the findings of the study titled "A study of the molluscicidal and larvicidal activities of *Citrullus colocynthis* (L.) leaf extract and its main cucurbitacins against the mollusk *Galba*

truncatula, intermediate host of *Fasciola hepatica*" by Chawech *et al*. (2017), proved the relationship between terpenoids and molluscicidal activity for the first time.

Numerous studies have evaluated the phytochemical content of *Blumea balsamifera* as a preliminary pharmacology and toxicology screening using both qualitative and quantitative techniques. More than 100 volatile or non-volatile components, such as monoterpenes, sesquiterpenes, diterpenes, flavonoids, organic acids, esters, alcohols, dihydroflavone, and sterols, have been extracted from sambong (Pang *et al*., 2014) [12]. L-borneol is the most significant component of Sambong. The two main volatile chemicals were alcohols and terpenoids, with terpenoids accounting for a significant proportion. *B. balsamifera* leaves extracted using ethanol and ethyl acetate solvents were found to have saponins, flavonoids, phenolics, tannins, and steroids as determined by the phytochemical screening. Notably, it was found that *B. balsamifera*'s phytochemical components using leaf extract in combination with ethanol were identical to with leaf extract in combination with ethyl acetate. The compound terpene has been linked to the molluscicidal effects of essential oils. Furthermore, other investigations have shown that flavonoids, terpenoids, and saponosides are responsible for the cercaricidal activity (Chifundera *et al*., 1993; Perret & Whitefield, 1995; Lahlou *et al*., 2002). Additionally, it was noted that the toxicity of the *Solanum genera* was attributed to the fruits' flavonoids and steroidal alkaloids (Silva *et al*., 2002 [6]; Bhattacharyya, 1984; Valverde *et al*., 1993).

There are currently no electronically available studies on the molluscicidal efficacy of *Blumea balsamifera* leaves. However, there are several studies about its potential as a pesticide. According to Chu *et al*. (2013) [3] in their study titled "Fumigant Compounds from the Essential Oil of Chinese *Blumea balsamifera* Leaves against the Maize Weevil (*Sitophilus zeamais*)," *Blumea balsamifera* leaves, a Chinese medicinal herb, were discovered to have fumigant toxicity against the *Sitophilus zeamais*. The primary elements of *B. balsamifera*'s essential oil are 1,8-cineole (20.98%), borneol (11.99%), -caryophyllene (10.38%), camphor (8.06%), 4-terpineol (6.49%), -terpineol (5.91%), and caryophyllene oxide (5.35%). Five component chemicals of the essential oil were isolated using bioactivity-guided chromatographic separation on several silica gel columns: 1,8-cineole, borneol, camphor, α-terpineol, and 4-terpineol. 1,8-Cineole, 4-terpineol, and α-terpineol. The mentioned components showed strong fumigant toxicity against S. zeamais adults (LC50 = 2.96 mg/L, 4.79 mg/L, and 7.45 mg/L air, resp.) and were more harmful than camphor (LC50 = 21.64 mg/L air) and borneol (LC50 = 21.67 mg/L air). Additionally, the crude essential oil was extremely toxic to S. zeamais adults (LC50 = 10.71 mg/L air).

According to Luo *et al*., *B. balsamifera* acetone extracts had an inhibition rate of over 90% against *Pryicutaria oryzae*, *Fusarium oxysporum sp.*, *Colletorichum musae*, *C. gloeosporioides*, *C. capsici*, and *F. oxysporum f. sp*. *in vitro*. *Aeromonas hydrophila*, *F. graminearum*, and *Magnaporthe grisea* were suppressed by the volatile oil of *B. balsamifera*. Wang *et al*. also revealed that the adult *Aleurodicus dispersus* were susceptible to a 60.8% insecticidal activity from the leaf extract of *B. balsamifera*. The findings suggested that the extracts of *B. balsamifera* could be employed as new potential plant pesticides due to their strong disease and insect resistance capabilities.

The first and most important step in the examination of medicinal plants is extraction since it is vital to separate and characterize the desired chemical components from the plant materials. Plant extraction separates the solid, insoluble plant residue from the liquid plant metabolites, such as phytochemicals. Maceration is an example of an extraction procedure in which the plant material is frequently shaken while being soaked in a particular solvent for a long time. The compounds that will be dissolved in the final yield will be greatly influenced by the solvents used in the maceration process. In research utilizing plant material, hydroalcoholic and ethanolic extracts appear to have the greatest phytochemicals.

In a study titled "A review on the extraction methods used in medicinal plants, principle, strength, and limitation" published in 2015, Azwanida claimed that the phytochemical Plant extract yields' content varies depending on a number of variables. Due to time constraints in the experimental design, dried samples are preferable over fresh samples. Additionally, it is demonstrated that, despite dried samples having higher flavonoid content, there is no discernible change in the total phenolic content between dried and fresh samples. Additionally, it has been discovered that the ground sample's particle size has a sizable impact on the extraction's yield. Generally speaking, smaller particle sizes will have more extraction surface area and result in a higher yield.

Open-dish evaporation is one of the many solvent extraction techniques. In solvent extraction the solute will be extracted into another solvent to remove it from a solution, the process is also known as liquid-liquid extraction. Essential immiscibility between the two solvents is required. According to Towler and Sinnott (2013), it is possible to purify the original solvent by removing impurities by using solvent extraction or to recover a valuable substance from the original solution. To recover a solid or a highly flammable liquid, it is required to remove solvent from a solution.

Open-dish evaporation involves putting the solution in an open container such as an Erlenmeyer, evaporating dish, beaker, or vial, a solvent can be evaporated. A heat source like a steam bath, hot plate, heating mantle, or sand bath is used to place the container, and the solvent is then boiled off. The drawback of open-dish evaporation is that the solvent is released into the atmosphere if the solvent is water, utilizing a heat source other than a steam bath. Any solvent other than water should always be evaporated in a hood when using an open dish.

A toxic substance is one that is harmful to living organisms or cells. Toxicity is the level of harm a toxin can cause. It may also be defined as a measurement of the dosage needed for a particular substance to damage a living organism. A single cell, a collection of cells, an organ system, or the entire body may be affected by these said effects. A toxic effect could manifest as physical harm or as a decline in functionality that can only be detected through testing. Three things affect a substance's toxicity: the chemical makeup of the material, how much of it is absorbed by the body, and the body's capacity to detoxify the substance (turn it into less harmful compounds) and discard it. The most pertinent theory in toxicology, however, is the dose-time relationship to toxicity, which links toxicity to the quantity of the substance involved (dose) and the length of exposure

(time), both of which have a direct bearing on toxicity and thus mortality; that is, an increase in one of the factors will result in an increase in toxicity and consequently an increase in mortality (Ottoboni, 2011).

Opposite to expectations, there is no straightforward linear model for the dose-mortality relationship. However, as demonstrated by numerous toxicology studies, the dose-response curve—a monotonic S-shaped curve—can be used to transform and predict the link between dose and mortality (Robertson *et al*., 2017). This makes sense because a substance won't be powerful enough to kill an organism below a particular threshold.

The component that the current research examined was the dose, which is synonymous with the concentration in solutions since it has been noted that the mortality rate changes with dose, but does so gradually until it reaches a point where it is strong enough to kill every creature in a population. The current study's use of the probit analysis approach is supported based on these theories in the literature and is in line with prior toxicity investigations. The comparison of LC50 and other toxicological dose descriptors is also said to be necessary when comparing the toxicities of different molluscicide candidates (WHO, 2019) [10]. The toxicity and molluscicidal efficacy of a molluscicide candidate are typically inversely correlated with the LC50 and LC90 values.

In an effort to combat environmental contamination, researchers are currently looking for molluscicidal products with plant origins. These are potentially biodegradable in nature because they are biosynthesis-derived compounds. The presence of phytochemicals in plants gives them the ability to fend off foreign intruders. Using plants as molluscicides instead of synthetic chemicals has a number of benefits. One of which is that plant-based molluscicides don't pose threats to the environment, especially to the crops themselves. The extracts made from secondary metabolites in plants are what give them their molluscicidal effects (Thakur *et al*., 2019). In the case of *B. balsamifera*, most of its secondary metabolites are found in its leaves (Wang *et al*., 2023). Furthermore, due to their availability, lower cost, eco-friendly nature, and lesser parameter chances of acquiring resistance by the mollusks, they seem to be the most reliable methods in controlling the overpopulation of mollusks. Hence, this became the basis of the present study, alongside the previously explained dose-mortality relationship, for the treatments used where the researchers tested the difference in the molluscicidal efficacy of the different concentrations and investigated the lowest concentration with high mortality.

The main goal of this study is to determine the molluscicidal efficacy of the ethanolic crude extract of the leaves of Sambong (*Blumea balsamifera*) on Golden Apple Snail (*Pomacea canaliculata*). Specifically, seeks the following: the percent mortality of *P. canaliculata* after 24 hours of exposure to various concentrations of the ethanolic crude extract of *B. balsamifera* leaves and another 24 hours of recovery period for the *P. canaliculata*; how well the probit model fits the concentration-mortality relationship among the treatments derived from the molluscicidal bioassay; the lowest concentration of *B. balsamifera* leaf extracts that would yield the mortality rates in the snail populations equal to Fifty percent (LC50) and Ninety percent (LC90); and the significant difference between the molluscicidal efficacy of the *B. balsamifera* leaf extracts, and the commercial molluscicide (positive control) in terms of mortality.

This study potentially benefits the agricultural sector, environmental preservation, community, stakeholders and manufacturers, field of toxicology, and future researchers. For the agricultural sector**,** Golden Apple Snails pose a significant threat to rice cultivation, a staple food for millions of people. If Sambong (*B. balsamifera*) is proven effective as a molluscicide, it can help reduce crop losses, increase agricultural productivity, and enhance food security in regions where rice is a primary dietary staple. For environmental preservation, the study's focus on finding an eco-friendly alternative to chemical pesticides aligns with the global push for environmentally sustainable agricultural practices. Reducing the use of harmful chemicals benefits our whole environment, which can have a wide range of ecological protection. For the community, the outcome of this research is expected to be helpful to the community since it will potentially discover a way to decrease the population of the agricultural pest and vector of parasites which is safer for humans than their commercial and artificial counterparts. For stakeholders and manufacturers, this study will be useful to people who are interested in making programs and policies on the use of natural plant-based molluscicides to control snail populations. It will also be helpful to molluscicide manufacturers that seek new innovations for their products. For the field of toxicology and future researchers: This study will fill an existing gap in research. Relatedly, it will serve as a reference for future researchers who wish to conduct a related study.

Materials and Methods

1. Research Design

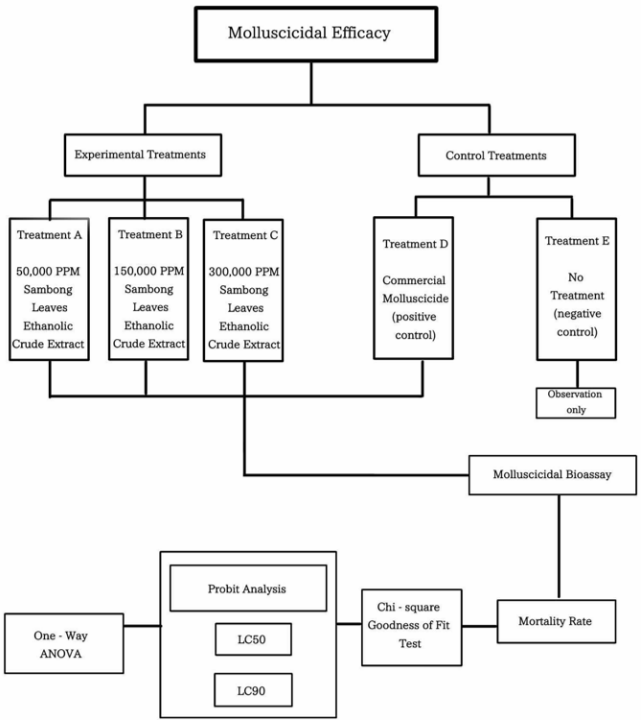

Fig 1: Research Design

The study used an experimental design with three experimental groups and two control groups. The study aimed to measure the molluscicidal efficacy of the three experimental groups-the varying concentrations of the ethanolic crude extract *B. balsamifera* leaves. There were

two control groups: a commercial molluscicide that acted as the positive control and an untreated dechlorinated water that acted as the negative control for observation only. The methodology was adapted from the modified version of the methodologies prescribed by the World Health Organization (2019) and Alibo *et al*. (2021). Statistical analysis of data includes the use of the chi-square goodness of fit test and the probit analysis method with ANOVA test for significance.

2. Research Procedure

The study's methodology was based on the World Health Organization's (2019) recommendations for molluscicide field and laboratory testing. The experiment was carried out in the biology laboratory of Bantayan Science High School in Barangay Ticad, Bantayan, Cebu (11°10'20"N, 123°43'16"E). All throughout the investigation, laboratory protocols were followed properly.

Collection and Preparation of Materials

First, the researchers collected *B. balsamifera* leaves one week before the experiment. They were collected from a property of one of the researcher's family located in Barangay Ticad, Bantayan, Cebu. Other materials and instruments needed for the study were already available at the school's laboratory while some were purchased or borrowed.

The leaves of the *B. balsamifera* were separated from their stems. Right after, they were washed thoroughly 3 times using distilled water.

Extraction of the Ethanolic Crude Extract

First, the *B. balsamifera* leaves were left to dry at room temperature for 24 hours. Then, it was placed in the drying oven for 3 hours with the temperature set at 70 ºC. Then the dried leaves were powdered using a blender.

Materials for the extraction process were prepared and assembled at the laboratory. For this, absolute ethanol was used in a 4:1 ratio with the powdered *B. balsamifera* leaves. The samples were then left for 24 hours with frequent stirring for the first 5 hours. The solid parts of the suspension were then filtered out using a grade 0905 crepe filter paper. The filtered B. *balsamifera* ethanolic crude extract was allowed to stand for 72 hours.

Making of Stock Solution

For the molluscicidal bioassay, stock solutions were created. In creating the stock solution for the plant-based molluscicide, 5 mL of the extract was mixed with 95 mL of water to create a 50000 PPM solution. Then, 15 mL of the extract was also mixed with 85 mL of water to create a 150000 PPM solution. Lastly, 30 mL of the extract was mixed with 70 mL of water to create a 300000 PPM solution.

Similarly, a stock solution was then made for the commercial molluscicide by dissolving 1.1 grams of the Niclosamide with 500 mL of water according to its instructions.

Molluscicidal Bioassay

A molluscicidal bioassay was then set up that follows the guidelines set by the World Health Organization (2019) with modifications. The *P. canaliculata* snails were randomly divided into five groups: two control groups and three test groups.

For the two control groups, one control group was the negative control which was only up for observation while the other control group was the positive control which was treated with a commercial molluscicide (Niclosamide). For the three test groups, the concentrations were 50000 PPM, 150000 PPM, and 300000 PPM. This is based on the study of Otnawi (2017) titled "Evaluating phytochemical profiles, molluscicidal and schistosomicidal activity of aqueous and ethanol extracts of vernonia amygdalina and harrisonia abyssinica," which utilized the amount 50, 150 and 300 mg/l. It was then added to each container to yield a specific concentration. The molluscicidal bioassay was conducted in the Bantayan Science High School Biology Laboratory on a photoperiod of 24 hours in light and 24 hours in darkness.

Data Collection and Disposal of Waste

At the conclusion of the 24-hour exposure period, snails were taken from treated or controlled water, cleaned, and transferred to containers containing standard snail water. Following a 24-hour recovery period, mortality was calculated. When a snail does not move after being stimulated with a needle to check for a contractile response, it is assumed to be dead.

Snails that were still alive after 24 hours were placed in freshwater with food and watched for an additional 24 hours (a total of 48 hours). Snails that were suspected of being dead were moved to different containers since, if they were, their decomposition may impair the survival of other snails. Suspected dead snails had their deaths verified at the conclusion of the recovery time.

The waste generated from the molluscicidal bioassay was then stored in sealed containers and disposed of in the laboratory disposal facility for biohazardous waste.

3. Statistical Treatment

Mortality of the molluscicidal bioassay was recorded after 24 hours of administering the treatment. The snail was counted as dead or moribund if it did not show signs of locomotion or reaction after the researcher touched it. The mortality rate will then be calculated using the following equation.

$$mortality\ rate = \frac{number\ of\ dead\ or\ moribund\ snails}{number\ of\ initial\ snails} \times 100$$

The mortality rates for each test concentration of the treatments were illustrated using bar graphs. The LC50 and LC90 values with their respective 95% confidence intervals for each of the treatments were then calculated by probit analysis using IBM SPSS Statistics 27 software. The statistical significance of the mortality of the snails in each treatment was then determined using the One-way ANOVA test.

Research Hypotheses

The study used a five-group experimental design. To determine the statistical significance of the findings, the researchers used One-way ANOVA (f-test). The use of the probit model was justified using the Chi-square Goodness of Fit test. An alpha level of $\alpha = 0.05$ was used for all statistical tests.

One-way ANOVA Test

H_o: There is no significant difference between the mortality of snails in treatments with the varying concentrations of the ethanolic crude extracts of Sambong leaves, and the commercial molluscicide.
H_a: There is a significant difference between the mortality of snails in treatments with the varying concentrations of the ethanolic crude extracts of Sambong leaves, and the commercial molluscicide.

Chi-square Goodness of Fit Test

H_o: The probit model adequately fits the mortality rates observed from the molluscicidal bioassay.
H_a: The probit model does not adequately fit the mortality rates observed from the molluscicidal bioassay.

Results and Discussion

The effect of the *B. balsamifera* extract on the *P. canaliculata*'s mortality was evaluated using a One-way Analysis of Variance (ANOVA). On the other hand, the LC50 and LC90 values of the extract were determined using probit analysis using IBM SPSS Statistics 27 and were justified using the Chi-square Test. All analyses incorporated a 0.05 alpha level.

Mortality

Mortality is a fundamental concept in biology, demography, epidemiology, and ecology, referring to the occurrence of death within a population or group of organisms.

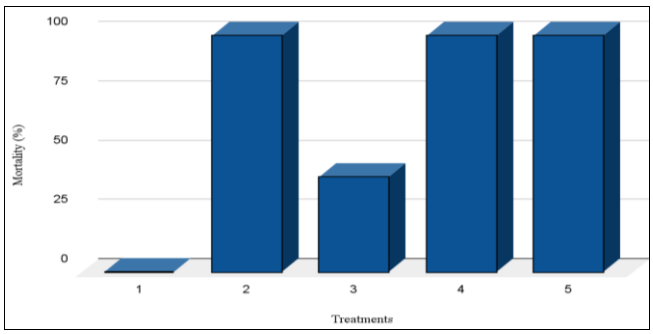

Fig 2: Mortality of Snails in Each Treatment

Fig 2 shows the mortality of *P. canaliculata* in treatments 1, 2, 3, 4, and 5 after 24 hours of exposure period and another 24 hours of recovery period. The graph shows a significant increase in mortality from Treatment 3 to Treatment 5. The positive control group and Treatments 4-5 showed 100% mortality while only Treatment 3 with 50000 PPM concentration could reach 40% mortality.

Table 1: The ANOVA Table for the Mortality of Snails in each Treatment

Source of Variation	Sum of Squares	*df*	Mean Square	*Fcomputed*	*Ftabular*	Interpretation
Between	8.48	4	2.12	39.75	2.58	Significant
Within	2.4	45	0.0533			
Total	10.88	49				

The ANOVA table shown above reports that the null hypothesis is rejected since the $F_{computed}$ value which is 39.75 is greater than the $F_{tabular}$ value which is 2.58. Therefore, there is a significant difference between the mortality of snails in Treatments 1, 2, 3, 4, and 5.

LC50 and LC90 Values of the Extract

LC50 is the concentration of a substance at which it causes mortality in 50% of the exposed population. LC50 is commonly used in toxicology to assess the potential harm of chemicals to organisms, including aquatic life, insects, or mammals. Researchers often determine LC50 through controlled laboratory experiments or field studies by exposing organisms to varying concentrations of the substance and recording mortality rates.
LC90 is similar to LC50 but represents the concentration at which 90% of the exposed population experiences mortality. It indicates a higher level of mortality compared to LC50. LC90 is used when a more stringent measure of toxicity is required, such as in situations where minimizing harm to organisms is a priority.

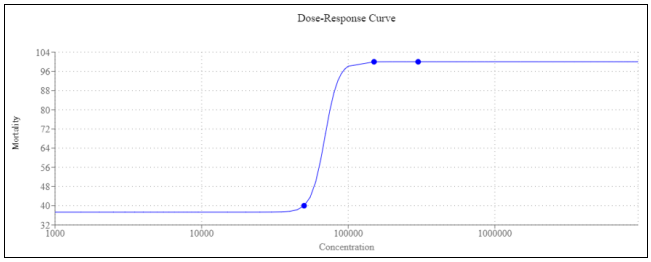

Fig 3: Dose-Response Curve

Table 2: Linear Regression Results (ANOVA Table) for the Mortality in each Treatment

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	10.04775	10.04775063	5.79609	0.2506
Residual	1	1.7335	1.733538993		
Total	2	11.781			

Table 3: Linear Regression Results for the Mortality in each Treatment

	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	-21.67949	12.16580	-1.78200275	0.32555194	-176.261	132.9017
X Variable 1	5.71228728	2.372698	2.407507322	0.25062671	-24.43567	35.860271

The coefficients of the Intercept and the X Variable 1 from the Results of Linear Regression Analysis was used to determine the LC50 and LC90 values of the extract with the formula $y = ax + b$ where y is the probit value equivalent to 50 and 90, a is the X Variable 1 coefficient, and b is the Intercept coefficient. Then, the antilog of the x value was computed.

Table 4: LC50 and LC90 Values of the *B. balsamifera* Extract

LC50	**LC90**
69344.219	79432.82

After calculating, the results showed that the LC50 value of the extract is 69344.219 while its LC90 value is 79432.82.

Chi-Square Goodness of Fit Test

The Chi-square goodness of fit test is a statistical hypothesis test used to determine whether a variable is likely to come from a specified distribution or not. The Chi-square goodness of fit test checks whether your sample data is likely to be from a specific theoretical distribution. The test can be used when counts of values for a categorical variable are available.

Table 5: The Chi-Square Goodness of Fit Table for the Expected Values in the Probit Model and Observed Mortality Rates

	O	*E*	X^2	*Critical Value*	*Significance Level*
Probit Pearson's goodness of fit Test	4	3.949	0.00067	3.841	0.920
	10	9.991			
	10	10			

a. Since the significance level is greater than .050, no heterogeneity factor is used in the calculation of confidence limits.
b. Statistics based on individual cases differ from statistics based on aggregated cases.

The Chi-Square Goodness of Fit table shown above reports that the alternative hypothesis is rejected since the X^2 value which is 0.00067 is less than the *Critical Value* which is 3.841. Therefore, the probit model adequately fits the mortality rates observed from the molluscicidal bioassay.

As to the mortality of snails from groups 1-5, groups 2 (positive control), 4 (150000 PPM concentration of ethanolic crude extract of *B. balsamifera*), 5 (300000 PPM concentration of ethanolic crude extract of *B. balsamifera*) has the most number of dead snails with 10 out of 10 snails found dead. It was followed by Group 3 (50000 PPM concentration of ethanolic crude extract of *B. balsamifera*) with 3 of the snails dead, 1 moribund, and the other 6 were still alive. While in group 1 (negative control), all of the snails were still alive.

As to the recovery of the snails after being applied with the different treatments, no snail from Groups 1-5 was able to recover after the 24-hour recovery period.

As to the LC50 and LC90 values of the *B. balsamifera* extract, the intercept and X variable 1 coefficients from the outcomes of the linear regression analysis were utilized to calculate the values of the extract. The calculations revealed that the extract's LC50 value is 69344.219 and its LC90 value is 79432.82.

As to the Chi-Square Goodness of Fit Test, the mortality rates obtained by the molluscicidal bioassay are well-fitted by the probit model. The significance level is 0.920 which is greater than the alpha level (0.05), therefore, there is no account for heterogeneity in the calculation of limits. Statistics based on aggregated cases are distinct from statistics based on individual cases. The alternative hypothesis is therefore rejected because the X2 value, which is 0.00067, is lower than the Critical Value, which is 3.841. Thus, the probit model successfully accounts for the mortality rates determined by the molluscicidal bioassay.

Scope and Delimitation

The objective of the study was to determine the molluscicidal efficacy of the ethanol extract of the *B. balsamifera* leaves against semi-adult *P. canaliculata*. Specifically, the study determined the mortality rates from each treatment with varying concentrations to calculate the LC50 and LC90 values for each of the samples. The study looked at the effect of the various treatments on the mortality of the target organisms and how changes in concentration would modify these relationships. The study utilized a five-group experimental design that used the molluscicidal bioassay method as recommended by the World Health Organization (2019). Moreover, statistical methods such as the chi-square goodness of fit test, probit analysis, and One-way ANOVA test were used by the researchers to analyze the data.

This study may not cover all regions affected by snail infestations worldwide, specifically focusing only on the Golden Apple Snail (*Pomacea canaliculata*), and may not extensively explore the molluscicidal effects of *B. balsamifera* on other snail species or pests. The study was done with no replication of the experiments due to time and financial constraints. Moreover, due to financial and instrumental limitations, the researchers did not conduct qualitative or quantitative phytochemical screenings for the molluscicide candidates. The researchers did not also look into the toxicity of these samples to non-target organisms.

This study was conducted to determine the effectiveness of the ethanolic extract of Sambong (*B. balsamifera*) as molluscicidal against Golden Apple Snails (*P. canaliculata)*. Five groups were tested: group 1 (negative control group), group 2 (positive control group), group 3 (experimental group), group 4 (experimental group), group 5 (experimental group).

Conclusions

After two 24 hours of treatment and 24 hours of observation, the results proved that the B. *balsamifera* ethanolic crude extract was effective in terms of killing the invasive Golden Apple Snails. However, lower concentrations of *B. balsamifera* extract may not be an efficacious molluscicide.

First, it was determined from the results that the 150000 PPM and 300000 PPM concentration of B. *balsamifera* ethanolic crude extract was effective in terms of killing all the population of the test subjects. However, the 50000 PPM concentration was not able to kill 50% of the snails. The commercial molluscicide on the other hand killed all the snails. It was calculated through one-way ANOVA that there was a significant difference between the five treatments suggesting that the commercial molluscicide and higher concentration of *B. balsamifera* is still more effective compared to lower concentrations of *B. balsamifera* extract.

Second, the LC50 and LC90 values of the extract showed that the 150000 PPM and 300000 PPM concentration of B. *balsamifera* extract is effective in eliminating 100% of the test subject since the LC90 value is 79432.82, however, the 5% concentration cannot eliminate half the population of the snails since the LC50 value is 69344.219.

Lastly, the results also showed that the obtained data from the molluscicidal bioassay is reliable since the significance level is greater than 0.05, thus there is no heterogeneity in the calculation of limits. Furthermore, individual case data and statistics based on aggregated cases are two different. This suggests that the results collected are credible and that *B. balsamifera* is indeed an effective molluscicide.

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References

1. Bevans R. One-way ANOVA | When and how to use it (With Examples). Scribbr, 2023. [updated 2023 June, 13 cited 2023, Oct. 2] Retrieved from: https://www.scribbr.com/statistics/one-way-anova/
2. Chawech R, Njeh F, Hamed N, Damak M, Ayadi A, Hammami H, *et al*. A study of the molluscicidal and larvicidal activities of Citrullus colocynthis(L.) leaf extract and its main cucurbitacins against the molluscGalba truncatula, intermediate host fasciola hepatica Pest Management Science, WILEY. 2016; 73(7):1473-1477.
3. Chu SS, Du SS, Liu ZL. Fumigant Compounds from the Essential Oil of Chinese Blumea balsamiferaLeaves against the Maize Weevil (Sitophilus zeamais). Journal of Chemistry. 2013; Article ID 289874, 7 pages.
4. Coelho PMZ, Caldeira RL. Critical analysis of molluscicide application in schistosomiasis control programs in Brazil. Infectious Diseases of Poverty, 2016. 1186/s40249-016-0153-6
5. Evans WC, Evans D. Pesticides of natural origin. In Elsevier eBooks, 2009, 533-536.
6. Giovanelli A, Da Silva CLPaC, Medeiros L, De Vasconcellos MC. The molluscicidal activity of niclosamide (Bayluscide WP70®) on *Melanoides tuberculata* (Thiaridae), a snail associated with habitats of *Biomphalaria glabrata* (Planorbidae). Memorias Do Instituto Oswaldo Cruz. 2002; 97(5):743-745.
7. Gohar AA. Molluscicidal Activity of the Methanol Extract of *Callistemon viminalis* (*Sol. ex Gaertner*) G.Don ex Loudon Fruits, Bark and Leaves against Biomphalaria alexandrina Snails. PubMed Central (PMC), January 1, 2014. Cited 2023, Oct. 2. Retrieved from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4157025/
8. Iyer M, Pal K, Upadhye V. Recent Frontiers of Phytochemicals. Elsevier Inc, 2023, 295-308.
9. Liu C, Zhang Y, Ren Y, Wang H, Li S, Jiang F, *et al.* The genome of the golden apple snail Pomacea canaliculata provides insight into stress tolerance and invasive adaptation. GigaScience. 2018; 7(9).
10. Madsen H, Oxborough R. Guidelines for laboratory and field testing of molluscicides for control of schistosomiasis. Geneva, World Health Organization (WHO), 2019, p27.
11. Mougabure-Cueto G, Sfara V. The analysis of dose-response curve from bioassays with quantal response: Deterministic or statistical approaches? Toxicology Letters. 2016; 248(1):46-51.
12. Pang Y, Wang D, Fan Z, Chen X, Yu F, Hu X, *et al*. *Blumea balsamifera*-A Phytochemical and Pharmacological Review. Molecules. 2014; 19(7):9453-9477.
13. Prabhakaran G, Bhore SJ, Ravichandran M. Development and Evaluation of Poly Herbal Molluscicidal Extracts for Control of Apple Snail (*Pomacea maculata*). Agriculture. 2017; 7(3):22.
14. Pum J. A practical guide to validation and verification of analytical methods in the clinical laboratory. Advances in Clinical Chemistry. 2019; 90(1):215-228.
15. Quaternary M. Compounds, P. A., Aromatic Hydrocarbons, & their salts. (n.d.). U.S. army CORPS OF ENGINEERS building strong®. Anl.gov. cited September 21, 2023, Retrieved from: https://glmris.anl.gov/documents/docs/anscontrol/Molluscicides.pdf
16. Schneiker J, Weisser WW, Settele J, Nguyen VS, Bustamante JV, Marquez L, *et al*. Is there hope for sustainable management of golden apple snails, a major invasive pest in irrigated rice? NJAS Wageningen Journal of Life Sciences. 2016; 79(1):11-21.
17. Silva YRR, Silva LD, Rocha TL, Santos DBD, Bezerra JCB, Machado KB, *et al*. Molluscicidal activity of *Persea americana Mill.* (Lauraceae) stem bark ethanolic extract against the snail *Biomphalaria glabrata*: A novel plant-derived molluscicide? Anais Da Academia Brasileira De Ciencias. 2020; 92(4).
18. Solvent removal. Boulder; University of Colorado Department of Chemistry and Biochemistry, n.d. [cited 2023 Sept. 25]. Retrieved from: https://www.orgchemboulder.com/Technique/Procedures/SolventRemoval/SolventRemoval.shtml#:~:text=Open%2DDish%20Evaporation,and%20the%20solvent%20boiled%20off.
19. Yang T. The Apple Snail Pomacea canaliculata, a Novel Vector of the Rat Lungworm, Angiostrongylus cantonensis: Its Introduction, Spread, and Control in China. PubMed Central (PMC). 2013; 72(6):23-25.
20. Zheng L, Deng L, Zhong Y, Wang Y, Guo W, Fan X. Molluscicides against the snail-intermediate host of Schistosoma: A review. Parasitology Research. 2021; 120(10):3355-3393.