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RESEARCH ARTICLE

Molluscicidal Activity of Ethanolic *Dieffenbachia seguine* (Dumb Cane) Leaf Extract Against *Pomacea canaliculata* (Golden Apple Snail)

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Abstract: The golden apple snail (*Pomacea canaliculata*), an invasive species that originated in South America, has caused significant damage to rice plantations in Southeast Asia, notably the Philippines. Because of its voracious appetite for aquatic vegetation and quick reproduction rate, it poses a significant agricultural problem. Conventional control approaches, which mostly use chemical molluscicides, have increased environmental issues and threats to non-target organisms. This study evaluates the molluscicidal activity of *Dieffenbachia seguine* leaf extract against *P. canaliculata*. The extract demonstrated dose-dependent mortality, achieving 100% mortality at 100 mg/L. However, the extract should be regarded as a potential plant-based alternative requiring further ecotoxicological and field validation rather than a confirmed environmentally safe substitute. According to the results, the lethal levels (LD_{50} and LD_{90}) were 12.46 mg/L and 61.13 mg/L, respectively. The findings indicate that the crude *D. seguine* extract produced significant laboratory mortality *in P. canaliculata* at increasing concentrations. Statistical analyses verified that concentration had a substantial impact on the extract's molluscicidal activities. These results support further investigation of *D. seguine* leaf extract as a possible component of Integrated Pest Management (IPM), provided that extract standardization, formulation stability, degradation behavior, non-target ecotoxicity, and field efficacy are established before practical application.

Keywords: Molluscicide, *Dieffenbachia*, *Pomacea*, Agriculture, Sustainability

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Introduction

Native to South America, in areas that produce rice, the golden apple snail (*Pomacea canaliculata*), an invasive freshwater gastropod, has grown to be a significant agricultural and ecological nuisance, particularly in Southeast Asia and the Philippines. Following its introduction to Asia for aquaculture and food, the species quickly spread throughout freshwater habitats because of its aggressive feeding habits, high reproductive capability, and adaptability to a variety of environmental circumstances. In rice agroecosystems, *P. canaliculata* causes extensive damage by feeding on young seedlings and plant

tissues, leading to poor crop establishment, reduced yields, and substantial economic losses. Consequently, the species is acknowledged as one of the most damaging invasive mollusks that impact rice-based agricultural systems [1-3].

Along with agricultural losses, *P. canaliculata* is a public health concern because it serves as an intermediate host for medically significant parasites such as *Angiostrongylus cantonensis* and *Echinostoma* species, which are linked to gastrointestinal infections and eosinophilic meningitis [4, 5]. These interrelated effects on agriculture and health highlight the necessity of safe, efficient, and long-lasting control measures.

Botanical extracts are increasingly explored as sources of pest-management agents because they are locally available and biodegradable. However, their natural origin should not be equated with environmental safety. Each extract still requires evaluation for toxicity, persistence, degradation behavior, and possible effects on non-target organisms before practical use.

In her research, [6] mentioned various ethnobotanical traditions of the Ilocano people and their strong dependence on plant-based remedies. Conversely, [7] established the ethnobotanical potential of medicinal plants in controlling such diseases as dengue. Similarly, [8] discovered the insecticidal potential of the leaf extracts of *Rhaphanus sativus* on *Drosophila melanogaster.*

Manual collection, biological control employing natural predators, water management, and the use of artificial molluscicides like metaldehyde and niclosamide are some of the methods used in the Philippines to manage golden apple snail infestations. Chemical molluscicides are effective, but their frequent and prolonged use has raised concerns because they are poisonous to animals that are not their intended targets, have environmental persistence, pose possible health risks to people, and contaminate aquatic ecosystems [9]. As a result, alternative control strategies that adhere to integrated pest management principles and are ecologically friendly are gaining popularity.

Recent international and local studies support the continuing investigation of plant-derived molluscicides as alternatives to synthetic pesticides. In general, botanical extracts may offer advantages of local availability and biodegradability; however, their safety must be demonstrated through standardized toxicity, ecotoxicity, and field-based evaluations. Corpuz and colleagues' local research has shown that plant extracts have potent dose-dependent bioactivity against agricultural pests and disease vectors [10].

However, despite these advances, a critical research gap remains in the standardization of the *D. seguine* leaf extract and the establishment of its dose-dependent relationship specifically against *P. canaliculata* under controlled laboratory conditions. Previous studies have reported general molluscicidal activity of plant extracts [11, 12] but limited data exists on the toxicity thresholds (LD50 and LD90), comparative efficacy with standard molluscicides such as Niclosamide [13], and reproducibility using a completely randomized experimental design. Furthermore, the selection of *P. canaliculata* as the model organism is justified due to its high agricultural relevance in rice ecosystems [14], thereby providing practical applicability of the findings.

The tropical ornamental plant *Dieffenbachia seguine* (dumb cane), which is rich in secondary metabolites such as alkaloids, calcium oxalate crystals, and proteolytic enzymes, has also been shown to have molluscicidal efficacy against major snail species used in agriculture and medicine. These bioactive substances cause physiological and cellular disturbances in mollusks, which ultimately result in death [15]. The examination of *D. seguine* as a possible botanical molluscicide against *P. canaliculata* is further supported by the proven efficacy of bioassay-guided plant extract generation in earlier investigations, including those carried out by [16].

The current study assesses *D. seguine* leaf extract's molluscicidal potential against *P. canaliculata* as a plant-derived candidate for further laboratory and field evaluation. By generating dose-response data under controlled conditions, the study contributes preliminary evidence that may support future development of safer and more sustainable rice pest-management approaches, consistent with the United Nations Sustainable Development Goal No. 2 on food security and sustainable agriculture.

Study Objectives

The molluscicidal efficacy of various concentrations of *D. seguine* leaf extract against *P. canaliculata* was evaluated in this study. Specifically, it addressed the following research objectives:

a) It determined the presence of selected phytochemical groups in the leaf extract of *D. seguine* through qualitative screening methods [17]

b) It determined the molluscicidal activity of *D. seguine* leaf extract at concentrations of 25 mg/L, 50 mg/L, 75 mg/L, and 100 mg/L, as exhibited by the mortality of *P. canaliculata*

c) It determined whether there are significant differences in the molluscicidal activity among the different concentrations of *D. seguine* leaf extract and control treatments using statistical analysis

d) It determined the lethal dose concentrations that result in 50% and 90% mortality (LD_{50} and LD_{90}), respectively, in *P. canaliculata* to ascertain the acute toxicity levels of the *D. seguine* leaf extract

e) It proposed a potential plant-based molluscicidal agent that is derived from *D. seguine*

Methods and Procedures

In this section, the investigation's methodology is presented. It provides a detailed account of the research design, study location, materials and tools utilized, and the sequential methods executed during the experimentation. The molluscicidal efficacy of *D. seguine* leaf extract against *P. canaliculata* snails is evaluated using a systematic methodology that ensures repeatability, reliability, and precision.

Research Design

To evaluate ethanolic *D. seguine* leaf extract's effectiveness as a natural molluscicide against *P. canaliculata*, the study used an experimental research design. The acaricidal bioassay protocols used by [18], who also used randomized controlled methods to assess toxicity in arthropods, served as the basis for the experimental setting. Additionally, it was conceptually consistent with the laboratory bioassay method employed in [10] larvicidal study, which used standardized mortality observation across graded extract concentrations and controlled treatments. A Completely Randomized Design (CRD) was used to reduce the possibility of bias.

Different concentrations of *D. seguine* leaf extract represented the independent variable, whereas *P. canaliculata* mortality was the dependent variable. The experiment included several treatment groups with different extract concentrations as well as a control group for comparison. The replication of each treatment increased the validity of the results.

Study Locale

The study was carried out at the University of Northern Philippines' College of Health Sciences Laboratory, which is located in Vigan City, Ilocos Sur. The *P. canaliculata* snails used in the molluscicidal tests were gathered from rice fields in Barangay Quimmarayan, Santo Domingo, Ilocos Sur, Philippines. According to local farmers, this area is infamous for regular infestations. To ensure the accuracy and repeatability of the findings, *D. seguine* extracts were put through controlled laboratory tests with constant temperature, light, and water quality regulation. The location was chosen due to the high frequency of golden apple snails in the area as well as the availability of the test organisms and plant materials needed for the investigation.

Materials

Materials employed in the investigation were classified into four categories: reagents, test organisms, glassware, and apparatus. Among the apparatus were automatic pipettes, a weighing scale, and a rotary evaporator. Test tubes, beakers, Erlenmeyer flasks, graduated cylinders, watch glasses, stirring rods, magnifying glasses, and other standard laboratory items were included in the glassware. *P. canaliculata* snails were employed as the test organisms. The reagents consisted of a Niclosamide solution, distilled water, 95% ethyl alcohol, and varying concentrations of *D. seguine* leaf extract.

Procedure

Plant Identification

The Resident Botanist of the Department of Environment and Natural Resources (DENR) Sub-Office in Bantay, Ilocos Sur, Philippines, Antonio P. Ridulme, identified the plant as *D. seguine*. Plant identification was based on morphological characteristics and expert taxonomic verification following standard botanical identification protocols [19] however, no molecular confirmation (e.g., DNA barcoding) was conducted, which is acknowledged as a limitation of the study.

Plant Preparation

Two hundred (200) grams of pulverized *D. seguine* leaves were immersed in 95% ethyl alcohol at a 1:2 ratio (one part leaf material to two parts alcohol). In order to optimize extraction, the mixture was permitted to remain in storage for 48 hours with stirring. Subsequently, the solid residues were eliminated through filtration, and the resultant alcoholic solution was concentrated using a rotary evaporator.

Phytochemical Screening Test

Qualitative analysis was used to identify the phytochemicals found in the leaf extract of *D. seguine*. This was only applicable to Wagner's test, which involved mixing 1 milliliter of the extract with an equivalent volume of the reagent. The reagent was made by dissolving 2 grams of potassium iodide (KI) and 1.27 grams of iodine (I_2) in 100 milliliters of distilled water. Alkaloids were indicated by the production of a brown precipitate. The presence of saponins was assessed using the froth test. Five milliliters of the extract and a few drops of sodium carbonate (Na_2CO_3) solution were used to accomplish this. After vigorously stirring the liquid, it was left undisturbed for approximately five minutes. The development of a steady froth signified the existence of saponins. The lead acetate test was conducted by combining 2 mL of the extract with a few drops of a 10% lead acetate solution. The existence of flavonoids was validated by the formation of a yellow precipitate. The presence of phenolic compounds was assessed using a gelatin test, which involved mixing 5 mL of the extract with 5 mL of distilled water. Two milliliters of a 1% gelatin solution with 10% sodium chloride were added after thorough mixing. The presence of phenolic compounds is shown by the formation of a white precipitate.

Preparation of the Different Concentrations of *D. seguine* Leaf Extracts and Niclosamide Solution

The *D. seguine* leaf extract was prepared in four concentrations for this study: 100 mg/L, 75 mg/L, 50 mg/L, and 25 mg/L. These concentrations refer to the mass of crude ethanolic extract per liter of final solution and do not represent purified or quantified active phytochemical constituents. Thus, the exact concentration of bioactive compounds within each treatment remains undetermined, which is a common limitation in crude extract-based bioassays [24]. These concentrations were designated as T4, T3, T2, and T1, respectively. To prepare the 100 mg/L solution, 10 mg of the crude extract was dissolved in 100 mL of distilled water, which is equivalent to 100 mg/L (100 mg/L = 10 mg/100mL). The remaining concentrations were prepared proportionally: 7.5 mg/100 mL (75 mg/L), 5 mg/100 mL (50 mg/L), and 2.5 mg/mL for 25 mg/L. All calculations were verified using the standard dilution equation (C1V1 = C2V2) to ensure accuracy and reproducibility [24]. The positive control was a 4 mg/L Niclosamide solution, which is a molluscicide that is widely recognized and has been shown to be effective against freshwater snails, including Oncomelania species [13]. Niclosamide, which is a dependable benchmark in molluscicidal investigations, disrupts snail metabolism and respiration, as recommended by the WHO. Surekill was the commercial product employed in this investigation, and it contains 0.814 g of Niclosamide per gram of product. An ethanol control was not included in the present study. Although the extract was concentrated prior to dilution, trace amounts of ethanol may have remained. However, based on the literature, ethanol at low residual concentrations is not expected to produce significant molluscicidal effects [24]. This is acknowledged as a limitation, and future studies are recommended to include solvent controls to isolate the effects of the plant extract.

Molluscicidal Bioassay

The molluscicidal bioassay was conducted at the University of Northern Philippines' College of Health Sciences Laboratory. The *P. canaliculata* snails were properly identified and acclimatized for three days to the laboratory environment. The WHO Guidelines for Laboratory and Field Testing of Molluscicides for Schistosomiasis Control [13] were used to evaluate the molluscicidal efficacy of *D. seguine* leaf extract. The test organisms (*P. canaliculata*) were selected based on uniform size (2-3 cm shell length), similar weight, intact shell condition, and active movement before exposure. Environmental conditions were controlled as follows: temperature (25-280C), pH (7.0), and clean, aerated water without feeding during exposure. The conditions were maintained to ensure reproducibility and minimize external variability [13]. The test setup included two control groups: A positive control (4 mg/L Niclosamide solution) and a negative control (distilled water), along with four concentrations of *D. seguine* extract (100 mg/L, 75 mg/L, 50 mg/L, and 25 mg/L). Each group received 100 mL of the suitable treatment solution in plastic basins covered with fine mesh netting to prevent the snails from escaping. The duration of exposure was 24 hours. The entire bioassay was replicated five times, with ten snails per replicate, to strengthen the reliability of the findings.

Snail mortality was tracked every hour during the 24-hour exposure period. The snails were taken out of the test solutions at the conclusion of the exposure, washed, and put in distilled water-filled containers for further examination. Using a magnifying glass, retraction and movement were studied in order to determine mortality. Dead snails were those that were totally encased in their shells and did not move at all. Each snail was positioned between two thick glass slides, and its shell was carefully crushed to look for any contractile reaction, which was used to confirm death. The absence of such a response indicated mortality.

Statistical Treatment of Data

Mean values and frequency distributions were employed to evaluate the molluscicidal activity according to the mortality of the test organisms following a 24-hour observation period. To find significant differences, the leaf extract's molluscicidal properties were examined using a One-Way Analysis of Variance (ANOVA) at a 5% significance level. To ascertain whether concentration pairs showed significant differences, the Tukey Honestly Significant Difference (HSD) test was used. Probit analysis was employed to determine lethal dose values (LD_{50} and LD_{90}).

LD50 AND LD90 Determination Procedure

To estimate the median Lethal Dose (LD50) and Lethal Dose (LD90), mortality data from each concentration group were entered into statistical software (SPSS version 26.0). Probit analysis was performed based on the number of snails that died at each concentration after 24 hours of exposure. The percentage mortalities were transformed into probit values, and a linear regression model was generated by plotting log concentration versus probit response. The resulting regression equation was used to calculate the corresponding lethal doses.

Results and Discussion

Phytochemicals Present in *D. seguine* Leaf Extract

According to phytochemical investigations as indicated in Table 1, the presence of many bioactive components, including saponins, alkaloids, phenolic compounds, and flavonoids, is responsible for the molluscicidal activity of *D. seguine* leaf extract. These chemicals promote mortality in mollusks through several ways that impact their specific physiological systems. Saponins are acknowledged for their capacity to compromise cell membranes by interaction with sterols, resulting in enhanced membrane permeability and ultimately, cell lysis. This leads to epithelium and gill damage in snails, hindering respiration and osmoregulation. [20] documented that extracts of *Camellia oleifera* saponins have membranolytic properties in mollusks, resulting in tissue damage and mortality in slug models, suggesting analogous mechanisms in related gastropods. This outcome aligns with their findings.

Table 1: Results of the Phytochemical Screening Test on *D. seguine* Leaf Extract

Analyte Tested	Test Conducted	Positive Result
Alkaloids	Wagner's test	Presence of brown precipitate.
Saponins	Froth test	Formation of stable foam.
Flavonoids	Lead Acetate test	Yellow solution with precipitate.
Phenolics	Gelatin test	Presence of white precipitate.

Alkaloids found in *D. seguine* demonstrate neurotoxic properties by inhibiting cholinesterase enzymes and disrupting neurotransmitter function, potentially resulting in paralysis and subsequent death. Likewise, oxidative stress caused by flavonoids and phenolic substances leads to metabolic malfunction and tissue damage in mollusks. Similar phytochemical-mediated mechanisms such as membrane disruption, neuromuscular interference, and enzymatic inhibition were observed in plant-derived acaricidal formulations from *A. indica* and *C. papaya*, reinforcing the wider relevance of these compounds among invertebrate pest taxa [16].

This procedure is consistent with the results of [21], who found that alkaloid-rich plant extracts had a substantial impact on the motor activity and survival of aquatic snails. Likewise, oxidative stress is provoked by phenolic chemicals and flavonoids, which play a role in molluscicidal activity. These substances generate Reactive Oxygen Species (ROS) that interfere with metabolic enzymes, hinder mitochondrial activity, and cause damage to cellular components. This observation

is consistent with research by [22], which showed that plant extracts high in flavonoids caused oxidative imbalance in *Biomphalaria glabrata*, resulting in metabolic failure.

The results of this study are consistent with those of [15], who observed similar molluscicidal action and phytochemical contents in *D. picta*, a closely related plant. Their research confirmed the presence of alkaloids, flavonoids, and saponins, which were accountable for significant mortality rates in mollusk populations. [22] noted that the effectiveness of plant-derived molluscicides can significantly differ depending on the extraction technique and the particular environmental circumstances of the plants' growth, leading to divergent results. This diversity underscores the importance of standardizing extract preparation and testing techniques in future investigations.

The phytochemicals detected in *D. seguine* may collectively contribute to the observed molluscicidal activity. However, because the present study used only qualitative screening and did not test isolated compounds or combinations of compounds, any claim of synergistic interaction remains preliminary. In line with earlier studies, this study supports further evaluation of plant-based molluscicide candidates as possible alternatives to synthetic chemical agents.

However, while the qualitative screening confirms the presence of the above-mentioned phytochemicals, it does not establish their individual or relative contributions to the observed molluscicidal activity. The attribution of toxicity to these compounds is therefore based on previously reported mechanisms in the literature rather than direct experimental validation in the study [20-22]. Likewise, no quantitative standardization of active compounds (e.g., total alkaloids or saponins) was conducted. Variability due to plant age, environmental conditions, and extraction efficiency is acknowledged as a limitation [17]. Future studies should include marker compound quantification to ensure batch consistency.

Moreover, any discussion of mechanisms is based on previously reported literature rather than direct experimental evidence from this study [23, 24].

Lastly, no fractionation or isolation of individual compounds was performed; hence, claims of synergistic interaction among phytochemicals remain hypothetical and require further experimental confirmation [24].

The *D. Seguine* Leaf Extract's Molluscicidal Effect on *P. canaliculata* at Various Concentrations

Table 2: Molluscicidal Activity of the *D. seguine* Leaf Extract in Terms of Mortality of the *P. canaliculata* at Different Concentrations Within a 24-Hour Observation Period

D. seguine Leaf extract	Number of Snails	Mean Mortality	Std. Deviation
25 mg/L	10	6.4	1.14018
50 mg/L	10	8.4	0.54772
75 mg/L	10	9.4	0.54772
100 mg/L	10	10	0.00000
Dist. Water (Negative Control)	10	0	0.00000
4mg/L Niclosamide Solution (Positive Control)	10	10	0.00000

D. seguine leaf extract exhibited a distinct dose-dependent molluscicidal activity against *P. canaliculata* in the study, as manifested by Table 2. The mean mortality rates increased as the concentration of the extract increased, from 6.4 at 25 mg/L (T1) to complete mortality (mean = 10.0) at 100 mg/L (T4). The efficacy of the positive control, 4 mg/L Niclosamide (T6), was matched by the maximum concentration (100 mg/L), both of which achieved 100% mortality with no variation (SD = 0.000), indicating a consistent and potent effect. But although 100 mg/L *D. seguine* extract achieved mortality comparable to 4 mg/L Niclosamide, this comparison is based solely on observed mortality outcomes and does not imply equivalent potency on a per-dose basis. The substantially higher concentration required for the plant extract indicates lower potency relative to the synthetic molluscicide. Conversely, the negative control group (T5 distilled water) demonstrated 0% mortality, thereby verifying that the observed effects were a result of the treatments rather than environmental factors. Furthermore, the zero standard deviation observed in the 100 mg/L treatment and Niclosamide control reflects complete uniformity of response (100% mortality across all replicates). This outcome is expected in highly effective treatments and does not indicate error but rather consistent biological response [25]. Moreover, each treatment group was replicated five (5) times, and the reported mean

mortality values represent the average across replicates. While individual replicate data were not tabulated, the use of mean +/- standard deviation is consistent with standard experimental reporting practices [25].

The results of the experiment are in agreement with those of [12], who reported that Dieffenbachia spp. extracts possess substantial molluscicidal properties, with snail mortality increasing as concentrations increased. [18] found similar dose-dependent trends in acaricidal mortality when they utilized higher amounts of papaya and neem extracts. This is similar to how the effectiveness of *D. seguine* treatments against mollusks consistently increased. In the same vein, [26] discovered that botanical extracts containing alkaloids, saponins, and flavonoids - phytochemicals that are present in *D. seguine* - significantly contribute to molluscicidal activity by interfering with respiratory and neural functions.

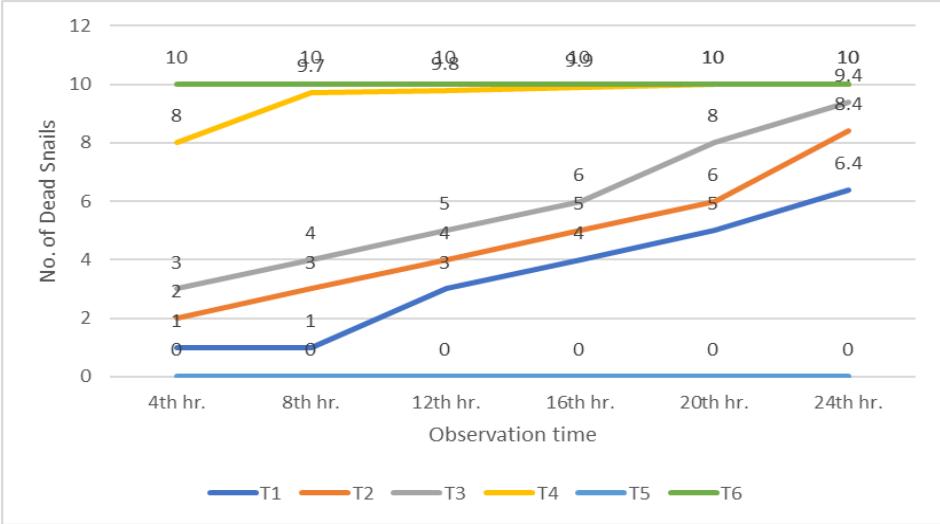

Fig. 1: Time-dependent molluscicidal effectiveness of *D. Seguine* leaf extract against *P. canaliculata* at different concentrations (T1–T4). The positive control is niclosamide (T6), whereas the negative control is distilled water (T5)

The results are also consistent with the [13] guidelines, which recognize niclosamide as a gold standard for molluscicidal testing. The observation that 100 mg/L *D. seguine* extract produced the same final mortality endpoint as 4 mg/L niclosamide under controlled laboratory conditions supports its potential as a locally available plant-derived candidate. Nevertheless, this result should be interpreted as preliminary laboratory evidence and not as proof of equal potency, environmental safety, or field-level effectiveness.

In contrast, a handful of studies have found lesser efficacy for *D. seguine* extracts. This is usually ascribed to differences in the extraction techniques or the bioassay environment, including temperature, pH, and snail strain. However, the modest standard deviations found in this investigation, especially at higher dosages, imply that the effects of the treatment are consistent and repeatable in the carefully monitored laboratory environment.

In conclusion, the findings provide compelling evidence for the molluscicidal potential of *D. seguine* leaf extract, particularly at concentrations of 75 mg/L and higher. However, further studies are necessary to determine standardized active constituents, formulation stability, non-target safety, and field efficacy before the extract can be recommended for practical application.

Figure 1 illustrates the result of the study using a graphical presentation. The graph shows the *D. seguine* leaf extract's time-dependent molluscicidal effectiveness against *P. canaliculata* at different concentrations (T1–T4). The positive control is niclosamide (T6), whereas the negative control is distilled water (T5). A distinct dose-response trend is demonstrated by the graph. The mortality rate was more rapid and extensive at higher concentrations (T3 and T4), with T4 (100 mg/L) attaining 100% snail mortality as early as the 12th hour. This is comparable to Niclosamide (T6), which maintained full mortality throughout the observation period.

T1 (25 mg/L) and T2 (50 mg/L) exhibited slow, progressive increases in mortality, with 6.4 and 8.4 deaths, respectively, by the 24th hour. The test reliability was validated by the absence of mortality in the negative control (T5) at any stage. In general, the data support the molluscicidal efficacy of *D. seguine* extract in a concentration- and time-dependent manner.

A key limitation of this study is the inability to identify which specific phytochemical constituents are primarily responsible for the observed toxicity. The use of crude extract prevents determination of individual compound potency and mechanism of action. Further studies involving fractionation, compound isolation, and targeted toxicity assays are necessary [11, 24].

Considerable Variation in the Molluscicidal Activity of the Leaf Extract at Various Concentrations Compared to the Controls Against *P. canaliculata*

Table 3 presents the mean mortality of *P. canaliculata* exposed to varying concentrations of *D. seguine* leaf extract, including positive and negative controls, and is compared in Table 3, which includes the One-Way ANOVA analysis. The treatment groups exhibit a statistically significant difference, as indicated by the analysis.

Table 3: Comparative Analysis of *D. seguine* treatment Mean Mortality of *P. canaliculata* using One-Way ANOVA

	Sum of Squares	df	Mean Square	F	Sig.	Re-marks
Between Groups	371.367	5	74.273	234.547	.000	Significant
Within Groups	7.600	24	.317			
Total	378.967	29				

Table 4: Pairwise Comparison Result of the Different Treatments of *D. seguine* Leaf Extract

Variable 1	Variable 2	Mean Difference	p-value[a]	Decision	Interpretation
T1 (25 mg/L)	T2	-2.000*	0.000	Do not accept H0.	Significant
	T3	-3.000*	0.000	Do not accept H0.	Significant
	T4	-3.600*	0.000	Do not accept H0.	Significant
	T5	6.400*	0.000	Do not accept H0.	Significant
	T6	-3.600*	0.000	Do not accept H0.	Significant
T2 (50 mg/L)	T3	-1.000	0.090	Accept H0.	Not Significant
	T4	-1.600*	0.002	Do not accept H0.	Significant
	T5	8.400*	0.000	Do not accept H0.	Significant
	T6	-1.600*	0.002	Do not accept Ho.	Significant
T3 (75 mg/L)	T4	-0.600	0.553	Accept H0.	Not Significant
	T5	9.400*	0.000	Do not accept H0.	Significant
	T6	-0.600	0.553	Accept H0.	Not Significant
T4 (100 mg/L)	T5	10.000*	0.000	Do not accept H0.	Significant
	T6	0.000	1.000	Accept H0.	Not Significant
F-value: 234.547 p-value: 0.000[b]					

Tukey HSD at 0.05 level of significance

One-way ANOVA at 0.05 level of significance

The majority of the variation in mortality rates is attributable to the differences among the interventions, rather than random error, as indicated by the between-group sum of squares (SS = 371.367) and the within-group sum of squares (SS = 7.600). The result is highly significant at the 0.05 level, as evidenced by a p-value (Sig.) of 0.000 and a high F-value of 234.547. This implies that there is substantial evidence to oppose the null hypothesis, which presupposes that all treatment means are equivalent.

The substantial outcome confirms that the mortality of *P. canaliculata* is significantly influenced by the differences in concentrations of *D. seguine* extract and the controls. As previously demonstrated in the descriptive statistics and time-based mortality graph, the observed mortality is directly influenced by the increasing concentrations of the extract. The results confirm that *D. seguine* is a promising natural molluscicide, with effects that are comparable to those of the conventional chemical molluscicide, Niclosamide.

In order to ascertain which specific treatment pairings exhibit significant differences, particularly between the extract concentrations and the control groups, a post hoc analysis, such as Tukey's HSD, would be required.

The statistically significant differences in the molluscicidal activity across various concentrations were revealed by the pairwise comparison of the varied treatments of *D. seguine* leaf extract as shown in Table 4. The 25 mg/L treatment (T1) was significantly less effective than all other treatments, including the higher concentrations (T2, T3, and T4), and the positive control (T6 – 4 mg/L Niclosamide). This low dose suggests a minimal molluscicidal effect. Furthermore, T1 was significantly more effective than T5 – distilled water. The 50 mg/L treatment (T2) did not exhibit a significant difference from 75 mg/L (T3), suggesting similar observed mortality between these two concentrations under the conditions of the assay. However, T2 was substantially less effective than 100 mg/L (T4) and Niclosamide (T6). The 75 mg/L extract (T3) did not differ significantly from either the 100 mg/L or niclosamide, in terms of observed mortality, suggesting that it approached the final laboratory mortality endpoint of the standard comparator. The 100 mg/L treatment (T4) was statistically comparable to niclosamide in observed mortality, with complete mortality recorded in both groups. This finding should be interpreted as comparable laboratory mortality outcome, not equivalent potency, environmental safety, or field efficacy. These results demonstrate a distinct dose-dependent trend in molluscicidal activity, with snail mortality increasing as concentrations increase. The findings are in accordance with prior research that has emphasized the molluscicidal properties of plant-based compounds, including those reported by [11]. These studies support the further evaluation of botanical extracts as possible alternatives to synthetic molluscicides, subject to ecotoxicological and field validation.

Lethal Dose Concentrations (LD_{50} and LD_{90}) of the *D. Seguine* Leaf Extract Against *P. canaliculata*

Figure 2 presents the dose-response curve of *D. seguine* leaf extract. The dose-response curve of *D. seguine* leaf extract exhibits a clear, concentration-dependent rise in death among the test species, suggesting the extract's significant toxicological attributes. Logistic dose-response relationships exhibit a sigmoidal mortality pattern that escalates with increasing concentration. The extract has significant toxicity at relatively low concentrations, demonstrated by the 12.46 mg/L LD_{50}, which is the amount required to cause 50% mortality. This indicates the potential for considerable bioactivity and effectiveness in insect or parasite control applications. The concentration required to eliminate 90% of the population, referred to as LD_{90}, is 61.13 mg/L. The response curve exhibits a pronounced slope owing to the substantial disparity between LD_{50} and LD_{90}. This indicates that even slight dosage increments over the LD_{50} lead to a fast rise in fatality rates. This response indicates that accurate dosage regulation is crucial for maximizing efficacy and minimizing environmental or non-target dangers.

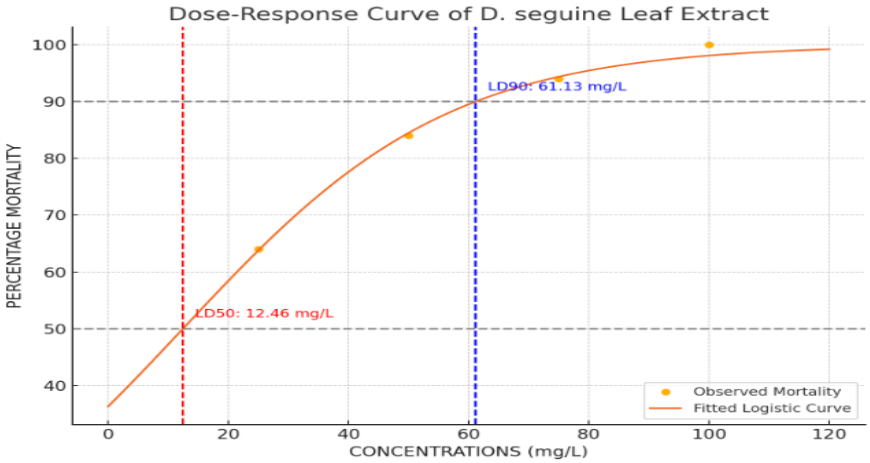

Fig. 2: Dose-response curve of D. seguine leaf extract against the test species

The logistic curve fitted to the experimental data closely aligns with the observed mortality data, depicted as yellow spots on the graph. This affirms the dependability and precision of the data. Mortality is mild at concentrations under 10 mg/L; however, a sharp increase occurs above this threshold, with nearly complete mortality reported at concentrations above 100 mg/L, indicating a plateau or saturation point in the extract's impact. This trend indicates the existence of a threshold beyond which the mortality rate is not significantly elevated by the inclusion of this extract. The findings confirm that *D. seguine* leaf extract possesses significant bioactive activities, supporting its potential as a natural molluscicide against *P. canaliculata*. These encouraging findings require further investigation of its mechanism of action, quantitative standardization, formulation

stability, degradation kinetics under natural conditions, safety for non-target organisms, and practical utility in agricultural contexts.

Although the extract demonstrated significant toxicity to *P. canaliculata*, the safety profile of non-target organisms was not evaluated in this study. Therefore, no definitive conclusions can be made regarding its ecological safety, and further ecotoxicological studies are required [9].

Based on the Results of the Investigation, Propose a Potential Plant-Based Molluscicidal Agent That Is Derived from *D. seguine*

D. seguine leaf extract exhibits significant potential as a plant-based molluscicidal agent, notably at higher concentrations, as indicated by the investigation's findings. Within 24 hours of exposure, the 100 mg/L crude extract produced 100% mortality of P. canaliculate, which was the same observed mortality endpoint obtained with 4 mg/L niclosamide positive control under the conditions of the experiment. This result suggests that *D. seguine* contains bioactive phytochemical groups, possibly including alkaloids, saponins, flavonoids, and phenolic compounds, that may contribute to its molluscicidal activity. However, because the extract was crude and the phytochemical screening was qualitative, the specific active compounds, their concentrations, and their individual or combined mechanisms of action remain undetermined.

Based on these findings, the ethanolic leaf extract of *D. seguine* may be further developed and evaluated as a potential plant-based molluscicidal formulation for the management of golden apple snails. Before practical application in rice fields or irrigation systems, however, future studies must establish standardized raw-material collection, extract preparation procedures, marker-compound quantification, formulation stability, degradation kinetics under natural environmental conditions, toxicity to non-target organisms, and field-level efficacy. Thus, the extract should be presented as a promising candidate requiring further validation, rather than as a confirmed environmentally safe substitute for synthetic molluscicides.

Conclusion

This research demonstrates that ethanolic *D. seguine* leaf extract exhibits significant laboratory molluscicidal activity against *P. canaliculata*. The extract showed concentration-dependent lethality, achieving 100% snail mortality within 24 hours at a dosage of 100 mg/L. This final mortality endpoint was similar to that of the 4 mg/L niclosamide positive control under controlled laboratory conditions of the study. However, this does not establish equivalent potency, field effectiveness, or environmental safety. The variations in molluscicidal efficacy across concentrations were statistically significant, as evidenced by One-Way ANOVA and pairwise comparisons. Furthermore, Probit regression analysis showed LD50 and LD90 estimated values of 12.46 mg/L and LD_{90} 61.13 mg/L, respectively, indicating measurable acute toxicity to the target snail species.

Overall, the study supports *D. seguine* leaf extract as a potential plant-based molluscicidal candidate. However, because the study used crude extract, qualitative phytochemical screening, no ethanol control, and no non-target necotoxicity assessment, the findings should be limited to controlled laboratory efficacy against *P. canaliculata*. Further studies are required to identify and quantify active compounds, validate mechanisms of action, standardize extract preparation, determine formulation stability, assess degradation kinetics under natural conditions, evaluate non-target ecotoxicological safety, and conduct field validation before practical application. This study confirms the molluscicidal activity of crude ethanolic *D. seguine* leaf extract against *P. canaliculata* under controlled laboratory conditions. However, due to the use of crude extract and qualitative phytochemical screening, further studies are required to identify active compounds, validate mechanisms of action, and assess environmental safety before application.

Recommendations

Based on the findings and limitations of the study, future research should first standardize the raw plant material and extraction process by defining the maturity of leaves, collection site and season, drying conditions, extraction solvent ratio, extraction time, and yield. Quantitative phytochemical analysis should also be conducted to identify marker compounds and determine batch-to-batch consistency of the crude extract.

Future studies should include an ethanol or solvent control to rule out any possible residual solvent effect. Fractionation, isolation, and compound-specific assays are also recommended to identify the active constituents responsible for molluscicidal activity and to determine whether the observed effect is due to individual compounds or combined phytochemical action.

Before practical agricultural application, formulation studies should be conducted to determine the most appropriate product form, concentration, shelf life, storage stability, and application frequency. Degradation kinetics under natural rice-field conditions, including exposure to sunlight, temperature changes, pH variation, organic matter, and water movement, should likewise be evaluated to determine persistence and possible environmental residues.

Ecotoxicological assessment is necessary to evaluate possible adverse effects on non-target organisms, including fish, aquatic invertebrates, amphibians, beneficial snails, soil organisms, and other fauna associated with rice agroecosystems. Field validation should then be conducted to determine efficacy, safety, optimal dosage, and practicality under actual rice-field and irrigation conditions. Only after these additional studies should *D. seguine* extract be considered for integration into broader Integrated Pest Management (IPM) programs. Community awareness and farmer-orientation activities may also be developed once field efficacy and safety have been adequately established.

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Author's Contributions

Alfredo V. Corpuz: Designed and planned the study, carried out the experiments, evaluated and interpreted the data, composed the report, and adhered to the reviewers' recommendations.
Carmela M. Florentino: Gathered the plant samples, took part in every experiment, examined the information, and helped revise the paper.
Ace Danielle C. Avero: Participated in every experiment, set up the facility for testing, and communicated with the publishing journal.
Sherwin V. Alvaro: Participated in every experiment, set up the lab for testing, and edited the manuscript.

Ethics

This article is completely distinctive. The corresponding author attests that there are no ethical inconsistencies and that all other authors have reviewed and approved the work.

Biosafety Clearance

The research protocol was submitted to the University of Northern Philippines' Institutional Research Ethics Committee prior to the study. The committee granted ethical clearance confirming compliance with institutional and national standards.

All experimental procedures involving snails were conducted following ethical laboratory handling practices. After the experiment, all test organisms were disposed of in accordance with biosafety and environmental safety protocols to prevent unintended release into the environment [13].

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