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MOLLUSCICIDAL ACTIVITY OF GROUND CHERRY (*PHYSALIS MINIMA LINN*.) AGAINST GOLDEN APPLE SNAIL (*POMACEA CANALICULATA LAM*)

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Abstract
The search for efficient, organic, and environment-friendly molluscicide that could minimize the spread of the invasive Golden Apple Snail (*Pomacea canaliculata*) in the Philippines is still on going. This study was conducted to identify the lethal concentrations of the crude extracts of *P. minima* as potential molluscicides. Snail mortalities were compared between each plant part and snail species and LC_{50} and LC_{100} values for the plant part tested were recorded. The most toxic part of *P. minima* extract against the Golden Apple Snail was the roots (7.43 ml l^{-1}) followed by the stem (9.65 ml l^{-1}) and the least toxic was the leaves (10.93 ml l^{-1}) after 24-hr median lethal concentration (LC_{50}). Based on the 24-hours lethal concentration (LC_{100}), the part with the greatest molluscicidal effect on Golden Apple Snail was the roots (17.47 ml l^{-1}) followed by the stem (21.02 ml l^{-1}) and the part with the least molluscicidal effect was the leaves (24.84 ml l^{-1}). The result obtained showed that the roots extract was more susceptible to the death of snail species *Pomacea canaliculata*. Comparing the LC_{50} and LC_{100} the roots extract showed the highest molluscicidal activity followed by the stem bark and leaves extracts. Therefore, this study indicated that ground cherry plant extracts possessed molluscicidal effect for controlling the golden apple snail.
Keywords: golden apple snail, ground cherry, molluscicidal activity, lethal concentration

INTRODUCTION

The quest for botanicals with molluscicidal activity became an essential research when the golden apple snail *(P. canaliculata)* became a pest which invaded the rice fields and caused a significant decreased in harvested rice in the Philippines. To overcome this serious threat, synthetic organotin (Brestan, Aquatin, Telustant) and niclosamide (Bayluscide) compounds were recommended. Considering the toxic hazards of these synthetic chemicals especially the organotins, the Department of Entomology, College of Agriculture, University of the Philippines at Los Baños undertook the search for plants toxic to golden apple snails since 1988 [4]. The highest proportion of molluscicidal species were in the family *Phytolaccaceae* (all five species tested), and *Polygonaceae* (seven of nine). Highly potent plants were also most commonly found in these families. An unusually large proportions (25%) of the 57 *Euphorbiaceae* tested at concentration of 100 ppm or lower killed 90- 100 of snails [7].

To date, there is still a continuous effort to search for the most potent organic biological agents that could minimize invasion and infestation of golden apple snails (local name “Golden kuhol”) in the rice granaries of the Philippines. Recent botanicals used as organic molluscicide include lemongrass, *Cymbopogon citratus* [5]; santol, *Sandoricum vidalii* ; fruit and barks of Tulipwood tree, *Harpulia arborea* and locust bean, *Parkia* sp. [18]; tobacco dust [2]; powderpuff tree, *Barringtonia racemose* [14]; and honey locust, *Gleditsia* sp. [3]. The main target of these organic, plant–based molluscicides is to reduce the population of the golden apple snails, but not harm non-target organisms and their environment. Farmers who aspire to use organic molluscicide for health and environment protection support this initiative with the claim that it is cheaper, locally available and will bring more good than harm. This farmer’s initiative supports the Philippines’ Organic Agriculture Act of 2010, which promotes the use of organic fertilizer and organic pesticides in organic farming [12].

Physalis minima Linn belongs to the family *Solanaceae* and commonly known as “Leletup” in Malaysia. This herb is found throughout India, Afghanistan, Africa, Indonesia, Malaysia and Australia [16]. The flowers are hermaphrodite (have both male and female organs) and pollinated by insects. The fruit is edible, yellowish and

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encapsulated in papery cover. The plant majorly contains phenolics, alkaloids, steroids and flavonoids [10]. The infusion of *P. minima* is said to relieve pain, lower fever, relieve indigestion, relieve cough with phlegm, be diuretic and relieve oral thrush [15].

The quest for locally available plants with a beneficial bioactive compounds extracts is of recent interest. Several plant extracts have been used to eradicate unwanted snails in aquaculture. Farmers will be able to control and eradicate unwanted species such as golden apple snails using plants, which they can grow in their backyards. They will not only be able to maximize their production cost but also decrease environmental risks. Moreover, the researchers will be provided with added information on the ways to control unwated species, which they can use for future reseaches. Furthermore, the result of this study will contribute to the existing literatures on botanicals which can be used as natural molluscicides and piscicides for aquaculture management.

MATERIALS AND METHOD

Plant Extract Preparation

Leaves, stem and roots of *P. minima* were collected along the municipality of Echague, Isabela. The leaves, stems and roots were cut pieces and air-dried at room temperature in a well ventilated room. The dried leaves, stem and roots were ground into a fine powder, placed in conical flask and soaked with 95% Ethanol [9]. The extraction was repeated three times and the combined extracts were then filtered through Whatmann No. 1 paper and evaporated in a vacuo using a rotary evaporator (Stuart RE 301).

Molluscicidal activities of the crude extracts of *P. minima*

Experimental snail. Snails were measured using a vernier caliper and the snails 25 mm in size were selected and used as test organisms. The test snails were acclimatized to the laboratory in basins half fille with water for 1 day. Furthermore, it is the time for the snails to lessen fecal accumulation before introducing the toxicant. The snails were not feed throughout the duration of the bioassay test.

Experimental set-up. A 2 L capacity plastic container were cut and used in the experiment, provided with net covers. Each container was filled with different volume of water to obtain 1 L. The water was pre-aereted for 24 hours to full oxygen saturation before the different volumes of the volumes of the plant extract was added. During collection, transfer, weighing and stocking, care was observed to minimize the occurence of damage to the shell of the snail. The test snails were stocked at ten pieces per container, and then addition of the pre- aereted water followed by the measured extract.

Bioassay. The static bioassay procedure used was a modification of the standard methods by American Public Health Association (APHA), American Water Works Association (AWWA), and Water Pollution Control Federation (WPCF) [20]. In a static bioassay, test medium is left undisturbed for the duration of the test after preparation and introduction to the test snails. Mollusk mortality was observed at 1, 2, 3, 6, 12, 24, 36, 48, 60, 72 and 96 hours after introducing the toxicant to the snails. Dead snails were removed immediately. A snail is considered affected by the plant toxicant when it manifests avoidance to the concentration, paralysis or having no response and settled at the bottom, floating at the surface with bubbles, and decreased capacity to respond when the operculum retract and sometimes floating with its flesh out.

Observation of snail mortalities. Mortalities of the test snail were observed and recorded at 1, 2, 3, 6, 12, 24, 36, 48, 60, 72 and 96 hours after stocking. The dead snails were removed immediately after observation. Snail mortalities at 12, 24, 36, 48, 60, 72 and 96 hours are cummulative.

RESULTS AND DISCUSSION

Molluscicidal activities of the crude extracts of *P. minima*

The leaves, stem and root extracts of *P. minima* were tested for its potential molluscicidal activity against the Golden Apple Snail (*Pomacea canaliculata*). The concentrations used were: 0, 1.25, 2.5, 5, 10 and 20 ml l-1 of the extract per liter of water. The percent mortality of *P. canaliculata* exposed to different concentrations of *P. minima* extracts at different time duration is shown in Figures 1-3, respectively.

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

Toxicity expressed as LC_{50}. Based on the 24-hr median lethal concentration, the most toxic part of the *P. minima* against the Golden Apple Snail was the roots (7.43 ml l^{-1}) followed by the stem (9.65 ml l^{-1}) and the least toxic was the leaves (10.93 ml l^{-1}).

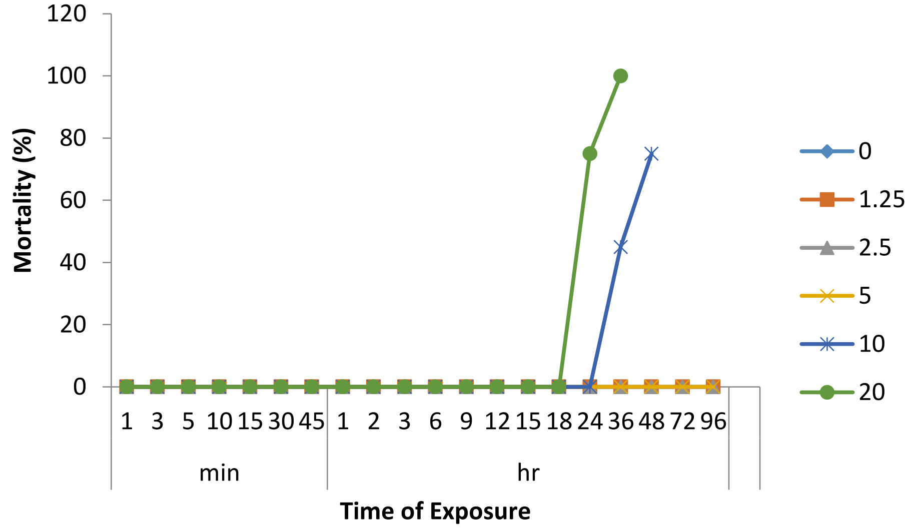

Figure 1. Percent mortality of *P. canaliculata* exposed to different concentrations of *P. minima* leaf extracts within 1 minute to 96 hours.

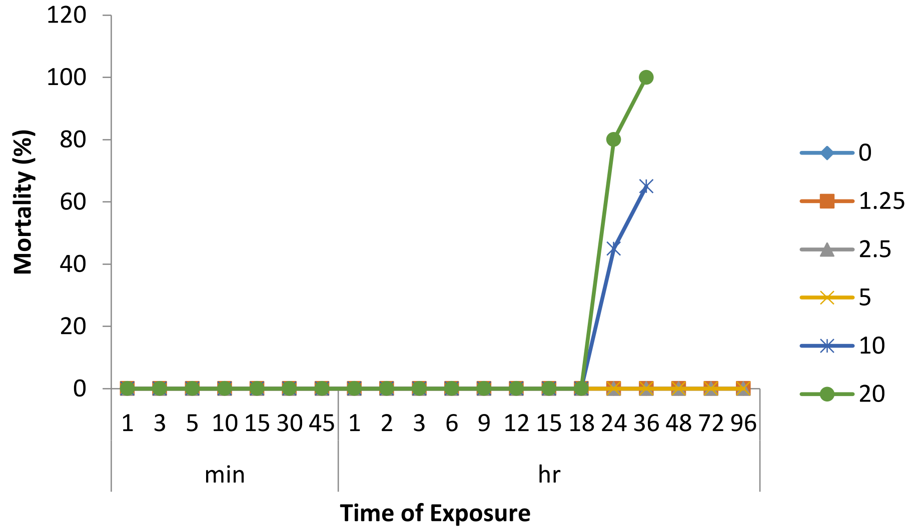

Figure 2. Percent mortality of *P. canaliculata* exposed to different concentrations of *P. minima* stem extracts within 1 minute to 96 hours.

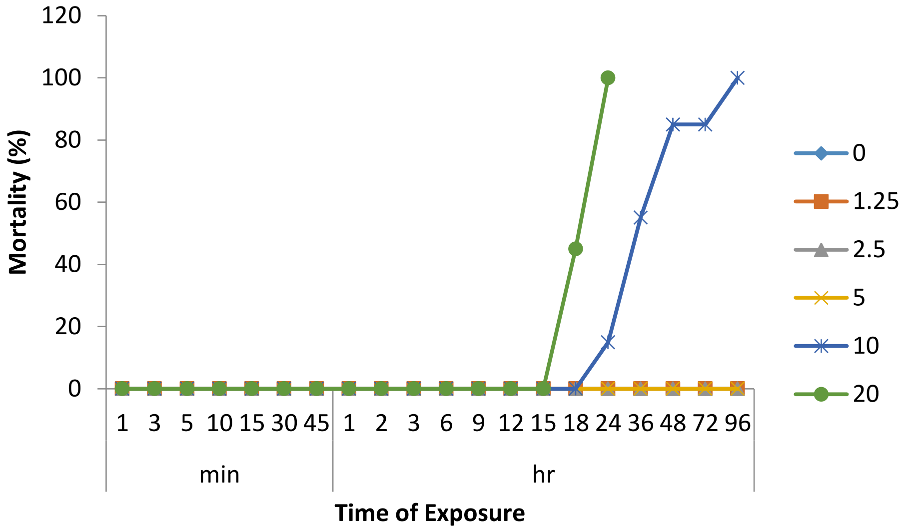

Figure 3. Percent mortality of *P. canaliculata* exposed to different concentrations of *P. minima* root extracts within 1 minute to 96 hours.

Molluscicidal activity expressed as LC_{100}. Based on the 24-hours lethal concentration, the part with the greatest molluscicidal effect on Golden Apple Snail was the roots (17.47 ml l^{-1}) followed by the stem (21.02 ml l^{-1}) and the part with the least molluscicidal effect was the leaves (24.84 ml l^{-1}).The mean lethal concentration (LC_{50} and LC_{100}) values of the crude extracts of *P. minima* with 24-48 hr after exposure is presented in Table 1.

Table 1. LC_{50} and LC_{100} of the different parts of the test plant at different durations

$LC_{50}LC_{100}$	24hrs	48hrs	24hrs	48hrs
Leaves	10.93 y = 4.9806x – 13	7.35 y = 5.8581x – 7	24.84 y = 4.9806x – 13	16.07 y = 5.8581x – 7
Stems	9.65 y = 4.3982x - 7.5714	7.36 y = 5.6074x - 8.7143	21.02 y = 4.3982x - 7.5714	16.28 y = 5.6074x - 8.7143
Roots	7.43 y = 3.5945x - 10.714	7.34 y = 5.7327x - 7.8571	17.47 y = 3.5945x - 10.714	15.87 y = 5.7327x - 7.8571

Treatments[1]	Mortality
1	13.1[d]
2	17.0[c]

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

3	12.7[d]
4	24.4[b]
5	31.0[a]
6	22.7[b]

Table 2. Molluscicidal activity of Ground cherry (*Physalis minima)* crude extracts on Golden Apple Snail (*Pomacea canaliculata)*.

Means without same superscript are significantly different (*p≤0.05*).

[1]Treatment 1 LC_{50}– stem part

2LC_{50}– leaf part

3 LC_{50}– root part

4 LC_{100} – leaf part

5 LC_{100}– root part

6 LC_{100}– stem part

Table 3. Molluscicidal activity of Ground cherry (*Physalis minima)* crude extracts on Golden Apple Snail (*Pomacea canaliculata)*.

	Concentration (C)		**Parts (P)**			**Interaction C x P**	**M.S.E.**[1]
	LC_{50}	LC_{100}	Leaves	Stem	Roots		
Survival	14.2[b]	26.0[a]	23.9[a]	18.8[b]	17.8[b]	0.16	4.0 x 10

Under the same main factor, means without same superscript are significantly different (*p≤0.05*).

[1] Means of standard error.

Table 2 shows the One-way Anova analysis of molluscicidal activity of Ground cherry (*Physalis minima)* extracts on Golden Apple Snail (*Pomacea canaliculata)*. Treatment 5 (LC_{50} – root part) was the most toxic and it was significantly different among the treatments. No significant difference was found on treatment 4 (LC_{100}-leaf part) to treatment 6 (LC_{100}–stem part) and on treatment 1 (LC_{50} – stem part) to treatment 3 (LC_{50}-root part). Moreover, LC_{50} and LC_{100} showed a significant difference. Disregarding concentration effect, leaves was most toxic than the stem and roots (Table 3).

The LC_{50} and LC_{100} values presented reveal that most of these extracts have good potential for future use in integrated control of snail infestation in rice fields. Difference in the slope functions of the extracts was noted in the analyzed mortality data and it indicates the extent to which increase in concentration of the extract should be made, to secure an increase in mortality. Sub lethal doses apparently irritate the snails as it was observed by the desire of the snails to crawl out of the test solution in order to avoid contact with the treated water. This is a protective behavior of *P. canaliculata* to avoid contact with treated water. One of the problems envisaged in the use of plant extracts, in the control of *P. canaliculata,* is the choice of solvent for extracting the plant materials.

The findings in these study conforms to the results of the previous studies on the use of natural plant extracts as potential molluscicides.

Abdullahi [1] investigated the molluscicidal activities of aqueous extract of leaves stem back and roots of *Balanite aegyptiaca* against adult *Lymnea natalensis*, using standard methods. The intermediate host of the helminth *Fasciola hepatica*. Snail mortalities were compared between each plant part and snail species and LC_{50} and LC_{90} values for the plant part tested were recorded .The result obtained showed that the leave extract was more susceptible to the death of snail species *Lymnea natalensis*. Comparing the LC_{50} and LC_{90} the leaves extract showed the highest

ISSN- 2394-5125 VOL 7, ISSUE 04, 2020

molluscicidal activity followed by the stem bark and root extracts. Desert date plant shows mollucicidal activities however it is found to be concentration- depended.

Picardal [17] also investigated the molluscicidal activity of garlic (*Allium sativum*) towards the rice pest (*Pomacea canaliculata*). This study employed Complete Randomized Design in 3 trials (n=630 snails) to test the efficacy of Aqueous Garlic Extract [AGE] under five experimental treatments (T_1=10ppm AGE; T_2=8.75ppm AGE; T_3=7.5ppm AGE; T_4=6.25ppm AGE and T_5=5.0ppm AGE) and two control groups (Positive Control=Niclosamide [Snail ShatterTM] and Negative Control= distilled H2O. After 48h experimental period, mortality data were analyzed using One Way ANOVA ($p<0.05$) and post hoc analysis (Tukey's test) while Probit analysis was employed to determine toxicity level of AGE at LD_{50} and LD_{90}. Results showed that there is a direct relationship between AGE and snail mortality, suggesting that all treatments exhibited molluscicidal properties. However, T_1 and T_2 were reported to have a comparable molluscicidal effect to that of the commercially-available molluscicide (NiclosamideTM). Toxicity level (LD_{50}) was found to be at 4.007ppm while LD_{90} is 7.602ppm. As evidenced by the laboratory experiment results, this study concludes that the best AGE concentration against the target mollusk *(Pomacea canaliculata*) is T_3=7.5ppm.

CONCLUSION

Based on the results of the study, it can be inferred that *P. minima* plant extracts primarily exhibited a repelling effect against the golden snail which could be attributed to the presence of bioactive constituents in the plant. These bioactive constituents can provide a basis for considering *P. minima* as a promising natural molluscicides because plants containing more bioactive constituents are among the most effective for the control of molluscs [6], [11], [18]. The root extracts of *P. minima* had a stronger molluscicidal activity than the other extract, and therefore it is the most suitable for biological application which offers a potentially simple, readily available, inexpensive and environmentally safe molluscicidal agent of plant origin.

REFERENCES

1. Abdullahi, Y; Muhammad, C ; Yerima, M. (2018). Molluscicidal Activity of Aqueous Extract of Leaves, Stem Back and Roots of Desert date (*Balanite Egyptiaca Del*.) against common Liver Fluke (*Fasciola Hepatica*) found in the Snail (*Lymnea natalensis*). Journal of Applied Science, Environment and Management Vol. 22 (3) 409 – 413. March 2018
2. Borja AO. (2012). Tobacco dust as organic molluscicide. The Philippine Star Jan. 22, 2012.
3. Crebassa X, Caruel H, Mercier F. (2012). Use of *Gleditsia* saponins for controlling mollusc pests. US Patent application number: 20120321688:
4. Cruz, J.J. (1989). Chemical Control of Golden Snail. In: B.O. Acosta and R.S. Pulins eds. Environmental Impact of the Golden Snail (*Pomaceae species*) on Rice Farming System in the Philippines. Summary Report Workshop held at the Freshwater Aquaculture Center, Central Luzon State University, Nueva Ecija, Phlippines, 9-10 Nov. 1989. Pp. 14-15
5. Demetillo MT, Baguio ML, Limitares DE, Madjos GG, Abrenica-Adamat LR. (2015). Effect of *Cymbopogon citratus* (lemon grass) crude leaf extracts on the developmental stages of *Pomacea canaliculata* (golden apple snail). Advances in Environmental Sciences – International Journal of the Bioflux Society 7(3), 460-467.
6. Dibyendu,S.,Damiki,L.,Smrti Rekha Chamda,D.,Suvakanta,D.(2019). Pharmacognostical and Phytochemical Standardization of *Physalis minima L*. Leaf. International Journal of Pharmacy and Pharmaceutical Sciences Print ISSN: 2656-0097 | Online ISSN: 0975-1491 Vol 11, Issue 11, 2019
7. Farnsworth, N. R., Henry, L. K., Svoboda, G. H., Blomster, R. N., Yales, M. J., Euler, K. L. (2000). Biological and Phytochemical Evaluation of Plants. Biological Test and Results from Two hundred Accession. Loydia 29: 01 – 122.
8. Guevarra, B. (2005). "A Guidebook to Plant Screening: Phytochemical and Biological". Research Center for the Natural Sciences, University of Santo Tomas. Copyright 2005.
9. H. N. Krishna Kumar and Jyoti Bala Chauh (2016) Phytochemical screening and anticancer activity of leaf extracts of *Physalis minima*. Journal of Advances in Natural Sciences. Vol. 3, pp. 283-287
10. Khare, CP. (2007). Indian Medicinal Plants An illustrated Dictionary. New York: Springer Science Business Media, LLC; 2007. p. 483
11. Kumar S, Yadav JP. (2016). Ethnobotanical and pharmacological properties of *Aloe vera*: a review. J Med Plants Res. 2014;48(8):1387–98.

12. Manila Bulletin [MB]. (2010). Republic Act No. 10068, otherwise known as "Organic Agriculture Act of 2010".
13. Marchee T. Picardal1,Kinberly T. Panlaan, Paula Marianne L. Castaño, Leandro G. Peña, Krisska T. Abella, Jay P. Picardal (2015) . Molluscicidal activity of the aqueous extract of garlic (*Allium sativum L*.) bulb against golden apple snail (*Pomacea canaliculata L*.) . International Journal of Biosciences | IJB | ISSN: 2220-6655 (Print), 2222-5234 (Online) http://www.innspub.net Vol. 13, No. 2, p. 75-87, 2018
14. Musman M, Kamaruzzaman S, Karina S, Rizqi R, Arisca F. (2013). A preliminary study on the anti-hatching of freshwater golden apple snail *Pomacea canaliculata* (Gastropoda: *Ampullariidae*) eggs from *Barringtonia racemosa* (Magnoliopsida: *Lecythidaceae*) seed extract. AACL Bioflux 6(4), 394-398.
15. Nathiya M, Dorcus D. (2012).Preliminary phytochemical and antibacterial studies on *Physalis minima Linn*. Inter J CurrSci, 2012;24-30.
16. Patel T, Shah K, Jiwan K, Shrivastava N. (2011). Study on the antibacterial potential of *Physalis minima Linn*. Indian J Pharm Sci, 2011;73:111-115
17. Picardal et. al. (2015). Molluscicidal activity of the aqueous extract of garlic (Allium sativum L.) bulb against golden apple snail (Pomacea canaliculata L.). International Journal of Bioscience (IJBS). ISSN: 2220-6655 (Print), 2222-5234 (Online) http://www.innspub.net Vol. 13, No. 2, p. 75-87, 2018
18. Singh,S.K. and Singh,A.(2010). The Ttoxicity of Leaf and Bark of *Thevetia Peruviana* plant to Fingerlings of *Labeo Rohita* (Hamilton) in Different Conditions. Malaysian Applied Biology · January 2010 39(1): 25–31.
19. Taguiling N. (2015). Effect of combined plant extracts on golden apple snail (*Pomacea canaliculata*) (Lam) and giant earth worm (*Pheretima sp*.) International Journal of Agriculture and Crop Sciences 8(1), 55-60
20. Greenberg, A.; Jenkins,D.; Connors,J.; American Public Health Association.; American Water Works Association. (1980). Standard methods for the examination of water and wastewater. Washington, D.C. : APHA-AWWA-WPCF, 1980. Available online at https://www.worldcat.org/title/standard-methods-for-the-examination-of-water-and-wastewater