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Histopathological effects of *Muntingia Calabura* leaf extract against *Pomacea Canaliculata* using Rice field Mimicking method

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Abstract

Pomacea canaliculata, also referred as the golden apple snail, is an invasive species significantly affecting agricultural crop production. Its rapid proliferation, secretion of contaminants like eggs and slime, and aggressive feeding habits have driven the search for safe, toxic-free alternatives to conventional pesticides. Among various plants, Muntingia calabura L., has gained attention for its potential as a natural pesticide. This study aimed to assess the molluscicidal activity of ethanolic leaf extract of M. calabura (ELEMC). Snail biossay was performed in simulated rice field, exposing the P. canaliculata to varying concentrations ranging from 200 to 1000 mg/L for 48 h. Mortality rates were recorded every 12h. Results showed that concentrations from 800 mg/L (T4) to 1000 mg/L (T5) were comparable to the commercial positive control. Probit analysis revealed that the LC50 was at 870.96 mg/L. A very high positive correlation (r=0.8358) was also observed between the concentration and mortality. Histopathological analysis of gills of ELEMC-treated snails revealed severe damage to the gills showing vacuolization, complete loss of cilia, degenerated columnar cells, reduced hemocyte quantity, splitting and degeneration of gill filaments. The molluscicidal activity of the extract may be accounted for from the presence of phytochemicals as revealed from literature. In conclusion, the ELEMC could be a potential source of molluscicide against P. analiculata.

Keywords: Histopathological, *M. calabura*, Phytochemical, *P. canaliculata*, Vacuolization.

Introduction

Invasive species lead to disruptions that impact the economy, environment, and agriculture. One such species is *Pomacea canaliculata* (golden apple snail), which causes severe damage to crop by reducing yields and increasing production costs. Native to South America, this pest has proliferated across nearly all regions of the Philippines. *P. canaliculata* infests approximately 1.6 million hectares of rice fields, leading to significant economic losses estimated between 425 and 1200 million USD annually[1]. The pest damages up to 85% of crops through egg deposition, slime contamination, and aggressive feeding[2], severely impacting farmers' earnings.

To manage this infestation, farmers have used synthetic molluscicides. However, concerns about their long-term effects on human health and the environment have prompted a search for plant-based alternatives[3]. Over 1,400 plant species with molluscicidal potential have been identified, particularly against *P. canaliculata* and related species[4]. *Muntingia calabura* L., an edible plant known for its antiseptic, anti-inflammatory, and antioxidant properties, has shown promising secondary metabolites such as flavonoids, tannins, and other compounds that are detrimental to pests. Research has identified toxic flavonoids in its leaves, including flavanones and flavones, which could serve as alternative molluscicidal agents[5]. Despite this, no studies have explored the molluscicidal potential of *M. calabura* L. This study, therefore, aims to conduct a preliminary evaluation of the molluscicidal effect of ELEMC extract against *P. canaliculata* in a rice-mimicking environment.

Materials and Methods

Plant Material: The collected leaves of *M. calabura* from the open field of Mabiga, Mabalacat City, Pampanga, Philippines. Three (3) kg of plant leaf samples were subjected to extraction and further concentrated at Department of Science and Technology (DOST), City of San Fernando, Pampanga, Philippines. A plant specimen was sent to Jose Vera Santos Memorial Herbarium, Institute of Biology, College of Science, University of the Philippines, Diliman, Quezon City, Philippines for plant authentication[6]. The specimen was labeled by the expert as *Muntingia calabura* L.

Rice Seed Germination and Rice Field Mimicking: Rice seeds acquired from Barangay Lawi, Capas, Tarlac, Philippines[7]. The healthy rice seedlings were transplanted into a small plastic basin full of pre-autoclaved garden lawn soil obtained from the Department of Agriculture, LGU office of Mabalacat City, Pampanga, Philippines at a depth of 2 inches. To mimic the rice field setting, 21 transparent containers with the following dimensions: 6 x 9 x 6.4 inch (W:L:H) were

utilized. Each container was designed to mimic a rice field, and each contained ten (10) pieces of rice seedlings, 2 kg of soil from the field, and 1 liter of per treatment extract. All samples were maintained and kept at Biology Laboratory, Mabalacat City College, Mabalacat City, Pampanga, Philippines.

Collection and Acclimatization of Snails: Adult snails of *P. canaliculata (n=300)* with shell lengths ranging from 35–60 mm was collected from the rice field in Brgy. Lawi, Capas, Tarlac, Philippines. The collected *P. canaliculata* were transported in a clean plastic container with distilled water. After collection, the snails were rinsed using aged water and allowed to acclimate at room temperature for three days before being used in the experiment. The 300 snails were divided into three large plastic containers, each accommodating 100 snails, and filled with 10 liters of aged water. Snails were provided with young taro leaves as food every 24 hours[8]. Snails were authenticated by Bureau of Fisheries and Aquatic Resources (BFAR) Regional Office No. III in Maimpis, City of San Fernando, Pampanga, Philippines. The experts then authenticated the specimen as *P. canaliculata.*

Molluscicidal Bioassay: Treatments with varying concentrations of 200 mg/L (T1), 400 mg/L (T2), 600 mg/L (T3), 800 mg/L (T4), and 1000 mg/L (T5), with three (3) replicates each, were prepared by mixing the respective amount of ELEMC with 1000 ml of aged water, yielding a concentration treatment solution of 1 liter. The treatment solution was administered into containers containing the mimicked rice field. Ten (10) snails with shell lengths ranging from 35 to 60 mm were exposed to the treatment. The containers were covered with plastic cling wrap to prevent the snails from crawling out with small holes poked, to allow airflow. For comparison, 1 L of aged water (the negative control – T-) was also provided, along with the niclosamide (the positive control – T+), with concentration of 625 mg/L. The snails were then exposed for 48 hours in different treatments. Snail mortality was recorded at 12, 24, 36, and 48 hours. To ensure the accurate determination of *P. canaliculata* mortality, forceps were used to move or poke the specimens. If a sample remained unresponsive, it was labeled as dead. The carcasses were removed from the containers to avoid contamination, after which the snails were extracted out of their shells, and gills dissected and trimmed, they were fixed in 10% formalin until utilized for histopathological preparation[9,10].

$$Snail\ mortality\ (\%) = \frac{number\ of\ deaths}{number\ of\ snails\ treated}\ x\ 100$$

Equation-1: Snail Mortality

Histopathological Screening and Analysis: To assess the histopathological aberrations after 48 hours of exposure to varying treatments, the organs of *P. canaliculata* were extracted from their shells, from which the gills were dissected and subsequently fixed in 10% formalin. The fixed gills were sent to Hi-Precision in Angeles City, Pampanga, Philippines, for histopathological preparation and stained with hematoxylin and eosin (H&E)[11]. The prepared samples were examined under a compound light microscope using scanner (40x), low power (100x), and high power (400x).

Statistical Analysis: Significant differences among the treatments were determined through parametric test of One-way Analysis of Variance (ANOVA). Tukey's post-hoc test is utilized to find significant differences between each treatment. A p-value of less than 0.05 (<0.05) was implied with significant difference. Additionally, median lethal concentration (LC50) was determined using probit analysis. The data was statistically analyzed using GraphPad Prism version 9.5.1 (available online at www.graphpad.com) and Microsoft Excel.

Results and Discussion

Mortality Rate of *P. canaliculata*: Table-1 presents the molluscicidal activity of the ELEMC against *P. canaliculata.* After 12 and 24 hours of exposure, the highest mortality rates were evidently observed in the positive control (Niclosamide), with a 70% mortality rate at 12 hours and an additional 30% mortality rate at 24 hours. At 36 hours, only the 1000 mg/L concentration showed a 20% mortality rate. After 48 hours, the concentrations of 600 mg/L, 800 mg/L, and 1000 mg/L exhibited mortality rates of 23%, 40%, and 57%, respectively. In contrast, a negligible mortality rate was recorded at the negative control, concentrations of 200 mg/L and 400 mg/L during the entire experimental period, which was due to the adaptability of *P. canaliculata* to artificially contained rice paddy water made of aged water and its resistance to lower concentrations of the extract. Moreover, after the entire experimental period (48 hours), the highest recorded mortality rate of *P. canaliculata* was registered at the positive control with a total average mortality of 100% and at concentrations of 800 mg/L and 1000 mg/L with 40% and 77% mortality rates, respectively. These results may further indicate that the molluscicidal activity of the ELEMC is highly time- and dose-dependent. These results supported by literature that *P. canaliculata* exposed to *Ipomoea batatas* leaf extract (aqueous, methanol, chloroform, and hexane) and *Allium sativum* (aqueous extract) showed increased mortality of *P. canaliculata* at higher concentrations with longer time exposure[3,14]. Similarly, the results of this study confirmed that mortality of *P. canaliculata* was observed and recorded at higher concentrations (600mg/L, 800mg/L, and 1000mg/L) with extended exposure times. This indicates the concentration and time exposure have significant and direct contribution to snail mortality.

Plants produce several naturally active compounds that have been found to induce a highly toxic and adverse effect on an organism's cellular and organ levels[12-14]. Bioactive compounds such as saponins, tannins, flavonoids, and terpenoids were all present in the phytochemical screening of the ethanolic leaf extract of *M. calabura*[12-19]. Such compounds have been reported

and are believed to possess a direct and significant role in the toxicity and molluscicidal effect against *P. canaliculat*[20]. Therefore, it is presumed that *M. calabura*'s phytochemical components directly contribute to its molluscicidal action in the current study against *P. canaliculata*. This is consistent with researches[14,21], which found a direct correlation between the mortality of *P. canaliculata* and the presence of bioactive compounds such as saponins, tannins, terpenoids, and flavonoids in plant extracts. Similarly, prior studies[23-25] have linked these chemical classes to molluscicidal activity against other snail species. Interestingly, a recent study revealed the existence of these secondary metabolites in the ethanolic, methanolic, and hexane leaf extract of *M. calabura*[25]. Theoretically, tannins and terpenoids inhibit food ingestion and growth rates in experimental animals[26,27], which has led to their use as plant-based pesticides[28]. Meanwhile, saponins disrupt cell membrane integrity, causing cell lysis[14,29]. Additionally, flavonoids are thought to inhibit the detoxification process in snails and increase saponin absorption, thereby enhancing toxicity and leading to rapid mortality[30,31].

Table-1: Molluscicidal Activity of ELEMC against *P. canaliculata.*

Treatments	% Mortality				Total % Mortality (48hrs)
	12h	24h	36h	48h	
T-	0	0	0	0	0
T+	70	30	0	0	100
T1	0	0	0	0	0
T2	0	0	0	0	0
T3	0	0	0	23	23
T4	0	0	0	40	40
T5	0	0	20	57	77

ANOVA revealed significant differences across treatment groups after 48 hours of exposure with p-value of <0.0001. *Post-hoc* Tukey's test identified the significant differences between treatments. T5 showed significant difference to T-. Although the treatments of T3 and T4 do not show significant difference to T-, high mortality rates were still observed in T3 and T4 in comparison to T-. Meanwhile, no significant differences were observed between the treatments of T4 and T5 to T+, indicating comparable results between the high concentrations of ELEMC and commercial product. Probit analysis of LC50 revealed that at 870.96 mg/L of ELEMC induces 50% mortality in *P. canaliculata* when subjected for 48 h. Correlation coefficient of r=0.8358 indicates a strong and positive relationship between the concentration and mortality. This confirms that it has a significant dose-dependent effect. Moreover, mortality in extracts were also observed at the later stage of observation indicating also that the extract is time dependent. The molluscicidal activity of ELEMC can be attributed to the presence of phytochemicals. Qualitative and quantitative analysis were conducted to detect and measure the phytochemical constituents of *M. calabura*. It was revealed that its leaves have presence of alkaloids, flavonoids, phenols, tannins, and saponins. Specifically, alkaloids are present in higher concentration in comparison with other phytochemicals[32]. In a review paper, alkaloid was highlighted with high efficacy to snail mortality. Physiological and biochemical effects of alkaloids can range from neurotoxicity to destabilization of cell membrane[33]. In one study, arecoline, an alkaloid, showed significant mortality rate to *P. canaliculata* by lowering oxygen intake, ammonia excretion, and inhibiting acetylcholinesterase[34].

Histopathological Examination: The histopathological observation of the gills of *P. canaliculata* revealed intact gill filaments in T- (Figure-1A). The gill of *P. canaliculata* consisted of numerous gill filaments, with a gill epithelium containing ciliated columnar cells. In Figure-1A, there were numerous cilia present outside the gill filaments, showing a double-layered arrangement of ciliated columnar cells positioned parallel to each other and partitioned by a narrow gap. Additionally, many hemocytes were observed in the hemolymph space, and the columnar cell structure appeared complete. In *P. canaliculata* treated with the positive control (T+), both snails showed complete cilia loss at 12 hours (Figure 1B) and 24 hours (Figure-1C). The snail exposed for 12 hours exhibited severe degeneration of columnar cells and minimal tissue disintegration (Figure-1B). In contrast, the snail exposed for 24 hours showed complete tissue disintegration, resulting in degenerated tissue (Figure-1C). Studies have shown that synthetic molluscicides, such as niclosamide, disrupt various cellular processes in snail tissues, compromising cell membrane integrity and ion channels, which are crucial for cell function. These disruptions lead to cellular breakdown, loss of tissue integrity, and eventual tissue disintegration[34]. Interestingly, tissue samples from live snails treated with 200 mg/L and 400 mg/L of ELEMC after 48 hours showed minimal aberrations. Histological changes in *P. canaliculata* treated with 200 mg/L included a reduction in cilia length and quantity, vacuolization, splitting of some columnar cells, and disorganized folding of the filaments (Figure-1D). Furthermore, *P. canaliculata* treated with 400mg/L of ELEMC exhibited histological alterations such as shedding of cilia and mild vacuolation or split columnar cells (Figure-1E).

On one hand, *P. canaliculata* treated with 600mg/L for 48 hours, exhibited a complete loss of cilia, vacuolated tissue and split columnar cells, and empty filaments with a reduced quantity of hemocytes were observed (Figure-1F). On the other hand, tissue from *P. canaliculata* treated with 800mg/L ELEMC for 48 hours exhibited a complete loss of cilia, severe

degeneration of columnar cells, and emptier filaments with a reduced quantity of hemocytes (Figure-1G). Lastly, *P. canaliculata* treated with 1000mg/L of ELEMC for 36-hour, manifested complete loss of cilia, degeneration of columnar cells (Figure-1H). In contrast, *P. canaliculata* treated with 1000 for 48-hour presented complete loss of cilia, Undulating folds of the gill filaments, vacuolization, splitting, and severe degeneration of columnar cells, and degeneration of some regions of the tissue (Figure-1I). These results of *P. canaliculata* treated with different concentrations of ELEMC resulted in varying degrees of aberration in the gills of snails. Consequently, this loss of cilia in the gills can disrupt the snail's essential physiological activities, including respiration and excretion. Research findings[35] have revealed a link between cilia shedding, and the inhibitory activity of the toxin could promote histological degeneration in *P. canaliculata*. Moreover, the results explain the direct relationship of concentration ELEMC led to more pronounced aberrations in the columnar cells. In lower concentrations of the plant extract, observed alterations included vacuolization and splitting of the columnar cells. Similar effects have been observed in studies involving the exposure of *P. canaliculata* to heavy metals[36] and copper[37]. Therefore, based on these previous studies and the results, it can be theoretically inferred that the observed vacuolization of columnar cells in response to ELEMC may be indicative of a cellular detoxification response to the plant extract.

Conclusion

In conclusion, the ELEMC has comparable molluscicidal activity with positive control at high concentrations after 48 h of exposure. Mortality rates were observed at 36 h to 48 h of the highest concentration (T5). It has LC50 of 870.96mg/L. Histopathological examinations of gills that were subjected to concentrations of 800mg/L to 1000mg/L ELEMC have shown progressive damage to the gills, as indicated by aberrations of vacuolization, cilia loss, and cell and tissue degeneration. Literature suggested that the molluscicidal activity was derived from its presence of bioactive phytochemicals. These findings suggest that ELEMC has promising molluscicidal activity at high concentrations and prolonged exposure.

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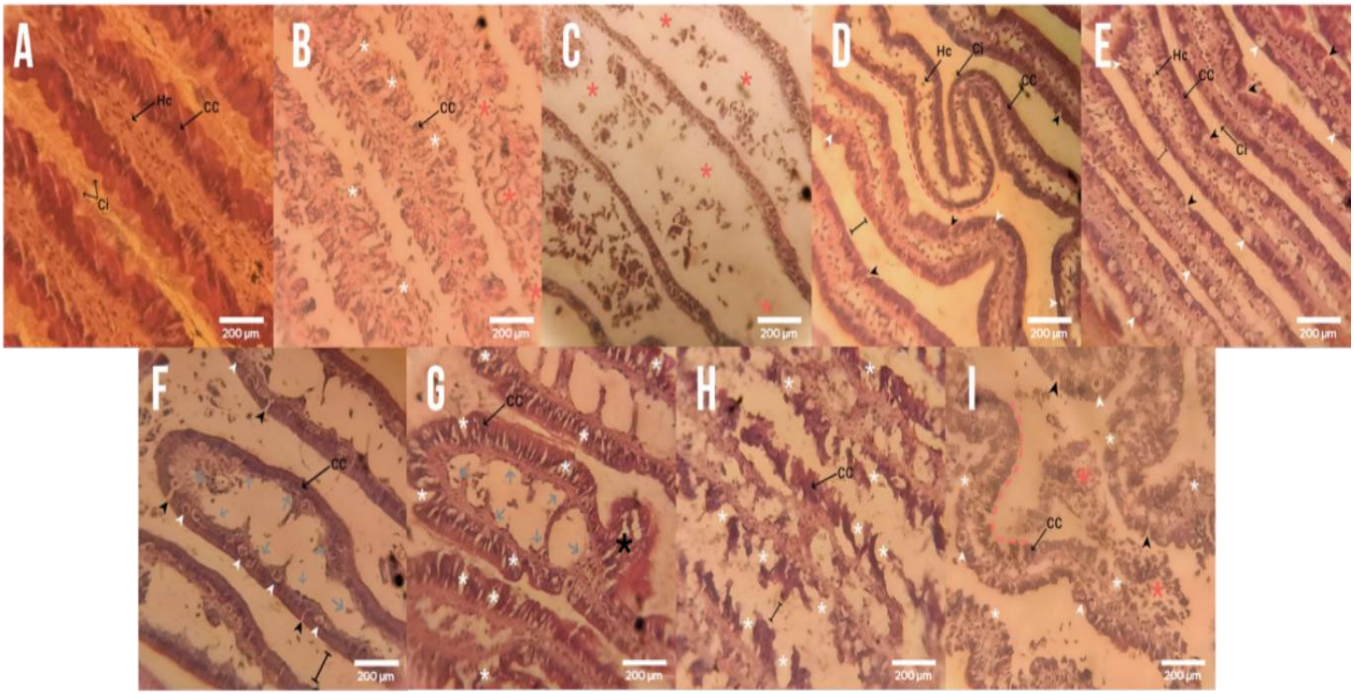

Figure-1: Histology of the gills of *P. canaliculata* subjected to different treatments (A-I) observed after 12-48 hours. (A) negative control (T-); (B) Positive control, 12hrs(T+); (C) Positive control, 24hrs (T+); (D) 200 mg/L ELEMC (T1); (E) 400 mg/L ELEMC (T2); (F) 600 mg/L ELEMC (T3); (G) 800 mg/L ELEMC (T4); (H) 1000 mg/L ELEMC, 36hrs (T5); (I) 1000 mg/L ELEMC, 48hrs (T5).*Ci=cilia; CC=columnar cells; Hc=hemocytes.* White asterisk=severe degeneration of CC; Red asterisk=disintegration of tissue; Gray arrow=dilated hemolymph space; Black arrow head=split; Red dashed line=wavy-like folding of gill filament; Measure line icon=larger gap in the interlamellar space. H&E. HPO 800x. Note: The black spots visible in the figure are artefacts.

References

1. de Brito, F. C., & Joshi, R. C. (2016). The golden apple snail Pomacea canaliculata: a review on invasion, dispersion and control. *Outlooks on Pest Management*, 27(4), 157-163.

2. Kumar, P. (2020). A review—on molluscs as an agricultural pest and their control. *International Journal of Food science and agriculture*, 4(4), 383-389.

3. Noorshilawati, A. A., Nur Suraya, A., & Siti Rossiyah, S. (2020). Molluscicidal activity of Ipomoea batatas leaf extracts against Pomaceacanaliculata (Golden apple snail). *Food Research*, 4(5), 131-137. http://dx.doi.org/10.26656/fr.2017.4(S5).003

4. Prabhakaran, G., Bhore, S. J., & Ravichandran, M. (2017). Development and evaluation of poly herbal molluscicidal extracts for control of apple snail (Pomacea maculata). *Agriculture*, 7(3), 22. https://doi.org/10.3390/agriculture7030022

5. Su, B. N., Park, E. J., Vigo, J. S., Graham, J. G., Cabieses, F., Fong, H. H., ... & Kinghorn, A. D. (2003). Activity-guided isolation of the chemical constituents of Muntingiacalabura using a quinone reductase induction assay. *Phytochemistry*, 63(3), 335-341. https://doi.org/10.1016/S0031-9422(03)00112-2

6. Yamauchi, F. A. B., Nolasco, G. S., David, L. F. S., Dizon, S. J. D., Tejano, A. C. V., Arcilla, M. S., et al. (2023). Anti-necrotic potential of ethanolic leaf extract of Ziziphus talanai against monosodium glutamate-induced cytoarchitectural alterations in the brain of albino mice. *Sciencetific Journal of Tan Trao University*, 9(1). https://doi.org/10.51453/2354-1431/2022/862

7. Sarsu, F., Ghanim, A., Das, P., Bahuguna, R. N., Kusolwa, P. M., Ashraf, M., ... & Ingelbrecht, I. (2018). *Pre-field screening protocols for heat-tolerant mutants in rice* (p. 39). Springer Nature.

8. Joshi, R. C., San Martín, R., Saez-Navarrete, C., Alarcon, J., Sainz, J., Antolin, M. M., ... & Sebastian, L. S. (2008). Efficacy of quinoa (Chenopodium quinoa) saponins against golden apple snail (Pomaceacanaliculata) in the Philippines under laboratory conditions. *Crop Protection*, 27(3-5), 553-557. https://doi.org/10.1016/j.cropro.2007.08.010

9. Shen, X., Wang, Z., Liu, L., & Zou, Z. (2018). Molluscicidal activity of Solidago canadensis L. extracts on the snail Pomaceacanaliculata Lam. *Pesticide biochemistry and physiology*, 149, 104-112. https://doi.org/10.1016/j.pestbp.2018.06.009

10. El-Sherbini, G. T., Zayed, R. A., & El-Sherbini, E. T. (2009). Molluscicidal activity of some Solanum species extracts against the snail Biomphalaria alexandrina. *Journal of Parasitology Research*, (1), 474360.

11. Nolasco, G., Escoto, G. A., David, L. F., & Yamauchi, F. A. (2023). Amelioration of Behavioral and Cognitive Impairment of Ethanolic Leaf Extract of Ziziphus Talanai Against MSG in Mice. *Journal of Healthcare and Biomedical Science*, 2(1), 24-34. https://doi.org/10.31098/jhbs.v2i1.1835

12. Twaij, B. M., & Hasan, M. N. (2022). Bioactive secondary metabolites from plant sources: types, synthesis, and their therapeutic uses. *International Journal of Plant Biology*, 13(1), 4-14. https://doi.org/10.3390/ijpb13010003

13. Madariaga-Mazon, A., Hernández-Alvarado, R. B., Noriega-Colima, K. O., Osnaya-Hernández, A., & Martinez-Mayorga, K. (2019). Toxicity of secondary metabolites. *Physical Sciences Reviews*, 4(12), 20180116. https://doi.org/10.1515/psr-2018-0116

14. Picardal, M. T., Panlaan, K. T., Castaño, P. M. L., Peña, L. G., Abella, K. T., & Picardal, J. P. (2018). Molluscicidal activity of the aqueous extract of garlic (Allium sativum L.) bulb against golden apple snail (Pomaceacanaliculata L.). *International Journal of Bioscience*, 13(2), 75-87. http://dx.doi.org/10.12692/ijb/13.2.75-87

15. Aarthi, N., Abinaya, S., Nithyasri, G. R. N., Thanga Brindha, T., Sneka, P., & Vijina, C. V. (2021) Phytochemical screening and Antivectoral activity of Muntingiacalabura. *Journal of Advanced Applied Scientific Research.* 3(5), 43–48. https://doi.org/10.46947/joaasr352021122

16. Gurning, K., & Sinaga, H. (2020). In vitro anti-diabetic potential extract test of seri (Muntingiacalabura, L.) leaves. *Asian Journal of Pharmaceutical Research and Development*, *8*(6), 39-41. https://doi.org/10.22270/ajprd.v8i6.874

17. Sari, S. A., Ernita, M., Mara, M. N., & AR, M. R. (2020). Identification of active compounds on Muntingiacalabura L. leaves using different polarity solvents. *Indonesian Journal of Chemical Science and Technology*, 3(1), 1-7. https://garuda.kemdikbud.go.id/documents/detail/1982061

18. Buhian, W. P. C., Rubio, R. O., Valle Jr, D. L., & Martin-Puzon, J. J. (2016). Bioactive metabolite profiles and antimicrobial activity of ethanolic extracts from Muntingiacalabura L. leaves and stems. *Asian Pacific journal of tropical biomedicine*, 6(8), 682-685. https://doi.org/10.1016/j.apjtb.2016.06.006

19. Surjowardojo, P., Sarwiyono, I. T., & Ridhowi, A. (2014). Quantitative and qualitative phytochemicals analysis of Muntingiacalabura. *Extraction*, 4(16).

20. Edis, J. K., Basay, F., Castillo, V., Alegado, D., Alicante, A., Alon, J., ... & Picardal, J. (2018). In vitro evaluation of the molluscicidal activity of Euphorbia tirucalli latex

extract against the mollusk rice pest Pomaceacanaliculata (Caenogastropoda: Ampullariidae). *Journal of Biodiversity and Environmental Sciences*, 13(2), 237-245.

21. Misnan, R., Aziz, N. S. A., Yadzir, Z. H. M., Abdullah, N., Bakhtiar, F., & Murad, S. (2016). Comparison of allergenic proteins of sea snail (Cerithideaobtusa) and freshwater snail (Pomaceacanaliculata). *Jurnal Teknologi*, 78(11). https://doi.org/10.11113/.v78.7940

22. Barreto-Linhares, L. P. M., Pereira, B. V. N., Dantas, M. K. G., Bezerra, W. M. D. S., Viana-Marques, D. D. A., de Lima, L. R. A., & Sette-de-Souza, P. H. (2022). Schinopsisbrasiliensis Engler—Phytochemical Properties, Biological Activities, and Ethnomedicinal Use: A Scoping Review. *Pharmaceuticals*, 15(8), 1028. https://doi.org/10.3390/ph15081028

23. Pereira, L. P. L. A., Ribeiro, E. C. G., Brito, M. C. A., Silveira, D. P. B., Araruna, F. O. S., Araruna, F. B., ... & Coutinho, D. F. (2020). Essential oils as molluscicidal agents against schistosomiasis transmitting snails-a review. *Acta tropica*, 209, 105489. https://doi.org/10.1016/j.actatropica.2020.105489

24. Cantanhede, S. P. D., Marques, A. D. M., Silva-Souza, N., & Valverde, A. L. (2010). Plant molluscicidal activity: A prophylactic alternative. *Revista Brasileira de Farmacognosia*, 20, 282-288. https://doi.org/10.1590/S0102-695X2010000200024

25. Chaudhari, R. N., Jain, A. K., & Chatap, V. K. (2020). Phytochemical Screening, Antioxidant and Antimicrobial Potential of Leaves Extract of Muntingia Calabura. *Journal of Advanced Scientific Research*, 11(04), 218-224.

26. Chung, K. T., Wong, T. Y., Wei, C. I., Huang, Y. W., & Lin, Y. (1998). Tannins and human health: a review. *Critical reviews in food science and nutrition*, 38(6), 421-464. https://doi.org/10.1080/10408699891274273

27. de Castilho, P. F., da Silva Dantas, F. G., de Araújo, R. P., Castro, L. H. A., de Araújo, F. H. S., Negri, M., ... & de Oliveira, K. M. P. (2021). General and genetic toxicology studies of Aleurites moluccana (L.) Willd. seeds in vitro and in vivo assays. *Journal of Ethnopharmacology*, 280, 114478. https://doi.org/10.1016/j.jep.2021.114478

28. Gillette, J. R., Heinzelman, R. V., Szmuszkovicz, J., Leemann, H. G., Stich, K., Thomas, M., ... & Khatoon, T. (1963). Biological activity of the terpenoids and their derivatives. *Progress in Drug Research/Fortschritte der Arzneimittelforschung /Progrès des recherches pharmaceutiques*, 279-346.

29. Wu, C., Wu, H. T., Wang, Q., Wang, G. H., Yi, X., Chen, Y. P., & Zhou, G. X. (2019). Anticandidal potential of stem bark extract from Schima superba and the identification of its major anticandidal compound. *Molecules*, 24(8), 1587. https://doi.org/10.3390/molecules24081587

30. Rosli, R., Latip, S. N. H. M., Othman, A. S. A. N., & Nawi, F. W. M. (2021). Potential control of Pomaceacanaliculata using botanical extracts in paddy field. *Nternational Transaction Journal of Engineering, Management, & Applied Sciences & Technologies*, *12*(9), 1-11. https://doi.org/10.14456/ITJEMAST.2021.181

31. Ballada, K. A., &Baoanan, Z. G. (2023). Molluscicidal properties of wild sunflower (Tithonia diversifolia) leaf extract fractions against invasive golden apple snail (Pomaceacanaliculata). *Environment, Development and Sustainability*, 1-18. https://doi.org/10.1007/s10668-023-03969-5

32. Suvarchala, V., Pavani, C., Bai, D. S., &Shasthree, T. (2022). Qualitative and quantitative determination of phytochemical contents of Muntingiacalabura. *Research Journal of Chemistry and Environment*, (26), 5.

33. Wszelaki, S., Podobiński, P., & Środoń, K. (2023). Molluscicidal activity of plant alkaloids. *Journal of Applied Toxicology*, 43(12), 1778-1792. https://doi.org/10.1002/jat.4466

34. Kengne Fokam, A. C., Sumo, L., Bagayan, M., Nana-Djeunga, H. C., Kuete, T., Nganjou, G. S. O., ... & Njiokou, F. (2022). Exposition of intermediate hosts of schistosomes to Niclosamide (Bayluscide WP 70) revealed significant variations in mortality rates: implications for vector control. *International Journal of Environmental Research and Public Health*, 19(19), 12873. https://doi.org/10.3390/ijerph191912873

35. Yang, C., Wang, Y., Ma, Y., Liu, J., Zhou, Y., Yan, X., ... & Chen, H. (2022). Research on the molluscicidal activity and molecular mechanisms of arecoline against Pomaceacanaliculata. *Ecotoxicology and Environmental Safety*, *246*, 114198. https://doi.org/10.1016/j.ecoenv.2022.114198

36. Dummee, V., Kruatrachue, M., Trinachartvanit, W., Tanhan, P., Pokethitiyook, P., & Damrongphol, P. (2012). Bioaccumulation of heavy metals in water, sediments, aquatic plant and histopathological effects on the golden apple snail in Beung Boraphet reservoir, Thailand. *Ecotoxicology and Environmental Safety*, 86, 204-212. https://doi.org/10.1016/j.ecoenv.2012.09.018

37. Peña, S. C., Pocsidio, G. N., & Co, E. L. (2017). Histological responses of golden apple snail (Pomaceacanaliculata) to copper. *Philippine Journal of Science*, *146*(3), 315-321.