Environment, Development and Sustainability
https://doi.org/10.1007/s10668-023-03969-5

Molluscicidal properties of wild sunflower (*Tithonia diversifolia*) leaf extract fractions against invasive golden apple snail (*Pomacea canaliculata*)

Karen A. Ballada[1] · **Zenaida G. Baoanan**[1]

Received: 22 July 2018 / Accepted: 25 September 2023

Abstract
Pomacea canaliculata is a serious invasive pest in the rice farms of the Philippines. Using botanical molluscicide is much favored for human health and environmental reasons. Crude leaf extracts of *Tithonia diversifolia*, also an invasive plant, were observed to have molluscicidal properties. This study aimed to fractionate the different bioactive compounds in the *T. diversifolia* crude leaf extract using solvent extraction and test these fractions for molluscicidal properties. The results showed that the alkaloid and saponin fractions exhibited an LC50 of 6000 ppm and 3000 ppm, respectively, at 24 h. Histological analyses of tissues from the test organisms showed deteriorated epidermal and subepidermal layers of the foot, highly vacuolated and deciliated epithelium of the gill filaments and osphradial leaflets, and deteriorated lining of the primary ducts of the digestive glands. These results are attributed to the presence of alkaloids and saponins in the different extracts, which are reported from literature to have detergent properties that can disrupt cell membranes and inhibit acetylcholinesterase activities in animals.

Keywords *Tithonia diversifolia* · *Pomacea canaliculata* · Molluscicide · Invasive species

1 Introduction

Golden apple snail (*Pomacea canaliculata*), popularly known in the Philippines as "*golden kuhol*" is considered as a notorious invasive species in the rice farms of the Philippines. Originally from South America, it was brought into the Philippines via Taiwan to serve as an alternative source of food protein and additional cash source to supplement the low income of Filipino farmers (Acosta and Pullin, 1991; Madamba & Camaya, 1987). However, these snails became pests through time and have caused more damage than benefits to agriculture (Joshi, 2006; Kannaujiya et al., 2023; Sin, 2003). The introduction of this species reduced rice production by as much as 5–100% depending on the locality (Naylor, 1996). The golden apple snail has also displaced the edible native species, *Pila conica*,

✉ Karen A. Ballada
kaballada@up.edu.ph

[1] Department of Biology, College of Science, University of the Philippines, 2600 Baguio City, Philippines

Published online: 10 October 2023

in the Philippines' northern island of Luzon (Pagulayan, 1997) that may lead to local extinction.

The negative impacts brought about by the snail made local farmers resort to using pesticides and other chemicals to try to control the pest population. This practice poses a threat to the environment as it adversely affects other native organisms in the area. Furthermore, it exposes the farmers to hazards that affect their health and well-being. It was reported that environmental exposure of humans to agrichemicals results in both acute and chronic health effects, including acute and chronic neurotoxicity, lung damage, chemical burns, cancers, immunologic abnormalities, and adverse reproductive and developmental effects (Devi et al., 2022; Weisenburger, 1993). Using plant-derived, naturally occurring compounds with molluscicidal activities is therefore a better alternative. In the Philippines, dried and milled leaves of the starflower, *Calotropis gigantea*, was found to have an antifeedant and lethal effect on *P. canaliculata* when administered in as high as 200 kg/ha (Lobo & Llagas 1991), while the powdered leaves of *balakat* tree (*Ziziphus talanai*) was found to be a feasible and farmer-accepted alternative to chemical molluscicide to control *P. canaliculata* in direct-seeded rice farms at 7 kg/ha (Bagunu et al., 2020).

Tithonia diversifolia, commonly known as wild sunflower, is an invasive weed that is widely distributed in the tropics, including the Philippines. It is an exotic plant commonly found in wastelands, banks of polluted city rivers, and roadsides in the Cordillera Region. This plant is commonly used as soil fertilizer and conditioner since it enhances the availability of phosphorus for crops. *T. diversifolia* is widely studied for its various properties such as nematicidal (Aswini et al., 2022), insecticidal (Adedire & Akinneye, 2004; Mukasa et al., 2008), acaricidal (Radhakrishnan & Prabhakaran, 2014), antimicrobial (Gutierrez et al. 2015; Susilowati et al., 2022), antioxidant (Di Giacomo et al., 2015; Kerebba et al., 2022), hypoglycemic (Zhao et al., 2012), anti-hepatoma (Lin et al., 2022), and anti-inflammatory and analgesic properties (Chagas-Paula et al., 2011; Owoyele et al., 2004). The bioactive properties of botanical biocide are usually attributed to the presence of various secondary plant metabolites which include sesquiterpenes, flavonoids, alkaloids, steroids, saponins, tannin, and polyphenols. However, there is no reported study yet on the specific identity and nature of the bioactive compound responsible for the molluscicidal activity of *T. diversifolia* against *P. canaliculata*. Hence, this study primarily aims to identify the different bioactive compounds present in the *T. diversifolia* leaves through fractionation and to test the molluscicidal properties by determining the mortality rate and histological effects on *P. canaliculata.*

Separation and identification of the best concentration of the bioactive compounds present in *T. diversifolia* that is capable of killing the invasive snail provides valuable information to help in managing the pest using natural methods. Using naturally occurring materials as the source of molluscicide is much safer than chemical ones. Furthermore, *T. diversifolia* is an invasive weed that is widely distributed in the Cordillera region. Thus, using this plant material in controlling the population of golden apple snails is doubly advantageous for the local community.

2 Methodology

2.1 Collection of the test organism

Adult male and female *P. canaliculata* were collected from the rice paddies of Suyo, Ilocos Sur in October 2016. Due to the considerably wide difference in the weights of the

snails collected, the more abundantly occurring snails weighing 2–7 g were selected. Snail identification was verified with the use of taxonomic keys (Burch, 1984; Estebenet et al., 2006). Males and females were separately contained in plastic containers with 15 cm × 10 cm × 5 cm dimensions. Each container contained ten snails of approximately the same weight and was allowed to acclimate for 24 h while submerged in 100ml distilled water. The setup was kept in a makeshift structure near the rice field.

2.1.1 Preparation of *Tithonia diversifolia* plant extract

Gutierrez et al. (2015) reported that as an adaptive mechanism, *T. diversifolia* tends to produce more kinds of bioactive compounds called secondary plant metabolites including alkaloids, saponins, polyphenols, and tannins when they are exposed to high vehicular traffic. Thus, robustly growing wild sunflower leaves (*T. diversifolia*) were collected from places that are highly exposed to vehicular emissions in Baguio City.

The collected leaves were washed with tap water, air-dried for 24 h, and oven-dried for 2–3 days at 40 °C. Dried leaves were powdered using a blender. The alkaloid and polyphenol fractions were extracted following the general extraction and fractionation procedure for the different classes of plant bioactive compounds according to polarity as described by Harborne (1984) with slight modification. Fifty grams (50 g) of powdered leaves were soaked for 24 h in 500 ml 95% methanol at room temperature and were filtered using Whatman filter paper no 1. The filtrate was concentrated to a thick, syrupy consistency in a pressurized rotary evaporator. The weight of the crude leaf extract was determined using a digital table top balance. Methanol measuring approximately 20% of the original volume of the filtrate was again added to the crude extract. The filtrate was acidified using 2 M sulfuric acid. Distilled water (twice the volume of the crude extract) and chloroform (thrice the volume of the crude extract) were added to the crude extract to separate the alkaloids from the polyphenols. The chloroform layer was separated from the aqueous acid layer and both were concentrated to dryness using pressurized rotary evaporator.

The saponins were extracted following the general procedures described by Claustro and Madulid (2005). Twenty grams (20 g) of powdered *T. diversifolia* leaves were soaked in 100 ml of petroleum ether in hot water bath for 30 min to de-fat the sample. The mixture was filtered using Whatman filter paper no. 1. Eighty milliliters (80 ml) of acetic acid and chloroform (99:1, v/v) was added to the residue in a hot water bath for another 30 min and again filtered. Eighty milliliters (80 ml) of chloroform, methanol, and acetic acid in 49.5:49.5:1 v/v ratio was added to the residue in a hot water bath for another 30 min and again filtered. Finally, 100 ml of methanol and distilled water (50:50 v/v) was added to the residue for 30 min in a hot water bath. The filtrate was concentrated in a pressurized rotary evaporator and further air-dried for two days. After taking the weight, the saponin fraction was re-dissolved in distilled water and the concentration was computed.

Each of the different fractions was stored in air tight bottles at 4 °C. Portions of the three extract fractions were sent to the Natural Science Research Unit (NSRU) of Saint Louis University, Baguio City, for phytochemical testing to confirm the presence of the desired bioactive components.

2.1.2 Determination of LC_{50} of the different fractions of the *T. diversifolia* leaf extract

LC_{50} or median lethal concentration refers to the concentration that was able to kill half of the population of the test organisms in a given time. In the determination of the $LC_{50,}$ the three fractions of *T. diversifolia* leaf extracts corresponding to alkaloid, saponin, and combined tannins and polyphenols were prepared in five different concentrations as follows: 2000 ppm, 4000 ppm, 6000 ppm, 8000 ppm, and 10,000 ppm. About 100 ml of the prepared solutions was poured into every container. For the negative control, only 100 ml of distilled water was poured into the container while the positive control was prepared as instructed in the packet of the molluscicide, niclosamide (Sure Kill™). Ten apple snails were immersed in separate containers containing each of the prepared solutions with two replicates (n = 320). Niclosamide (Sure Kill™) was used as the positive control because this is a commercial molluscicide used by farmers in Suyo, Ilocos Sur. Snail mortality was determined every 24, 48, until 72 h. Mortality was tested by observing the following parameters: (a) the absence of retraction reflex of the foot of the snail following tactile stimulation, (b) probing of the operculum, and (c) detachment from the vertical surface of the container.

2.2 Molluscicidal bioassay

The fractions of the *T. diversifolia* leaf extract that exhibited bioactivity against the test organism were further tested. Three concentrations consisting of the median lethal concentration (LC_{50}) and two lower concentrations were prepared. The same parameters were observed in checking the molluscicidal activity of each fraction concentration.

2.3 Histological analysis

To determine the possible mode of action of the different fractions tested for molluscicidal activities, tissue samples from both treated and untreated dead snails were processed for comparative purposes. Sections of the foot, gills or ctenidia, osphradium, and digestive gland (Fig. 1) were preserved in formalin. These tissue samples were sent for histological processing at a reputable service laboratory (Hi-Precision Diagnostics, Baguio City). Slides were analyzed and documented using Optika B-150 microscope with split camera attached to it (EC300ms).

2.4 Statistical analysis

Data from this study were analyzed using pairwise comparisons (Log Rank, Breslow and Tarone Ware Analysis) and T-test using SPSS to determine if there were significant differences in the molluscicidal activities of the different fractions and concentrations of *T. diversifolia* leaf extract as compared to the positive and negative control.

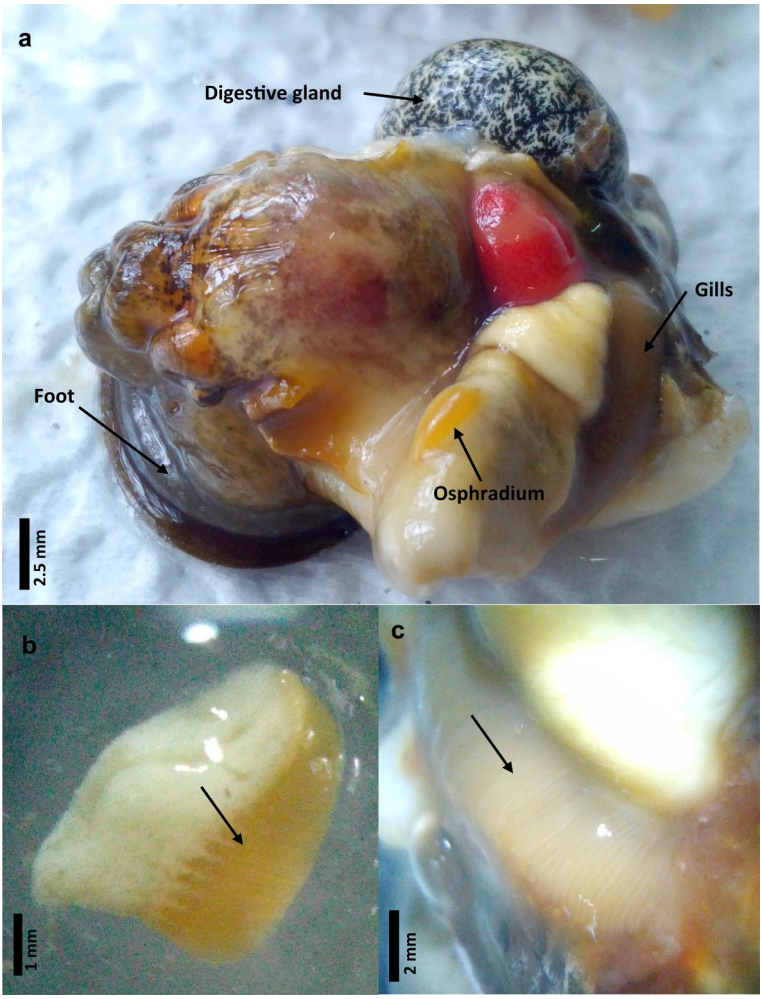

Fig. 1 Gross morphology of *Pomacea canaliculata.* **a** The mantle cavity was cut and deflected to the right to show organs used in the histopathological analysis. **b** Detached osphradium; **c** Ctenidium

3 Results

3.1 Phytochemical analyses of the different fractions of *T. diversifolia* leaf extract

The results of the phytochemical analysis of the crude methanol extract of *T. diversifolia* leaf revealed the presence of alkaloids, saponins, phenolics and tannins, and flavonoids (Table 1). The alkaloid fraction and the saponin fraction can be considered successfully fractionated as indicated by the presence of mainly alkaloids and saponins. The tannins and polyphenols fraction was not successfully separated as indicated by the presence of alkaloids and saponins in the phytochemical results. This could be due to the

Table 1 Result of the phytochemical analysis obtained from leaf extract fractions of *Tithonia diversifolia*

Test for constituents		Crude methanolic extract	Alkaloid fraction	Saponin fraction	Tannins and polyphenols fraction
Alkaloids	Mayer's test	−	−	−	−
	Dragendorff's test	+	+	−	+
	Hager's test	+	+		+
	Wagner's test	+	+	+	+
Saponins	Froth test	+	−	+	+
	Foam test	+	−	+	−
Phytosterols	Cupric acetate test for diterpenes	+	−	+	+
	Salkowski's test for triterpenes	−	−	−	−
	Liebermann–Burchard's test for phytosterols	−	−	−	+
Phenolics	Ferric chloride test for phenolics	+	−	−	−
	Gelatin test for tannins	+	−	−	−
Flavonoids	Alkaline reagent test			+	+
	Lead acetate test	+	−	+	+

The test was conducted at Natural Science Research Unit (NSRU) of Saint Louis University, Baguio City

Legend: (+) present; (−) absent

high amounts of alkaloids and saponins in the leaf extract and may require that the leaf extract first be diluted before the solvent fractionation process.

3.2 Determination of the LC_{50} of the different fractions of *T. diversifolia* leaf extracts against *P. canaliculata*

Mature snails weighing 2–7 g were used in this study. The weight rather than size as determining factor in grouping the samples for the bioassay was used because the treatment was dose dependent. Figure 2 presents the mortality of golden apple snails after the 24, 48, and 72 h treatment. In the negative control group, only 5% of the snails died after 24 h until 72 h. In the positive control, about 65% died after 24 h of exposure but all eventually died after 48 h of exposure. Statistical analyses show that all the different fractions exhibited molluscicidal properties as indicated by significantly higher activities when compared to the negative control ($p = 0.05$) but with varied molluscicidal activities when compared with the commercial molluscicide Surekill™ (positive control).

In the alkaloid fraction, mortality of golden apple snails after 24 h of exposure ranges from 25 to 35% in all the five different concentrations in no particular order. The mortality drastically increased after 48 h and slightly after 72 h to 75–100%. These results are comparable to the number of dead snails in the saponin fraction but in a dose-dependent manner. The snail mortality in the saponin fraction ranges from 60 to 100% with increasing concentration after 24 h of exposure. The mortality increased up to 75–100% after 48 and 72 h of exposure. In both alkaloid and saponin fractions, the observed concentration that is closest to the LC_{50} is 6000 ppm. Statistical analyses show that the molluscicidal activities of the five concentrations of alkaloid are comparable to the activity of the positive control ($p = 0.05$). For the saponin fraction, the 10,000 ppm exhibited the highest activity when compared to the other concentrations and the positive control, while only the activity of the 8000 ppm is equal to the activity of the positive control ($p = 0.05$).

In the tannin and polyphenols fraction, snail mortality ranges from 0 to 45% after 24 h of exposure in the different concentrations in a dose-dependent manner. The activity of

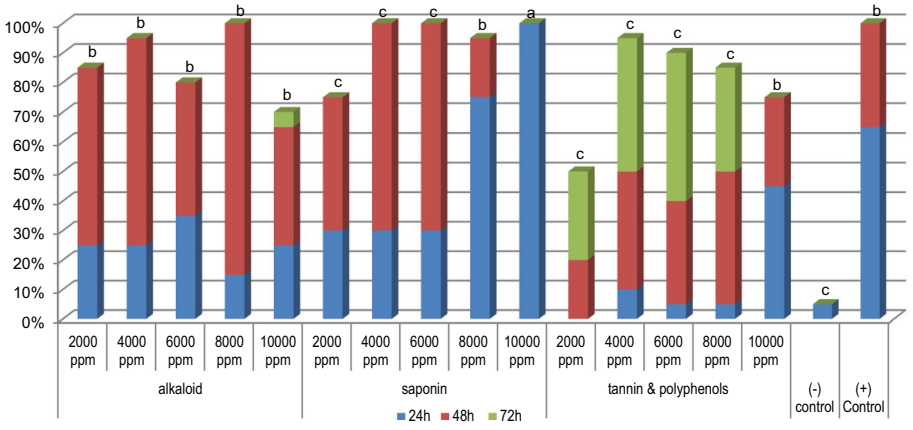

Fig. 2 Determination of LC_{50} based on the percentage mortality of golden apple snails (*P. canaliculata*) treated with the different fractions (alkaloid, saponin, and tannin and polyphenols) and concentrations (2000–10,000 ppm) of *T. diversifolia* leaf extract after 24, 48, and 72 h of exposure. The negative control was distilled water while niclosamide (Sure Kill™) served as positive control. Bars with different letters indicate significant differences

the different concentrations increased after 48 and 72 h, where mortality increased by up to 75% in the two highest concentrations (10,000 ppm and 8000 ppm). Statistical analyses show that only the activity of 10,000 ppm is comparable to the positive control ($p = 0.05$). Compared to the alkaloid and saponin fractions, the actual number of dead snails in the different concentrations of this fraction is lesser, where none of the different concentrations were able to kill all the test organisms. Thus, the observed LC_{50} of the tannins and polyphenol fraction is the highest concentration which is 10,000 ppm.

Based on the overall results of the LC_{50} test, the LC_{50} concentration and two lower concentrations were prepared for the molluscicidal assay. For the alkaloid and saponin fractions, 4000 ppm, 5000 ppm, and 6000 ppm were prepared, and 7000 ppm, 8000 ppm, and 9000 ppm for the tannin and polyphenols fractions.

3.2.1 Molluscicidal properties of the different *T. diversifolia* leaf extract fractions against *P. canaliculata*

Figure 3 presents the results of the molluscicidal assay using the two lower concentrations and the LC_{50} concentration of the three leaf extract fractions. In the negative control group, 5% of the snails died after 24 h and the rest remained alive up to 72 h. However, 55% of the snails in the positive control died after 24 h then all eventually died after 48 h. The alkaloid fraction exhibited snail mortality of 55–75% after 24 h exposure in the three different concentrations. The highest activity is noted in the 6000 ppm and 4000 ppm. All the three concentrations of the alkaloid fraction eventually exhibited 100% molluscicidal activity against adult snails after only 48 h of exposure. Statistical analyses using pairwise comparisons and *T*-test show that all the three concentrations of the alkaloid fraction have molluscicidal properties comparable to the activity of the positive control, niclosamide ($p = 0.05$).

In the saponin fraction, a concentration-dependent and time-dependent molluscicidal activity was observed. The mortality increased with increasing concentration and longer exposure time. A mortality of 10–45% was observed after 24 h, which increased up to 80% after 72 h of exposure. The 4000 ppm concentration has the least molluscicidal activity of

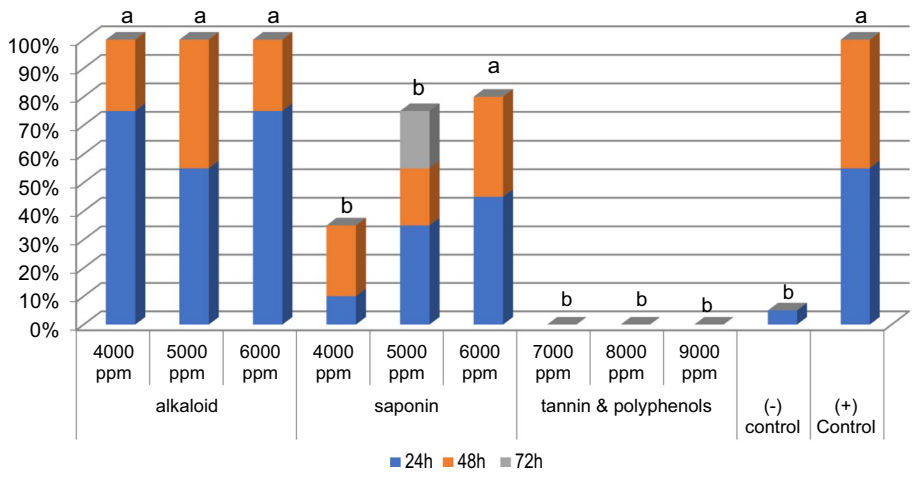

Fig. 3 Percentage mortality of adult golden apple snails (*P. canaliculata*) treated with the three different fractions and concentrations of *T. diversifolia* leaf extracts after 24, 48, and 72 h of exposure. Bars with different letters indicate significant differences

10–25% after 24 h and the 6000 ppm concentration exhibited the highest (up to 80%). Pairwise comparisons and *T*-test analyses show that the activity of the 6000 ppm is comparable to the activity of the positive control ($p = 0.05$).

All the three different concentrations of the tannins and polyphenols fraction did not show any molluscicidal activities. This is supported by the same statistical analyses wherein the activities of the different concentrations are not significantly different from the negative control.

3.2.2 Histological analyses of *P. canaliculata* tissues

The tissue samples obtained from normal (untreated) snails were compared to those treated with the different fractions of *T. diversifolia* leaf extracts. Generally, the histopathological effects were observable at the superficial tissues of the organs such as the epithelium and subepithelial layers.

Histological analyses of tissue samples from the foot showed that in the untreated group (Fig. 4a), the epithelial tissue is intact and dominated by ciliated pseudostratified columnar cells with few scattered secretory cells. The nucleus of the columnar cells lies in the basal part of the cells. The subepidermal glandular and muscular layers are also intact, and the muscular layer has distinct muscle fibers. In the alkaloid fraction (Fig. 4b), the epithelium is almost separated from the subepidermal layer and shows numerous gaps. The remaining epithelial cells appear highly vacuolated and devoid of cilia, or if present, appear to adhere together. The subepidermal glandular cells are more abundant and highly vacuolated. In the saponin fraction, histopathological changes are the same as in the alkaloid fraction with the epithelium also showing gaps between the cells, and appear slightly separated from the subepidermal glandular layer. The epithelial cells are devoid of cilia and are also highly vacuolated. The subepidermal glandular cells are more abundant and appear darkly stained. Those exposed to the tannin and polyphenols fraction show more distinctly degenerated epithelium and glandular cells (Fig. 4c). The epithelial layers have numerous cell gaps and some of the cells are entirely disrupted. The remaining epithelial cells are deciliated, or if present appear to adhere together. The subepidermal glandular layers appear more abundant and are highly vacuolated.

The gills or ctenidium serves as the respiratory organ of the snail when it is underwater. The gills are composed of a single succession of leaflets (monopectinate) attached to the longitudinal septum. Normal leaflets appear as a flattened structure with short to tall columnar epithelium (Fig. 5a, b). The epithelial tissue consists of tall ciliated columnar

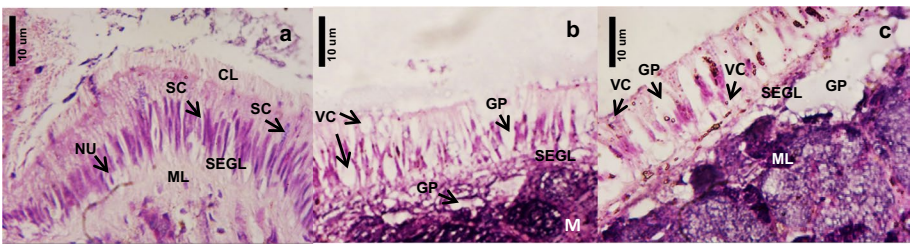

Fig. 4 Comparative histological changes in the foot tissues of *P. canaliculata* treated with the different fractions of *T. diversifolia* extract. **a** negative control, **b** alkaloid fraction, **c** Tannins and polyphenols fraction. *SC* secretory cell, *SEGL* subepidermal glandular layer, *NU* nucleus, *ML* muscle layer, *VC* vacuoles, *GP* gap

cells with few mucus-secreting goblet cells. Cilia are present all throughout the epidermis but are more prominent at the afferent edges. In the interior of leaflets are connective tissues outlining the blood lacunae and longer gill filaments are thrown into folds. The epithelium of snail gills exposed to the alkaloid and saponin fractions appear partially damaged as indicated by reduction in length and number of cilia, and high degree of vacuolation (Fig. 5c). A more severe damage is observed in the tannin and polyphenols fraction where the apical ends of the gill filaments are consistently damaged as indicated by degenerated subepidermal connective tissues and loss of epithelium (Fig. 5d). Ciliated epithelial cells at the basal parts of the filaments are intact and some goblet cells were observed.

The osphradium is a chemosensor organ that is bipectinate in structure and looks like the gills but is smaller in size, as observed in this species of snails. The epithelium of the osphradial leaflets contains various cell types such as ciliated supporting cells, mucus cells, gland cells, and pigment containing cells. These structures appear intact in the negative control group (Fig. 6a–c). In all the treated groups, the epithelia are deciliated (Fig. 6d–f). The leaflets are partially damaged in the alkaloid fraction, while the epithelium of the saponin group shows high degree of vacuolation.

The digestive gland, also known as midgut gland, contains cells that function for enzymatic secretion and absorption of digested materials. In between these glands are connective tissues. The glands have large and distinct excretory spherules (Fig. 7a). The large primary ducts are lined by epithelium and the aged cells contain a calcareous body (Fig. 7b). All these were observed in the untreated sample. All the treated tissues show deterioration in the lining of primary ducts (Fig. 7c). Excretory, glandular, and absorptive cells are

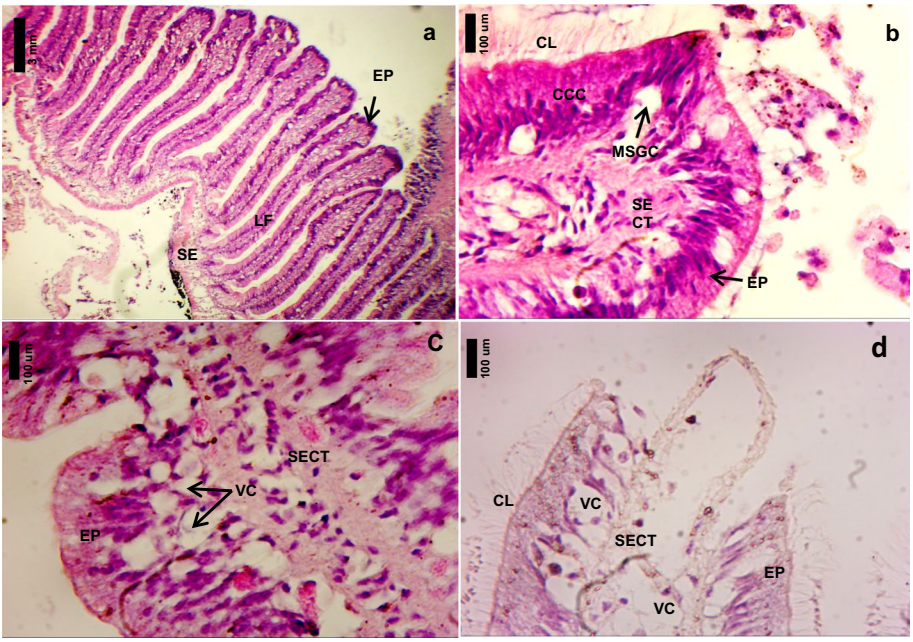

Fig. 5 Comparative histological changes in the gill or ctenidium tissues of *P. canaliculata* treated with the different fractions of *T. diversifolia* extract. **a** and **b** negative control, **c** alkaloid fraction, **d** Tannins and polyphenols fraction. *LF* leaflet, *SE* septum, *EP* epidermis, *CL* cilia, *CCC* ciliated columnar cell, *MSGC* mucus secreting goblet cell, *VC* vacuoles, *SECT* subepidermal connective tissue

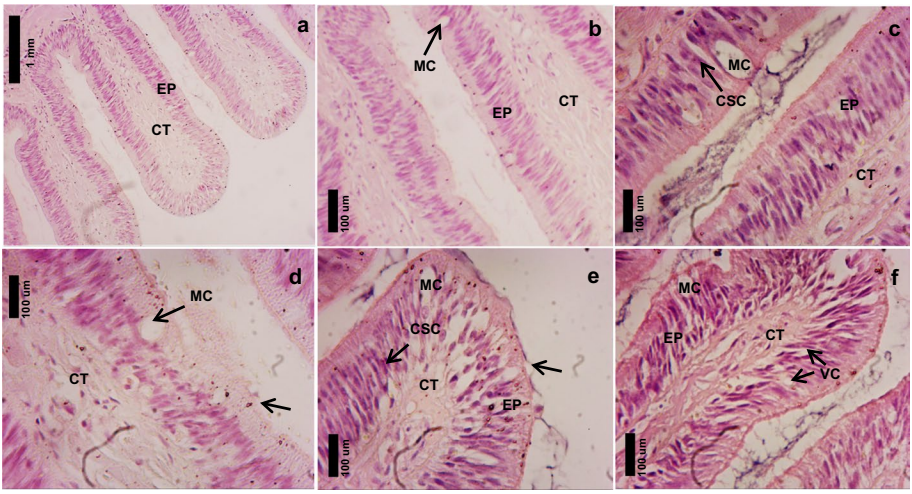

Fig. 6 Comparative histological changes in the osphradial tissues of *P. canaliculata* treated with the different fractions of *T. diversifolia* extract. **a–c** negative control, **d** alkaloid fraction, **e** Saponin fractions, **f** Tannins and polyphenols fraction. *EP* epidermis, *CT* connective tissue, *MC* mucus cell, *VC* vacuoles, *CT* connective tissue, *CSC* ciliated supporting cell

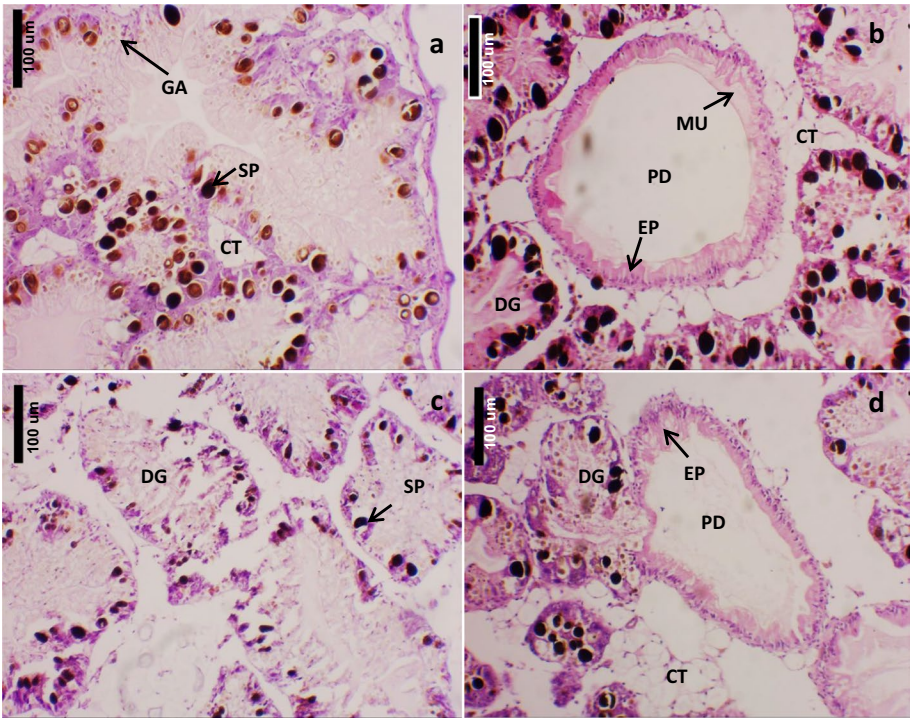

Fig. 7 Comparative histological changes in the digestive glands of *P. canaliculata* treated with the different fractions of *T. diversifolia* extract. **a**, **b** negative control, **c** alkaloid fraction; showing damaged gland cells, **d** Saponin fractions; showing damaged primary duct. *GAC* glandular and absorptive cell

also destroyed, and there are more excretory spherules compared to the untreated sample (Fig. 7d).

4 Discussion

Phytochemical analyses of the *T. diversifolia* leaf extract showed the presence of different bioactive compounds including alkaloids, saponins, diterpenes, phenolics and tannins, and flavonoids.

4.1 Molluscicidal activity of the alkaloid fraction of *T. diversifolia* leaf extract

Taiz and Zeiger (2002), Wszelaki et al. (2023) described alkaloids as a large family of alkaline nitrogen-containing secondary metabolites found in approximately 20% of the species of vascular plants and are noted for their striking pharmacological effects on vertebrate animals. They can be toxic, or even fatal, to ruminants and other animals that accidentally ingest alkaloid-containing plants in the field.

Most studies on plant alkaloids as molluscicides target problematic snails that pose threat to human health, economy, and the environment, especially those serving as intermediate hosts to human parasites. The exact mechanism of action of alkaloids is still being studied (Wszelaki et al., 2023) and the chemical diversity of alkaloids exhibited different activities, intensities, and damaging effects on snails. N-methylisosalsoline derived from *Hammada scoparia* has strong molluscicidal activity against *Galba truncatula*, an intermediate host of the liver fluke *Fasciola* in humans (Mezghani-Jarraya et al., 2009), while nicotine was found effective against *Cerithidea cingulata* (Borlongan et al., 1998) and *Planorbella trivolvis* (Kuhn et al. 2014). Caffeine was found deadly to *Veronicella cubensis* and *Zonitoides arboreus* snails (Hollingsworth et al., 2002), while capsaicin was documented to be an irritant to snails (Kalil-Gaspar et al., 2007). Sanguinarine derived from *Macleaya cordata* was found toxic to *Oncomelania hupensis* by damaging their hepatopancreas (Ke et al., 2017, 2019; Ming et al. 2011). Protopine derived from *Argemone mexicana* is capable of reducing the level of proteins and nucleic acids within the nervous system cells, as well as reduction in phospholipid levels in *Lymnaea acuminata* (Singh & Singh, 1999). Glycoalkaloids isolated from *Solanum sodomaeum* has molluscicidal activity against the snail *Bulinus truncatus*, the intermediate host of the human parasite *Schistosoma haematobium*, presumably through hemolytic and hemorrhagic damage of organs (Bekkouche et al., 2000). Molluscicidal alkaloids isolated from the stem of *Dysoxylum lenticellare* were found effective against the snail *Biomphalaria glabrata* due to their cardiodepressant activities (Adewunmi & Aladesanmi, 1988).

To date, the toxicity mechanism of alkaloids from *T. diversifolia* on golden apple snails is still unknown (Chagas et al. 2012). In this study, the observed LC_{50} for the alkaloid fraction after 24 h of exposure was 6000 ppm while the concentration that was able to kill all the test organisms only after 48 h was 8000 ppm. In the follow-up molluscicidal assay, the results showed that even concentration lower than the determined LC_{50} value, which included 5000 ppm and 4,000 ppm, was still effective molluscicides. However, their activities were more pronounced after 48 h. Thus, the molluscicidal activity of the alkaloid fraction appears to be time dependent rather than concentration dependent.

Histological analysis of tissue samples from the golden apple snails exhibited mostly damages to the superficial tissues of the organs such as the epithelium and subepithelial layers. This could be due to the limited time of exposure of the test organisms to the different treatments which only lasted 24–72 h. Snail tissues treated with the alkaloid fraction showed degenerated and vacuolated epithelia of the foot and in some gill filaments and increased occurrence of subepidermal glandular cells in the foot organ. Partially damaged and vacuolated epithelial cells of osphradial leaflets and deteriorated lining of primary ducts of the digestive glands were also noted. These indicate possible chemical damage to the epithelium by disruption of cell membrane integrity and evidenced by the increased secretory activity of the glandular layer of the foot, which is plausibly a defense mechanism. Other lethal physiological effects of the alkaloid fraction may include disruption of normal functioning of the hepatopancreas and nervous system as previously reported by Ke at al. (2017) and Singh and Singh (1999).

4.2 Molluscicidal activity of the saponin fraction of *T. diversifolia* leaf extract

The presence of the bioactive compound saponin accounts for the greatest number of results reported on the activity of plant molluscicides (Chengjun et al., 2021; Chuong Nguyen et al., 2022; Roonjho et al., 2021). Saponins are described as steroid and triterpene glycoside with detergent properties. Their toxicity is thought to be a result of their ability to form complexes with sterols and may interfere with sterol uptake from the digestive system or disrupt cell membranes after absorption into the bloodstream (Taiz and Zeiger 2002). According to Duncan (1987), saponin-containing molluscicides are believed to act by means of their pore-forming action. Hederagenin saponins isolated from *Sapindus mukorossi* (Huang et al., 2003) and triterpenoid saponins in *Sapindus saponaria* (Quijano et al., 2014) were found to be toxic to golden apple snails probably because of this detergent property.

In this study, the observed LC_{50} of the saponin fraction is similar to the alkaloid fraction, which is 6000 ppm. Only the highest concentration of 10,000 ppm was able to eliminate all the test organisms after 24 h. However, almost all the different concentrations were able to kill all the snails after 48 h of exposure. Based on these observations, the saponin activity of the fraction can be said to be initially concentration and time dependent, with activity increasing at increasing time and concentration.

A degenerated and highly vacuolated epithelial tissue devoid of cilia was observed in the foot epithelial tissue of snails treated with the saponin fraction. Subepidermal glandular cells were also more abundant. The epithelial layer of the gills was observed to have fewer cilia and is also highly vacuolated. Osphradial leaflet epithelium appeared deciliated and partially damaged and the lining of primary ducts of the digestive glands also appeared to be deteriorated. Seemingly, these observed effects may be attributed to the detergent properties of saponins which can affect cell membranes especially of the surface tissues.

4.3 Molluscicidal activity of the tannins and polyphenols fraction of *T. diversifolia* leaf extract

Tannins are considered defensive plant phenolic polymers due to their ability to bind proteins, inactivate herbivore digestive enzyme, and create complex aggregates that are difficult to digest, thus affecting the growth and development of herbivores (Taiz and Zeiger 2002).

Polyphenols are another group of plant metabolites known for their antioxidant properties. Due to this, plant polyphenols are generally considered as beneficial nutrients rather than toxic compounds.

The observed LC_{50} of the tannin and polyphenols fraction is higher (10,000 ppm) as compared to the alkaloid and saponin fractions. Furthermore, the molluscicidal assay of the three lower concentrations (7000 ppm, 8000 ppm, and 9000 ppm) did not show molluscicidal activities, and thus, this fraction may require a higher concentration to effect molluscicidal properties.

There is dearth of information on the molluscicidal properties of plant tannins extracted for molluscicidal bioassay. Reports on the activity of tannins are always in combination with other secondary metabolites in the plant extract such as alkaloids and saponins. For example, Abdullah and co-workers (2017) attributed the molluscicidal property of *Entada rheedii* against golden apple snail to the combined presence of flavonoids, glycosides, saponins, tannins, and terpenoids. In this study, foot epithelium and glandular cells of snails exposed to the tannin and polyphenols fraction were observed to be more distinctly degenerated. A notable deteriorated apical end of gill filaments and lining of primary ducts of the digestive glands were also observed. Osphradial leaflets exhibited deciliated epithelium. These observed damages in the tissue of the treated snails are not attributed to the tannin and polyphenols but to alkaloids and saponins. The result of the phytochemical analysis reported in Table 1 did not reveal tannin and polyphenols but alkaloids and saponins. This simply means that the combined effect of alkaloids and saponins may exhibit a synergistic effect magnifying the individual observed activities of saponins and alkaloids. The observed low activity of this fraction when compared to the alkaloid and saponin fractions may be due to lesser amounts of alkaloid and saponins since these two were not totally separated from the tannin and polyphenols fraction during the fractionation process.

Generally, the histopathological alterations of the foot and gills may lead to respiratory problems, disturbance of the normal osmoregulatory, and hemodynamic functions of the snails with open circulatory system (Rupert et al. 2004). The destruction of the epithelial lining would have allowed the transport of the secondary metabolites in the different organs of the treated snails resulting in their death. The osphradium is thought to have a chemosensory function and plays a role in detection of sediments in the water (Rupert et al. 2004). With the deterioration of the osphradial leaflets, the defense mechanism of the snail is most likely compromised. The digestive gland functions like the vertebrate kidney (Rupert et al. 2004) for the biochemical conversion of nutrients and wastes. Hence, damages on the lining of the digestive duct would suggest a failure in absorption and intracellular digestion.

To date, few literature in the Philippines showed promising results on the molluscicidal properties of crude plant extracts against the invasive golden apple snail. Taguiling (2015) reported that combined crude extracts of *Sandoricum vidalii* fruit and barks of *Harpullia arborea* and *Parkia sp*. were found effective against adult golden apple snail and giant earthworm (*Pheretima sp.*). This study therefore contributes to the potential of *T. diversifolia* as a natural source of molluscicide and provides scientific evidence for the local farming practice of spreading cut sunflower plants in rice paddies to control the invasive snails and sustain rice production.

5 Conclusion and recommendations

The results from this study proved that *T. diversifolia* has bioactive compounds, namely alkaloids and saponins, which exhibit molluscicidal properties. The observed LC_{50} of the alkaloid and saponin fractions against *P. canaliculata* is 6000 ppm at 24 h. The concentrations that were able to kill all the *P. canaliculata* are 8000 ppm of the alkaloid fraction at 48 h and 10,000 ppm of the saponin fraction at 24 h. The observed histological anomalies in treated snails can be attributed to bioactive compounds present in the extract, which are still the alkaloids and saponins. Alkaloids were observed to be capable of disrupting cell membranes and inhibiting the activity of the enzyme acetylcholinesterase, as well as having cardiodepressant properties. Saponins were previously reported to be toxic to golden apple snails due to their detergent property.

The observed LC_{50} values of the different fractions of *T. diversifolia* extract can be considered high when compared to other reported botanical molluscicides. This can be attributed to the crudeness of the fractions used. Since the alkaloid, saponin, and tannin and polyphenols fractions were prepared through solvent fractionation, the bioactive compound present in each fraction is not in its purest form. It is recommended that pure form of substances be used which can be obtained using sophisticated equipment such as high-performance liquid chromatography (HPLC). However, this equipment can only process little amounts of samples with very small amounts of yield (in micrograms), which may be not enough for bioassays requiring larger volume of the extracts or fractions.

Acknowledgements The researchers express their heart-felt gratitude to: the Cordillera Studies Center of the University of the Philippines Baguio for funding this research; Dr. Romeo M. Dizon for the statistical analysis of results; Dr. Roland M. Hipol for the use of his laboratory; and, the Department of Biology, College of Science, UP Baguio for the logistics and administrative support.

Funding The funding was provided by 2015 Cordillera Studies Center Research Grant.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Prior to the collection of plant material, a gratuitous permit to collect was secured from the office of the Department of Environment and Natural Resources (DENR) Regional Office- Cordillera Administrative Region in Baguio City, Philippines.

References

Abdullah, N. S., Aziz, N. A., & Mailon, R. (2017). Molluscicidal activity of *Entada rheedii* stem bark methanolic extract against paddy pest *Pomacea canaliculata* (golden apple snail). *Malaysian Journal of Analytical Sciences,21*(1), 46–51.

Acosta, B. O., & Pullin, R. S. (Eds.). (1991). Environmental Impact of the Golden Snail (*Pomacea sp*) on Rice Farming Systems in the Philippines. Central Luzon State University. http://pubs.iclarm.net/libinfo/Pdf/Pub%20CP6%2028.pdf.

Adedire, C. O., & Akinneye, J. O. (2004). Biological activity of tree marigold, *Tithonia diversifolia*, on cowpea seed bruchid, *Callosobruchus maculatus* (Coleoptera: Bruchidae). *Annals of Applied Biology,144*(2), 185–189.

Adewunmi, C. O., & Aladesanmi, A. J. (1988). Molluscicidal activities of *Dysoxylum lenticellare* gillespie constituents on *Biomphalaria glabrata* say. *Phytotherapy Research,2*, 104–106.

Aswini, B., Anita, B., & Sharmila, A. S. D. J. S. (2022). Nematicidal potential of mexican sunflower (*Tithonia diversifolia*) against the root-knot nematode *Meloidogyne incognita*. *The Pharma Innovation Journal,SP-11*(8), 963–967.

Bagunu, J. V., dM Gonzales, V., Totaan, E. V., Mula, M. G., Surban, N. C., Mallari, M., & Miclat, S. V. (2020). Balakat (*Ziziphus talanai*) leaf powder as an alternative molluscicide against golden apple snail (*Pomacea canaliculata* Lamarck) in direct seeded rice areas of Pampanga, Philippines. *Advances in Agriculture Horticulture and Entomology,5*, 1–7.

Bekkouche, K., Markouk, M., Larhsini, M., Jana, M., & Lazrek, H. B. (2000). Molluscicidal properties of glycoalkaloid extracts from Moroccan Solanum species. *Phytotherapy Research,14*(5), 366–367.

Borlongan, I. G., Coloso, R. M., Mosura, E. F., Sagisi, F. D., & Mosura, A. T. (1998). Molluscicidal activity of tobacco dust against brackishwater pond snails (*Cerithidea cingulata* Gmelin). *Crop Protection,17*(5), 401–404.

Burch, J. B. (1984). Freshwater snails of the Philippines. *Walkerana,2*, 81–112.

Chagas-Paula, D. A., de Oliveira, R. B., da Silva, V. C., Gobbo-Neto, L., Gasparoto, T. H., Campanelli, A. P., & Da Costa, F. B. (2011). Chlorogenic acids from *Tithonia diversifolia* demonstrate better anti-inflammatory effect than indomethacin and its sesquiterpene lactones. *Journal of Ethnopharmacology,136*(2), 355–362.

Chagas-Paula, D. A., Oliveira, R. B., Rocha, B. A., & Da Costa, F. B. (2012). Ethnobotany, chemistry, and biological activities of the genus *Tithonia* (Asteraceae). *Chemistry & Biodiversity,9*(2), 210–235.

Chengjun, M. A., Rui, WANG., Bin, L. I. U., Bin, H. U. A. N. G., Youming, H. O. U., & Baozhen, T. A. (2021). N. G. Molluscicidal activity of tea saponin to *Pomacea canaliculata* and its safety evaluation against three aquatic organisms. 农药学学报, *23*(1), 139–145.

Chuong Nguyen, T. H., Kim Lien, G. T., Yen, P. H., Ho, T. T., Van, T., Van Kiem, D. T., Huy Hung, P., Juo, N., & Setzer, W. N. (2022). Molluscicidal activity of compounds from the roots of *Aralia armata* against the golden apple snail (*Pomacea canaliculata*). *Natural Product Communications*. https://doi.org/10.1177/1934578X221144573

Claustro, A., & Madulid, R. (2005). Botany Section. In B. Q. Guevara (Ed.), *A guide book to plant screening: phytochemical and biological.* UST Publishing House, University of Santo Tomas.

Devi, P. I., Manjula, M., & Bhavani, R. V. (2022). Agrochemicals, environment, and human health. *Annual Review of Environment and Resources,47*, 399–421.

Di Giacomo, C., Vanella, L., Sorrenti, V., Santangelo, R., Barbagallo, I., Calabrese, G., & Acquaviva, R. (2015). Effects of *Tithonia diversifolia* (Hemsl.) A. Gray extract on adipocyte differentiation of human mesenchymal stem cells. *PLoS ONE,10*(4), e0122320.

Duncan, J. (1987). The biochemical and physiological basis of the mode of action of molluscicides. In K. E. Mott (Ed.), *Plant Molluscicides* (pp. 27–44). Wiley.

Estebenet, A. L., Martí´, N., & Burela, S. (2006). Conchological variation in *Pomacea canaliculata* and other south American Ampullariidae (Caenogastropoda, Architaenioglossa). *Biocell,30*, 329–335.

Gutierrez, R. M., Ballada, K. A., & Patacsil, M. (2015). The antibacterial property of *Tithonia diversifolia* (wild sunflower) extract from Baguio-Benguet areas in the Philippines, in response to exposure to Vehicular Traffic. *Asian Journal of Microbiology Biotechnology & Environmental Sciences,17*(1), 43–52.

Harborne, J. B. (1984). *Phytochemical methods: A guide to Modern Technologies of Plant Analysis*. Chapman and Hall.

Hollingsworth, R. G., Armstrong, J. W., & Campbell, E. (2002). Caffeine as a repellent for slugs and snails. *Nature,417*, 915–916. https://doi.org/10.1038/417915a

Huang, H. C., Liao, S. C., Chang, F. R., Kuo, Y. H., & Wu, Y. C. (2003). Molluscicidal saponins from *Sapindus mukorossi*, inhibitory agents of golden apple snails, *Pomacea canaliculata*. *Journal of Agricultural and Food Chemistry,51*(17), 4916–4919.

Joshi, R. C. (2006). Invasive alien species (IAS): Concerns and status in the Philippines. Food and Fertilizer Technology Center. http://www.fftc.agnet.org/htmlarea_file/activities/20110826121346/paper-729213301.pdf.

Kalil-Gaspar, P., Marcuzzo, S., Rigon, P., Goulart Molina, C., & Achaval, M. (2007). Capsaicin-induced avoidance behavior in the terrestrial Gastropoda *Megalobulimus abbreviatus*: Evidence for TRPV-1 signaling and opioid modulation in response to chemical noxious stimuli. *Comparative Biochemistry and Physiology Part A,148*, 286–291. https://doi.org/10.1016/j.cbpa.2007.04.019

Kannaujiya, D., Vishwakarma, D., Awasthi, S., & Shikha. (2023). Health implications of agrochemicals: Nexus of their impacts, sustainable management approaches and policy gaps. In M. C. Ogwu & S. Chibueze Izah (Eds.), *One Health Implications of Agrochemicals and their sustainable Alternatives. Sustainable Development and Biodiversity* (pp. 245–274). Springer. https://doi.org/10.1007/978-981-99-3439-3_9

Ke, W., Lin, X., Yu, Z., Sun, Q., & Zhang, Q. (2017). Molluscicidal activity and physiological toxicity of *Macleaya cordata* alkaloids components on snail *Oncomelania hupensis*. *Pesticide Biochemistry and Physiology,143*, 111–115. https://doi.org/10.1016/j.pestbp.2017.08.016

Ke, W., Tu, C., Cao, D., Lin, X., Sun, Q., & Zhang, Q. (2019). Molluscicidal activity and physiological toxicity of quaternary benzo[c]phenanthridine alkaloids (QBAs) from *Macleaya cordata* fruits on *Oncomelania hupensis*. *PLoS Neglected Tropical Diseases,13*(10), 1–16.

Kerebba, N., Oyedeji, A. O., Byamukama, R., Kuria, S. K., & Oyedeji, O. O. (2022). UHPLC-ESI-QTOF-MS/MS characterisation of phenolic compounds from Tithonia diversifolia (Hemsl.) A Gray and antioxidant activity. *Chemistry Select,7*(16), e202104406.

Kuhn, D. D., Smith, S. A., Mainous, M. E., & Taylor, D. P. (2014). Tobacco dust: A novel molluscicide for aquaculture applications. *Aquacultural Engineering,63*, 25–31. https://doi.org/10.1016/j.aquaeng.2014

Lin, C. Y., Liao, M. H., Yang, C. Y., Chang, C. K., Hsu, S. M., Juang, C. L., & Wen, H. C. (2022). Anti-metastatic activity of tagitinin C from *Tithonia diversifolia* in a xenograft mouse model of hepatocellular carcinoma. *Livers,2*(4), 400–411.

Lobo, P. P. G., & Llagas, M. A. (1991). Evaluation of starflower (*Calotropis gigantea*) against golden apple snail (*Pomacea canaliculata*) in lowland transplanted rice. *Philippine Journal of Crop Science,16*(3), 103–107.

Madamba, C. P., & Camaya, E. (1987). The golden apple snail: boon and bane? In *Proceedings 18th Anniversary and Annual Convention of the Pest Control Council of the Philippines, Davao City, Philippines*.

Mezghani-Jarraya, R., Hammami, H., Ayadi, A., & Damak, M. (2009). Molluscicidal activity of *Hammada scoparia* (Pomel) Iljin leaf extracts and the principal alkaloids isolated from them against *Galba truncatula*. *Memorias Do Instituto Oswaldo Cruz,104*(7), 1035–1038. https://doi.org/10.1590/S0074-02762009000700017

Ming, Z., Gui-Yin, L. I., Jian-Guo, Z., Li, Z., Ke-Long, H., Jin-Ming, S., Xiao, L. & Wang-Yuan, W. (2011). Evaluation of molluscicidal activities of benzo [c] phenanthridine alkaloids from Macleaya cordata (Willd) R. Br. on snail hosts of Schistosoma japonicum. *Journal of Medicinal Plants Research, 5*(4), 521–526.

Mukasa, D., Olila, D., Tinzaara, W., & Kagezi, G. H. (2008). Effects of *Tithonia* and *Phytolacca* extracts against the banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae). *Africa Journal of Animal and Biomedical Sciences,3*(1), 24–29.

Naylor, R. (1996). Invasions in agriculture: Assessing the cost of the golden apple snail in Asia. *Ambio,25*(7), 443–448.

Owoyele, V. B., Wuraola, C. O., Soladoye, A. O., & Olaleye, S. B. (2004). Studies on the anti-inflammatory and analgesic properties of *Tithonia diversifolia* leaf extract. *Journal of Ethnopharmacology,90*(2), 317–321.

Pagulayan, R. C. (1997). Update on freshwater mollusk research in the Philippines. In R. D. Guerrero III., A. Tisico-Calpe, & L. C. Darvin (Eds.), *Aquatic Biology Research and Development in the Philippines* (pp. 69–80). Laguna: PCARRD.

Quijano, M., Riera-Ruíz, C., Barragán, A., Miranda, M., Orellana, T., & Manzano, P. (2014). Molluscicidal activity of the aqueous extracts from *Solanum mammosum* L., *Sapindus saponaria* L. and *Jatropha curcas* L. against *Pomacea canaliculata*. *Emirates Journal of Food and Agriculture,26*(10), 871–877.

Radhakrishnan, B., & Prabhakaran, P. (2014). Biocidal activity of certain indigenous plant extracts against red spider mite, *Oligonychus coffeae* (Nietner) infesting tea. *Journal of Biopesticides,7*(1), 29–34.

Roonjho, A. R., Muhamad, R., & Omar, D. (2021). Determination of lethal and feeding deterrent activities of saponin from Phaleria macrocarpa against Pomacea maculata. *Journal of Animal & Plant Sciences,31*(4), 1070–1077.

Ruppert, E. E., Fox, R. S., & Barnes, R. D. (2004). *Invertebrate Zoology: A functional Evolutionary Approach*. Brooks/Cole.

Sin, T. S. (2003). Damage potential of the golden apple snail *Pomacea canaliculata* (Lamarck) in irrigated rice and its control by cultural approaches. *International Journal of Pest Management,49*(1), 49–55.

Singh, S., & Singh, D. K. (1999). Effect of molluscicidal components of *Abrus precatorius, Argemone mexicana* and *Nerium indicum* on certain biochemical parameters of *Lymnaea acuminata*. *Phytotherapy Research,13*, 210–213.

Susilowati, I. T., Soetjipto, H., & Hastuti, S. P. (2022). Testing the antibacterial activity of crude extracts of *Tithonia diversifolia* leaves, flowers, and roots in various solvent fraction. *EduChemia (Jurnal Kimia dan Pendidikan),7*(1), 124–136.

Taguiling, N. (2015). Effect of combined plant extracts on golden apple snail (*Pomacea canaliculata* (Lam.)) and giant earthworm (*Pheretima sp*). *International Journal of Agriculture and Crop Sciences,8*(1), 55–60.

Taiz, L., & Zeiger, E. (2002). *Plant physiology*. Massachusetts: Sinauer Associates.
Weisenburger, D. D. (1993). Human health effects of agrichemical use. *Human Pathology,24*(6), 571–576.
Wszelaki, S., Podobiński, P., & Środoń, K. (2023). Molluscicidal activity of plant alkaloids. *Journal of Applied Toxicology*. https://doi.org/10.1002/jat.4466
Zhao, G., Li, X., Chen, W., Xi, Z., & Sun, L. (2012). Three new sesquiterpenes from *Tithonia diversifolia* and their anti-hyperglycemic activity. *Fitoterapia,83*(8), 1590–1597.