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Research Article

Molluscicidal and Feeding Deterrent Activity of Crude Plant Extracts on *Pomacea maculata* Perry

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Abstract: This study was focused on molluscicidal effect and feeding deterrent activity of saponin from five different plant materials; *Andrographis paniculata*, *Entada spiralis*, *Ficus deltoidea*, *Furcraea selloa* and *Ipomoea batatas*. Crude plant extracts were prepared from plant powders using methanol. The crude extracts were then tested on *Pomacea maculata* using five different concentrations (5, 10, 15, 20 and 25 ppm, respectively) against niclosamide (control). After 72 h of exposure, the highest percentage of mortality of 80% was achieved from 15 ppm of *F. selloa.* Two analyses were conducted to observe the feeding deterrent activity and after 24 h, both analyses demonstrated the feeding deterrent activity in both crude extracts (*F. selloa* and *E. spiralis*) similar in niclosamide.

Keywords: Crude extract, feeding deterrent, molluscicidal, mortality, saponin

INTRODUCTION

Pomacea maculata Perry from Ampullariidae family is native to South America and occur throughout the humid tropics and subtropics. *Pomacea maculata*, also known as the black apple snail, is the largest living freshwater snail (San Martin, 2008). As the snail failed to serve its purpose, it was released into the rice field, thus enabling its spread from one place to another through irrigation or water canal, thus causing it to become a serious threat to rice production and the environment. To date, two species of apple snails have been identified in Malaysia and they are *Pomacea canaliculata* and *P. maculata* but according to Arfan *et al*. (2014), *P. maculata* is more abundant and widely distributed as compared to *P. canaliculata* (Chang, 1992; Mat Hasan and Abdul Kadir, 2002; Yahaya *et al*., 2006; Hayes *et al*., 2012). *Pomacea maculata* has also been identified as among the 100 worst Invasive Alien Species (IAS) because of its high reproductive rate, good adaptability to harsh environmental conditions, having the ability to move for a long distance through the water system and also having a voracious appetite (Lowe *et al*., 2000). In Malaysia, the infestation of the snails becomes a major concern among farmers and also the government because our rice growers have been practicing direct seeding cultivation system since a long time. Apple snails favour rice field as habitat because the snails are able to consume young rice seedlings during the day and night continuously. Due to lack of natural enemies in the rice field to limit their activities, snails then become a serious pest in many rice planting area. According to International Rice Research Institute (IRRI), snails can completely destroy 1 m^2 of field overnight if no control has been taken and this damage could lead to more than 50% yield loss (IRRI, 1991). Research conducted by Rice Institute in Central Java showed that under field condition, 12 snails per 2 m^2 areas could cause up to 11% damage and yield reduction of about 15% (Sudjono *et al*., 2000).

To fit the purposes of controlling the population of snails in all countries that have been seriously attacked by apple snail, different management methods had been applied including cultural, mechanical, biological and also chemical control but none has proven to be adequately safe and effective. However, majority of the local farmers most preferred chemical molluscicides instead of other control methods due to its fast effect and low cost. Niclosamide, metaldehyde and fentin acetate were being used widely all over the country (Yang *et al*., 2006). However, it is a known fact that chemical molluscicides are hazardous to human and also the environment, often killing non-target organisms effectively. However, these problems might be overcome using botanical molluscicides which are

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considered as green technology and safe. San Martin (2006) discovered that *Chenopodium quinoa*, a plant that contain saponin compound proved as an efficient control against golden apple snail at lower doses and directly showed no phytotoxicity towards rice. Saponin is reported to have diverse biological properties such as haemolytic properties and high toxicity to most cold-blooded animals (Sparg *et al*., 2004). Other than that, it can cause apoptosis which is uncontrolled cell death to the snails (De Geyter *et al*., 2007). Earlier studies have also shown the presence of activities such as molluscicidal, anti-inflammatory, antimicrobial and antifungal, anti-tumor and others (Sparg *et al*., 2004). Saponins also exert clear insecticidal activities which has a strong and rapid working action against a broad range of insects and the most observed effects are increased insect mortality, lowered food intake, weight reduction, retardation in development and decreased reproduction (De Geyter *et al*., 2007).

Given the above, the objectives of the study were to extract and quantified saponin bioactive compound from selected plants and to evaluate the toxicity of selected crude plant extracts on *P. maculata* through mortality bioassay and feeding deterrent bioassay.

MATERIALS AND METHODS

Study site: This study was conducted in a glasshouse unit at Field 2 and Laboratory C, Faculty of Agriculture, Universiti Putra Malaysia.

Preparation and quantification of saponin from plant material: Preparation of crude plant extract from the following plant materials, *F. deltoidea* (leaf), *A. paniculata* (leaf), *E. spiralis* (stem), *I. batatas* (tuber) and *F. selloa* (leaf) was done in the laboratory. Collected plants were washed, oven dried at 45°C for five days and ground into powder using table type smashing machine (FDS Model, Taiwan). The extraction was conducted using sequential extraction method adapted from Harpreet *et al*. (2011) with modification whereby the plant powder was suspended in methanol for 48 h. The suspended solid was filtered through Whatman No. 1 filter paper and the solvent in the filtrate was then removed using rotary evaporator (BUCHI, United Kingdom) to obtain the crude extract. The saponin in crude form was quantified and subjected to quantitative High-Performance Liquid Chromatography (HPLC) analysis with Photodiode Array detector against the analytical standard of saponin from Sigma-Aldrich, USA. The separation of saponin was performed by using C18 (5 µm particle size, 150×4.6 mm) column (Sigma-Aldrich, USA) and the detection wavelength was set at 280 nm. The temperature was maintained at 30°C, with the injection volume of 20 µL and flow rate of 1.5 mL/min. The standards and samples were separated using water and methanol as gradient mobile phase with the ratio of 50: 50 v/v for 10 min.

Mortality bioassay of crude plant extracts: Mortality bioassay was conducted using guideline for evaluation of molluscicides with modification by WHO (1983). The crude extracts were tested on apple snail using five different concentrations (5, 10, 15, 20 and 25 ppm, respectively). Niclosamide at a recommended rate was used as positive control while water as negative control. The hatchlings in size of 1 to 2 cm were used in this experiment. Five snails were used for each treatment and all snails were starved 24 h before experiment commenced. During experiment, snails were fed with 1 g of 20 days old rice leaves. The experiment was conducted in a Completely Randomized Design (CRD) with four replications. Observation was made every 24, 48 and 72 h, respectively. Snails were considered dead if they were unable to make a coordinated movement when gently prodded. Percentage of mortality for each treatment was calculated as below:

(Percentage of mortality (%) = (Number of dead snail) / (Total number of snail) × 100)

Data obtained were analysed by ANOVA using Least Significant Difference (LSD) test at 0.05 probability levels. To normalize, data were arcsine transformed before analysed using SAS 9.3 computer software.

Feeding deterrent activity of crude plant extract: The experiment for feeding deterrent activity was performed using leaf dip method adapted by Dawidar *et al*. (2012) with modification. Five different concentrations (5, 10, 15, 20 and 25 ppm, respectively) of each crude extract were tested on apple snails. Niclosamide at a recommended rate was used as a positive control while water as a negative control. Each treatment was tested on 1 cm snail with 10 replications. All snails were starved 24 h before the start of experiment. During the experiment, 0.3 g of 20 days old rice leaves were dipped into the respective treatments and provided to snails. The experiment was conducted in a Completely Randomized Design (CRD). The leaf area and leaf weight of rice leaves consumed by apple snails were recorded before and after the experiment using LI-3100 Area Meter (LI-COR, USA) and Sartorius BP221S analytical balance (Sartorius, Germany). The data obtained were analysed by ANOVA using Least Significant Difference (LSD) test at 0.05 probability levels.

RESULTS AND DISCUSSION

Quantification of saponin: From HPLC, the chromatogram of standard saponin displayed the peak of saponin detected at particular retention time (tR). The saponin quantification of *F. selloa*, *E. spiralis*, *A. paniculata*, *I. batatas* and *F. deltoidea* crude extracts

Table 1: Saponin content (ppm) in five different crude plant extracts (*F. selloa*, *E. spiralis*, *A. paniculata*, *I. batatas* and *F. deltoidea*) from HPLC analysis

Plant	Plant part	Saponin content (ppm)	(%)
F. selloa	Leaf	7971	0.79
E. spiralis	Stem	4779	0.47
A. paniculata	Leaf	4745	0.47
I. batatas	Tuber	2649	0.26
F. deltoidea	Leaf	2332	0.23

was shown in Table 1. Based on the HPLC analysis of standard, saponin can be detected at 1.159 min of retention time which means the corresponded time taken for saponin to travel through the column to detector was at about one minute, respectively. Kupiec (2004) has highlighted that the measurement of peak height or peak area for chromatogram in HPLC was the basic theory for quantification and both peak area and height are plotted versus the concentration of the substance in order to determine the concentration of the particular compound. Therefore, based on the peak area which covered 3427366/sec and 256193 µv of peak height, respectively, the chromatogram indicates 100% of saponin compound. From Table 1, highest saponin was found from the leaves extract of *F. selloa* (0.79%) with the retention time at 1.195 min.

According to Simmons-Boyce *et al*. (2004), four steroidal saponins were isolated from the leaves of *F. selloa* which were furcreafurostatin (furostanol saponin) and three spirostanol saponins which includes furcreastatin, yuccaloeside C and cantalasaponin-1. This result was also supported by Itabashi *et al*. (1999) who mentioned that a novel steroidal saponin known as furcreastatin was isolated from an ethanol extract of the leaves of *Furcraea foetida*. Most of the Agavacea family such as *Furcraea* and *Agave* possess saponin as the main bioactive compound because steroidal saponins and sapogenins are often found abundantly in the family of Agavacea (Simmons-Boyce and Tinto, 2007). This proves that the major bioactive compound in *F. selloa* is saponin. The stem extract of *E. spiralis* contained the second highest saponin content and it can be concluded that 4779 ppm out of 10,000 ppm of *E. spiralis* crude extract contained saponin after 1.227 min of retention time. The total amount of saponin bioactive compound in *E. spiralis* was lower as compared to *F. selloa* crude extract because saponin may not be the main compound of the genera Entada. Chemical analyses conducted by Barua (1955), Dai *et al*., (1991), Zhao *et al*. (2011) and Iwamoto *et al*. (2012) had proven that *Entada phaseoloides* possessed compound such as triterpene acid, phenylacetic acid ester, triterpene saponins and phenolic acids. Meanwhile, Aktar *et al*. (2011) reported that the methanolic crude extract of *E. phaseoloides* showed higher level of total phenolic content. The presence of phenolic compound in crude extract might affect the total content of other chemical constituents in the sample. In addition, the earlier research has highlighted that phenolic compounds were an important component in *Entada africana* extracts which possess strong antioxidant activity (Tibiri *et al*., 2007; Tibiri *et al*., 2010). The extract of *A. paniculata* contained the same amount of saponin concentrations as in *E. spiralis* extract at 0.47% but the detection of the compound was faster than *E. spiralis* which was at 1.199 min. As in before, the saponin compound was lower as compared to *F. selloa* crude extract because *A. paniculata* consisted andrographolide as major bioactive compound. Andrographolide is the primary bioactive component of *A. paniculata* and presents in all parts of the plant especially in leaves (Siripong *et al*., 1992; Cui *et al*., 2004). Results from previous researches had also emphasized on the andrographolide in *A. paniculata*. The ethanolic and methanolic extracts of the whole plant, leaf and stem of *A. paniculata* showed that over 20 diterpenoids and over ten flavonoids have been detected. Andrographolide ($C_{20}H_{30}O_5$), the major diterpenoid compound, making up about 4%, 0.8 to 1.2% and 0.5 to 6% in dried whole plant, stem and leaf extracts, respectively (Matsuda *et al*., 1994; Burgos *et al*., 1997; Kishore *et al*., 2003; Pholphana *et al*., 2004; Li *et al*., 2007; Chao and Lin, 2010). Saponin was also found in the tuber extract of *I. batatas* but in a small quantity which was 2649 of 10,000 ppm. Based on HPLC analysis conducted for *I. batatas* crude extract, it can be concluded that saponin can be detected at 1.173 min. The lower quantity of saponin in *I. batatas* is due to the presence of another major chemical constituent known as phenolic compounds. Pochapski *et al*. (2011) reported that 60% of phenolic compounds were present in 100 g of the dry sample of *I. batatas.* In addition, four different polyphenolic compounds known as 4,5-di-*O*-caffeoyldaucic acid, 4-*O*-caffeoylquinic acid, 3,5-di-*O*-caffeoylquinic acid and 1,3-di-*O*-caffeoylquinic acid were isolated from methanolic extract of *I. batatas* (Dini *at al*., 2006). Study by Truong *et al*. (2007) showed that chlorogenic acid, a type of phenolic acid, content was highest in root tissues of *I. batatas*. Referring to the HPLC analysis result of *F. deltoidea* crude extract, it has contained the least amount of saponin bioactive compound with only 2332 ppm (0.23%) with the retention time at 1.217 min. Even though the leave extract of *F. deltoidea* is proven to have saponin bioactive compound, the concentration is low because polyphenolic compounds and flavanoids that are responsible for strong antioxidant activities were reported to be the major chemical constituents in Ficus species (Sirisha *et al*., 2010; Zakiah and Hazlan, 2014).

Mortality bioassay of crude plant extracts: The observation for molluscicidal activity of the five selected crude extracts at 72 h is shown in Table 2. Niclosamide gave the highest percentage of snail's mortality whereas water did not affect mortality since there was no treatment applied. As expected,

Table 2: Percentage mortality (%) of *P. maculata* when exposed to *F. selloa*, *E. spiralis A. paniculata*, *I. batatas* and *F. deltoidea* crude extracts at 72 h

Treatment (ppm)	Percentage (%) mortality of *P. maculata*				
	F. selloa	*E. spiralis*	*A. paniculata*	*I. batatas*	*F. deltoidea*
5	5^{d}	0^{d}	0^{c}	0^{c}	15^{b}
10	40^{c}	20^{bc}	0^{c}	0^{c}	15^{b}
15	80^{ab}	25^{b}	0^{c}	0^{c}	20^{b}
20	45^{c}	5^{cd}	40^{b}	10^{bc}	25^{b}
25	60^{bc}	5^{cd}	0^{c}	20^{b}	20^{b}
Water	0^{d}	0^{d}	0^{c}	0^{c}	0^{b}
Niclosamide	100^{a}	100^{a}	100^{a}	100^{a}	100^{a}

Means with same letters in a column indicate no significant difference at $p<0.05$

niclosamide possessed better molluscicidal effect because of its chemical compound as compared to other treatments. Chemical such as niclosamide (Bayluscide), metaldehyde (Snailkil, Porsnail), endosulfan and fentin acetate have been used to control apple snail because of their fast effects (Joshi *et al*., 2001; Wada, 2004). From the table, there was a significant difference in the percentage of mortality between concentrations and niclosamide. The highest mortality (80%) at 72 h was observed in *F. selloa* crude extract at 15 ppm concentration. This was followed by 60, 45 and 40% mortality in the concentration of 25, 20 and 10 ppm, respectively. Only 5% of dead snails were recorded for 5 ppm saponin concentration as this concentration might be too low to kill the snail. As for the *E. spiralis* crude extract, the mortality of *P. maculata* recorded were 25% at 15 ppm, 20% at 10 ppm and 5% at both 20 ppm and 25 ppm. The percentage was lower as compared to *F. selloa* crude extract. This result is supported by the result of saponin quantification for *E. spiralis* by HPLC analysis, which highlighted that saponin content in *E. spiralis* sample was only 0.47% as compared to 0.79% for *F. selloa.* In the case of *A. paniculata* crude extract, the maximum mortality of 40% at 72 h which was slightly higher than *E. spiralis* crude extract. According to Tanwer and Vijayvergia (2010), the toxicity of *A. paniculata* crude powders was time dependant. Therefore, the mortality of *P. maculata* might increase by increasing the time of exposure more than 72 h. Meanwhile, the percentage mortality of *I. batatas* extract at 72 h after exposure was 20% for 25 ppm and 10% for 20 ppm concentrations. No mortality was recorded at 5, 10 and 15 ppm, respectively throughout the 72 h observation. The percentage mortality of *I. batatas* crude extract is relatively low due to its low saponin content which was 0.26%. Therefore, the molluscicidal activity of *I. batatas* required longer duration to enable effective killing. In addition, higher concentration of crude extract is needed to cause mortality on snails. Currently, there was no study done on the molluscicidal effect of *I. batatas* against apple snails in Malaysia. Nevertheless, it was reported that *I. batatas* may act as repellent or deterrent due to its secondary metabolite compound which is saponin (Hubbel *et al*., 1983). The molluscicidal effect of *F. deltoidea* at 72 h showed the highest percentage mortality of *P. maculata* achieved was 25% at 20 ppm concentration. This was followed by 20% mortality in both 15 and 25 ppm crude extract and 15% mortality in 5 and 10 ppm crude extract. The molluscicidal activity of *F. deltoidea* crude extract is increased by increasing the duration of exposure to saponin. From the observation, the mortality of *P. maculata* was increasing until the final day of observation at 72 h after exposure. The effectiveness of all crude extracts was also directly proportional to the exposure time to saponin concentration. The survival rate, growth rate and egg laying capacity of apple snail decreased when the time of exposure to saponin increased (Mahato *et al*., 1982). Extract of *F. selloa* at 15 ppm can be considered as an alternative to niclosamide since it caused 80% mortality. This was followed by 60 and 45% mortality at 25 and 20 ppm concentration of *F. selloa*, respectively.

Although other plants tested contained saponin as one of the bioactive compounds, the amount was relatively low. Nevertheless, they still showed some molluscicidal activity on snails. According to WHO (2009), Bagalwa *et al.* (2010), Mahmoud *et al*. (2011), Adewumi *et al*. (2013) and Bagalwa *et al.* (2014), the use of synthetic or natural origin molluscicides can be a promising means in controlling snails but the use of natural origin molluscicide is preferred for the purpose of human and environmental safety issues.

Feeding deterrent activity of crude plant extract: Results for mean leaf area consumed by *P. maculata* when treated with *F. selloa*, *E. spiralis*, *A. paniculata*, *I. batatas* and *F. deltoidea* crude extracts is shown in Table 3. The mean leaf area consumed by *P. maculata* for niclosamide treatment was the lowest (0.25±0.04 cm^2) after 24 h. A lower leaf mean area indicates better effect of crude extracts in interrupting the feeding behaviour of apple snail. All concentrations of *F. selloa* and *E. spiralis* crude extract showed no significant difference with niclosamide at $p<0.05$ which means, all concentrations of both plant crudes (5, 10, 15, 20 and 25 ppm, respectively) showed an anti-feedant effect as niclosamide. The lowest mean leaf area for *F. selloa* was 0.57±0.05 cm^2 from 20 ppm concentration whereas 0.44±0.13 cm^2 from 25 ppm for *E. spiralis* crude extract. Meanwhile, the concentrations of *A. paniculata* and *F. deltoidea* differed significantly from niclosamide at $p<0.05$.

Table 3: Mean leaf area (cm^2) consumed by *P. maculata* after treated with *F. selloa*, *E. spiralis*, *A. paniculata*, *I. batatas* and *F. deltoidea* crude extracts within 24 h

Treatment (ppm)	Mean (cm^2)				
	F. selloa	*E. spiralis*	*A. paniculata*	*I. batatas*	*F. deltoidea*
5	0.97^{b}	1.07^{b}	13.28^{abc}	3.05^{bc}	13.63^{a}
10	0.73^{b}	0.79^{b}	12.26^{bc}	3.34^{b}	11.56^{ab}
15	0.66^{b}	0.60^{b}	11.21^{bc}	3.10^{b}	12.31^{ab}
20	0.57^{b}	0.70^{b}	15.29^{ab}	3.10^{bc}	11.04^{ab}
25	0.62^{b}	0.44^{b}	17.52^{a}	2.78^{bc}	14.14^{a}
Water	9.27^{a}	9.27^{a}	9.27^{c}	9.27^{a}	9.27^{b}
Niclosamide	0.25^{b}	0.25^{b}	0.25^{d}	0.25^{c}	0.25^{c}

Means with same letters in a column indicate no significant difference at $p<0.05$

Table 4: Mean leaf weight (g) consumed by *P. maculata* after treated with *F. selloa*, *E. spiralis*, *A. paniculata*, *I. batatas* and *F. deltoidea* crude extracts within 24 h

Treatment (ppm)	Mean (g)				
	F. selloa	*E. spiralis*	*A. paniculata*	*I. batatas*	*F. deltoidea*
5	0.09^{b}	0.11^{b}	0.14^{abc}	0.26^{b}	0.10^{b}
10	0.09^{b}	0.09^{bc}	0.13^{bc}	0.26^{b}	0.11^{b}
15	0.08^{b}	0.06^{c}	0.12^{c}	0.27^{ab}	0.10^{b}
20	0.07^{bc}	0.08^{bc}	0.15^{ab}	0.28^{a}	0.10^{b}
25	0.04^{cd}	0.06^{cd}	0.17^{a}	0.29^{a}	0.11^{b}
Water	0.17^{a}	0.17^{a}	0.17^{a}	0.17^{c}	0.17^{a}
Niclosamide	0.02^{d}	0.02^{d}	0.02^{d}	0.02^{d}	0.02^{c}

Means with same letters in a column indicate no significant difference at $p<0.05$

Table 4 showed the mean leaf weight consumed by *P. maculata* after treated with five different concentrations of selected crude extracts. After 24 h of exposure, niclosamide gave the lowest means of leaf weight with 0.02±0.01 g. Lower mean of leaf weight indicates a better effect of crude extracts in interrupting the feeding behaviour of apple snail. There was a significant difference in mean leaf weight between concentrations of crude extract with niclosamide at $p<0.05$ except at 25 ppm of both *F. selloa* and *E. spiralis* extracts. The lowest mean leaf weight for *F. selloa* crude extract was 0.04±0.01 g which was 0.02 g more than niclosamide. Meanwhile, 0.06±0.01 g was the lowest mean leaf weight for *E. spiralis* crude extract and it was 0.04 g higher than niclosamide. This indicates that leaves treated with *F. selloa* and *E. spiralis* crude extract was less preferred by *P. maculata* as compared to other extracts. As for the other crude extracts of *A. paniculata*, *F. deltoidea* and *I. batatas*, significant difference in mean leaf weight between the concentrations with niclosamide at $p<0.05$ was observed. This shows that the crude extracts of *A. paniculata*, *F. deltoidea* and *I. batatas* has almost no anti-feedant effect on apple snail throughout the experiment. Based on the mean leaf area and leaf weight analyses, *F. selloa* and *E. spiralis* crude extracts gave better anti-feedant effect towards apple snails as compared to the different concentrations of *A. paniculata*, *I. batatas* and *F. deltoidea* crude extracts. Higher concentration of crude extract will reduce the feeding attractant of *P. maculata* towards rice leaves. This observation was directly related to the findings of Huang *et al*. (2003) whereby, the higher the saponin content, the slower the feeding as saponin had toxicological properties against apple snail which later lead to cell death. The toxic odor produced by saponin may also prevent snails from consuming the rice leaves. Besides having insecticidal and molluscicidal properties, saponin is also known for its anti-feedant activity (Chaieb *et al*., 2009). According to Mason *et al*. (1994), saponin containing plant extract with concentration of 0.05% by weight or higher may serve as an anti-feedant, thus preventing terrestrial molluscs from feeding on living plants.

CONCLUSION

Results from both mortality and feeding deterrent analyses conducted in this study, strongly concluded that *F. selloa* and *E. spiralis* crude extracts could be used to control the population of *P. maculata*.

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CONFLICT OF INTEREST

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