# Molluscicidal activity of *Ipomoea batatas* leaf extracts against *Pomacea canaliculata* (Golden apple snail)

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# Abstract

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This study was conducted to investigate the molluscicidal activity of four different Ipomoea batatas (I. batatas) leaf extracts (hexane, chloroform, methanol, and aqueous extracts) against Pomacea canaliculata (P. canaliculata) and screen the phytochemical compounds of *I. batatas* leaf extracts. The golden apple snails (size range: 20-40 mm) were exposed to three concentrations (1000, 5000, and 10,000 ppm) of each extract (hexane, chloroform, methanol, and aqueous extracts). The efficacy of the *I. batatas* extracts was laboratory-tested by submerging the P. canaliculata into paddy-filled water mixed with the extracts and the mortality was observed every 24 hrs for 3 days. The presence of phytochemical compounds such as terpenoids, flavonoids, saponins, tannins, alkaloids, and glycosides were tested. For all extracts, the results showed a positive relationship between the concentration and the golden apple snail mortality, suggesting that all extracts have molluscicidal properties. The study showed more than 50% mortality rate of P. canaliculata after 72 hrs for each treatment. Chloroform and methanol I. batatas leaf extracts at 10,000 ppm showed 100% mortality after 72 hrs due to the presence of saponin. Saponin causes the death of the snails by disturbing their feeding and growth and blocking their breathing process. For further research, it is suggested to conduct the study under field trials in the search for a natural and environment-friendly molluscicide.

# 1. Introduction

Pomacea canaliculata (P. canaliculata) or also known as golden apple snails has caused severe damage to the paddy industry in Malaysia. The snails were first introduced to South-East Asia in the 1980s for food purposes. Due to low demand, the snail farming was abandoned and the snail escaped to the rice fields (Ruma and Sanchez, 2016). The snail was first found in Malaysia in the 1990s and since then it has become one of the major pests in the paddy industry (Nur Suraya et al., 2017). P. canaliculata attacks and destroys young stems and leaves of plants and could eat 7-24 rice seedlings per day (Cagauan and Joshi, 2002). The damage done by the snails is sufficiently serious and is a major concern. The problem becomes more serious as P. canaliculata's populations can increase rapidly under favourable conditions (Ding et al., 2018).

In controlling the infestation of *P. canaliculata*, various methods such as cultural control, biological

control, chemical control, salt compounds, organic compounds, and molluscicides have been used (Liang et al., 2013). Handpicking and using ducks are among cultural and biological controls used. These methods have been proven to control the snails at a low cost. However, they require a large labour force and causes damage to the rice seedlings (duck control) (Liang et al., 2013). Chemicals are also widely used in controlling P. canaliculata as they are very effective. The principal chemicals used effectively against golden apple snails are metaldehyde, formulated as bait pallets, wettable powders or flowable suspensions, and niclosamide, formulated as either an emulsifiable concentrate, suspension concentrate or wettable powder. Even though they are effective, the use of chemicals is discouraged as they have long-term toxicity effects on humans and the environment. Musman (2010) reported that the usage of chemical fertilisers such as metaldehyde and niclosamide can pollute water sources and thus affect the ecosystem. Furthermore, the cost of chemical molluscicides is unaffordable to many farmers.

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An alternative in organic farming for snail control is by using biopesticides, which are derived from natural compounds such as plants, microorganisms, and minerals. Interest in plant molluscicides has increased in recent years as they have a significant impact (Quijano et al., 2014). Molluscicidal properties have been reported in many plant species. Previous studies have tested and reported the effectiveness of plant extracts in controlling P. canaliculata. Among the plants tested are Solanum mammosum L., Sapindus saponaria L. and Jatropha curcas L. extracts (Quijano et al., 2014), Agave filifera, Ammi majua and Canna indica leaves and flowers (Rawi et al., 2011), and Barringtonia racemosa L. kernel extract (Musman, 2010). The combinations of crude extracts of Sandorium vidalii, Harpulia arborea, and Parkia sp. have also proven to show toxicity towards P. canaliculata (Taguiling, 2015).

To date, there is still a continuous effort to search for the most potent organic biological agents that could minimise the invasion and infestation of *P. canaliculata* (Picardal *et al.*, 2018). This study has evaluated the effectiveness of *Ipomoea batatas* (*I. batatas*) leaf extracts for their molluscicidal activity against *P. canaliculata*. A screening for phytochemical compounds in the extracts was also conducted.

#### 2. Materials and method

#### 2.1 Preparation of Ipomoea batatas leaf extract

Four different *I. batatas* leaf extracts (hexane, chloroform, methanol, aqueous) were prepared for this study. Hexane, chloroform, and methanol *I. batatas* leaf extracts were prepared according to Ayoola *et al.* (2008). A hundred grams of air-dried leaves were grounded into fine powder and then macerated with 1 L of solvent (hexane, chloroform, and methanol) for 24 hrs consecutively. The extract was filtered and evaporated using a rotary evaporator. The crude extracts were stored for further use.

For aqueous extraction, the method by Safanah *et al.* (2012) was adapted. Air-dried leaves were soaked in water for 24 hrs, filtered, and centrifuged for 10 mins. The extracts then were dried in the oven at 40°C. The crude extracts were stored for further use. Three different concentrations (1000, 5000, and 10000 ppm) were prepared for each hexane, chloroform, methanol and aqueous *I. batatas* leaf extract.

# 2.2 Sampling of tested Pomacea canaliculata (golden apple snail)

The *P. canaliculata* were collected from Integrated Agriculture Development Area (IADA) Pekan, Pahang,

the snails were assorted into size class with the size range within 20-40 mm (Massaguni and Md Latip, 2012). The size of *P. canaliculata* was determined by the height of the shell. The snails were reared for 2-3 days in the laboratory and were fed with papaya leaves (Nur Suraya *et al.*, 2017).

#### 2.3 Molluscicidal assay test

The molluscicidal test was adapted from Musman (2010) and Reish and Oshida (1987) with a slight modification. A volume of 1 L paddy field water was poured into containers holding ten tested snails each. The tested snails were allowed to move freely for about 30 mins before 100 mL of I. batatas extracts were poured into the same containers. The mortality rate was observed every 24 hrs for 3 days. Mucus secretion (Musman, 2010), change in the shell colour and failure of the flesh portion to withdraw into the shell (Abdullahi et al., 2018), and the body hanging out of the shell (Massaguni and Md Latip, 2015) are signs that the snails have died. Three control tests were done where the P. canaliculata were treated with paddy field water added with dimethyl sulfoxide (DMSO) and methanol (1:1) (control test for hexane and chloroform extracts), 50% methanol (control for methanol extract) and distilled water (control for aqueous extract).

#### 2.4 Phytochemical screening

Phytochemical screening to determine the presence of alkaloids, flavonoids, glycosides, saponins, tannins, and terpenoids was performed using the methods described by Rauf et al. (2012) and Ayoola et al. (2008). To test for alkaloids, a 0.2 g sample of the extract was diluted with 2% H<sub>2</sub>SO<sub>4</sub> and then added with a few drops of Mayer's reagent. The appearance of a reddish-brown colour indicates the presence of alkaloids. To test for glycosides, a 0.2 g sample of the extract was hydrolysed with hydrochloric acid (HCl) and then neutralised with sodium hydroxide (NaOH) solution. After that, a few drops of Fehling's A and B solutions were added. A red precipitate is formed if glycoside compounds are present. To test for flavonoids, NaOH (1 mL) was added into 0.2 g extracts and the mixture then was added with a few drops of HCl. The presence of flavonoids is indicated by a change from a yellowish colour to colourless. To test for saponins, a 0.2 g sample of the extract was added into 2 mL distilled water in a test tube. The solution was shaken vigorously and observed for stable persistent bubbles. The bubbles' presence indicates the presence of saponins. To test for tannins, a small amount of the extract was diluted using distilled water and heated to boiling point using a water bath. The solution was then filtered and then added with a few drops of ferric

chloride. The appearance of a dark green colour indicates the presence of tannins. To test for terpenoids, a 0.25 g sample of the extract was added to 1 mL of chloroform. Concentrated  $H_2SO_4$  (1.5 mL) was carefully added to form a layer. A reddish-brown colouration of the interface indicates the presence of terpenoids.

# 2.5 Lethal concentration 50 (LC<sub>50</sub>)

Lethal concentration  $(LC_{50})$  was evaluated by means of Probit Analysis (Finney, 1971). Probit against log10 concentration was plotted and the concentration to the response ratio was determined.

## 3. Results and discussion

Four different *I. batatas* leaf extracts (hexane, chloroform, methanol, and aqueous extracts) with three different concentrations were evaluated for their molluscicidal activity against *P. canaliculata*. All extracts showed a positive relationship between the concentration and the golden apple snail mortality, suggesting that all extracts have molluscicidal properties.

Table 1 shows the molluscicidal activity of *I. batatas* leaf hexane extracts against P. canaliculata. After being exposed for 24 hrs, each concentration only shows between 10-20% mortality rate. The mortality rate increases with increased exposure time. This is in agreement with Abdullahi et al. (2018) and Musri (2010) who found that the molluscicidal properties of selected plant extracts are time and concentration-dependent. The highest mortality rate of golden apple snail is recorded at 10,000 ppm where the mortality rate is 70% after 72 hrs exposure. The lowest mortality rate is recorded at the negative control test (20%) after 72 hrs of exposure. The result indicates that the I. batatas hexane leaf extracts have a molluscicidal effect against P. canaliculata as shown by the increase in mortality rate with increasing concentration. This result is aligned with the finding by Musman et al. (2013) which showed the decreasing hatchability of P. canaliculata eggs with increasing concentration of B. racemosa extracts.

Table 1. Molluscicidal activity of *I. batatas* leaves hexane extracts *P. canaliculata* 

| Treatment Concentration | Percentage of Mortality (%) |                 |                  |
|-------------------------|-----------------------------|-----------------|------------------|
|                         | 24 hrs                      | 48 hrs          | 72 hrs           |
| 1000 ppm                | 10 <sup>a</sup>             | 20 <sup>a</sup> | 50 <sup>ab</sup> |
| 5000 ppm                | 20 <sup>a</sup>             | 20 <sup>a</sup> | $60^{ab}$        |
| 10000 ppm               | 20 <sup>a</sup>             | 30 <sup>a</sup> | 70 <sup>b</sup>  |
| Negative control        | $10^{a}$                    | 20 <sup>a</sup> | 20 <sup>a</sup>  |

\*\*Mean followed by the same letter along the column are not significantly difference at P > 0.05

I. batatas leaf chloroform extracts show good

molluscicidal activity against P. canaliculata especially at the 10,000 ppm concentration (Table 2). A previous study by Comia et al. (2018) suggested the same pattern in the result. This may be due to the presence of saponins, tannins, and anthraquinones. All concentrations show 50% to 100% mortality rates after being exposed to the treatment for 72 hrs. The treatment at a concentration of 1000 ppm has a 50% mortality rate, while 5000 and 10000 ppm show 70% and 100% mortality rates, respectively. For the negative control, a mortality rate of less than 50% is recorded (after 72 hrs). There is a significant difference in mortality rate between the negative control test and 10,000 ppm treatment concentration test at each recorded time. However, the mortality rate for negative control and 1000 ppm treatment concentration shows no significant difference at the exposures of 24 hrs, 48 hrs and 72 hrs. The varying results reflect the different amounts or types of active compounds present in the different concentrations. The results also reveal the linear relationship between concentration and mortality rate as shown in Figure 1. A similar result was reported by Prabhakaran et al. (2017) found a linear relationship between the who concentrations of six plant extracts (Azadirachta indica, Nicotiana tabacum, Nerium indicum, Pongamia pinnata, Zingiber officinale, and Piper nigrum) and the mortality

Table 2. Molluscicidal activity of *I. batatas* leaves chloroform extracts against *P. canaliculata* 

of Pomacea maculata.

| Treatment Concentration | Percentage of Mortality (%) |                  |                  |
|-------------------------|-----------------------------|------------------|------------------|
|                         | 24 hrs                      | 48 hrs           | 72 hrs           |
| 1000 ppm                | 20 <sup>a</sup>             | 30 <sup>a</sup>  | 50 <sup>ab</sup> |
| 5000 ppm                | $40^{ab}$                   | 50 <sup>ab</sup> | 70 <sup>b</sup>  |
| 10000 ppm               | 70 <sup>b</sup>             | 90 <sup>b</sup>  | 100 <sup>b</sup> |
| Negative control        | $10^{a}$                    | 20 <sup>a</sup>  | $20^{a}$         |

\*\*Mean followed by the same letter along the column are not significantly difference at P > 0.05

Based on the result in Table 3, after being exposed to the treatments for 24 hrs, 30% of the tested snails show the signs of death. The number of dead snails increases with an increased period of exposure to the treatment. *I. batatas* leaf methanol extracts at 10,000 ppm concentration promise good molluscicidal activity as they take only 48 hrs to show a 100% mortality rate. The methanol extract of *I. batatas* leaf extract was found to contain tannins, saponins, and flavonoid that contributed to these results (Anthoney and Omwenga, 2014). Anwar and Przybylski (2012) also found that the use of a polar solvent enables a significant amount of phenolics and flavonoid.

The result of the molluscicidal assay test (Table 4) shows that *I. batatas* leaf aqueous extracts possess good

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 Table 3. Molluscicidal activity of I. batatas leaves methanol

 extracts against P. canaliculata

| Treatment Concentration - | Percentage of Mortality (%) |                  |                  |
|---------------------------|-----------------------------|------------------|------------------|
|                           | 24 hrs                      | 48 hrs           | 72 hrs           |
| 1000 ppm                  | 30 <sup>a</sup>             | 50 <sup>ab</sup> | 70 <sup>b</sup>  |
| 5000 ppm                  | 30 <sup>a</sup>             | 80 <sup>b</sup>  | 100 <sup>b</sup> |
| 10000 ppm                 | 30 <sup>a</sup>             | 100 <sup>b</sup> | 100 <sup>b</sup> |
| Negative control          | 30 <sup>a</sup>             | 30 <sup>a</sup>  | $40^{a}$         |

 Table 4. Molluscicidal activity of I. batatas leaves aqueous

 extracts against P. canaliculata

| Treatment Concentration - | Percentage of Mortality (%) |                  |                 |
|---------------------------|-----------------------------|------------------|-----------------|
|                           | 24 hrs                      | 48 hrs           | 72 hrs          |
| 1000 ppm                  | $0^{\mathrm{a}}$            | $0^{\mathrm{a}}$ | $60^{ab}$       |
| 5000 ppm                  | 10 <sup>a</sup>             | 30 <sup>a</sup>  | $60^{ab}$       |
| 10000 ppm                 | 20 <sup>a</sup>             | 30 <sup>a</sup>  | 70 <sup>b</sup> |
| Negative control          | $0^{\mathrm{a}}$            | $0^{a}$          | 30 <sup>a</sup> |

\*\*Mean followed by the same letter along the column are not significantly difference at P > 0.05





significantly difference at P > 0.05

\*\*Mean followed by the same letter along the column are not



Figure 1. Molluscicidal activity of different *I. batatas* leaves extracts (hexane, chloroform, methanol and aqueous) against *P. canaliculata* 

molluscicidal activity as more than 50% mortality rate is observed for each treatment after 72 hrs. The mortality rates recorded after 72 hrs of exposure are 60% (1000 ppm), 60% (5000 ppm), 70% (10,000 ppm), and 30% (negative control). For negative control, there is no mortality rate recorded for the first (24 hrs) and second (48 hrs) readings. However, a 30% mortality rate is recorded after 72 hrs. A previous study by Liang *et al.* (2013) proved that the deaths are caused by environmental stress. An environment of stagnant water and warmer temperature can result in stress in the tested snails.

Plants are believed to have been commercialised as biopesticides because they contain various secondary metabolites that show superior anti-pest activities (Noorshilawati *et al.*, 2015). *I. batatas* have been proven to show a broad spectrum of larvicidal activity (Bharathi *et al.*, 2017). The phytochemical screening indicated that *I. batatas* leaf extract contains active compounds like flavonoids, saponins, tannins, and terpenoids (Table 5) which play an important role in controlling the snails' population (Noorshilawati *et al.*, 2018). This finding is in agreement with the finding by Picardal *et al.* (2018).

Flavonoids and saponins found in *I. batatas* may act synergistically in controlling *P. canaliculata* (Comia *et* 

al., 2018). A study by Koncic et al. (2013) also found a significant amount of phenols and flavonoids which can increase the rate of saponin absorption into the snail's hepatic cells and thus lead to death. Souza et al. (2013) revealed that flavonoids can inhibit the snail's detoxification system and allow saponins to induce greater toxicity. Saponins demonstrate the molluscicidal activity by disturbing the feeding and growth of the pest. The finding by Musman (2010) also support the molluscicidal effect of flavonoids and saponins where the mortality rate of the tested *P. canaliculata* is higher in extracts containing both flavonoids and saponins compared to the extracts containing only flavonoids. Taguiling (2015) also found that saponins exhibit pesticidal activity. Saponins also could lower the surface tension of water and block the breathing process (Musman, 2010).

Table 5. Phytochemical screening result

| Phytochemical Test | Result       |
|--------------------|--------------|
| Alkaloids          | Negative (-) |
| Flavonoids         | Positive (+) |
| Glycosides         | Negative (-) |
| Saponins           | Positive (+) |
| Tannins            | Positive (+) |
| Terpenoids         | Positive (+) |

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Figure 2. Lethal concentration (LC50) of different I. batatas leaves extracts

Meanwhile, the presence of tannins in the phytochemical screening shows a direct toxicity effect that leads to the mortality rate of *P. canaliculata*. Tannins are endogenous inhibitors of growth for numerous species of pests. Besides being good for pesticidal activity, tannins also can be used as bactericides (Vu *et al.*, 2017). Terpenoids screened in *I. batatas* leaf extracts also play an important role in plant defence against pests. Terpenoids' toxicity is by acting as an antifeedant and growth disruptor (Castilhos *et al.*, 2017).

Based on the probit analysis of  $LC_{50}$  (Figure 2), to control 50% of the golden apple snail population, about 1639 ppm *Ipomoea batatas* leaf hexane extracts are suggested for application. While the suggested concentration for *Ipomoea batatas* leaf chloroform, methanol, and aqueous extracts are about 6709 ppm, 4789 ppm, and 37549 ppm, respectively (Table 6).

Table 6. Lethal concentration 50 value of *Ipomoea batatas* leaves extracts with 95% confidence interval

| Extracts   | Suggested Concentration |
|------------|-------------------------|
| Hexane     | 1639 ppm                |
| Chloroform | 6709 ppm                |
| Methanol   | 4789 ppm                |
| Aqueous    | 37549 ppm               |

#### 4. Conclusion

*I. batatas* leaf extracts have potency in controlling golden apple snails especially *I. batatas* methanol leaf

extracts and can be used as alternative molluscicides. Therefore, the use of *I. batatas* extracts as molluscicides are highly recommended because they are environmentally safe, toxicological friendly, easy to use, and have a wide range of insecticidal activity. In addition, it is suggested that this study should be conducted on the paddy field to investigate the environmental effect caused by the *I. batatas* leaf extracts.

#### **Conflict of interest**

The authors hereby declare no conflict of interest.

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