THE MOLLUSCICIDAL EFFECT OF THE STEM EXTRACTS OF *Tinospora crispa* IN CONTROLLING THE GOLDEN APPLE SNAIL *Pomacea canaliculata*

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Abstract

This study investigated the molluscicidal effect of the stem extracts of *Tinospora crispa* in controlling the golden apple snail *Pomacea canaliculata*. Extracts were prepared in four solvents, i.e., hexane, chloroform, methanol, and distilled water at three concentrations (1,000, 5,000, and 10,000 ppm) per solvent. The phytochemical contents of the extracts were qualitatively identified, and the lethal concentration (LC50) of the extracts for molluscicidal potential was determined using the probit analysis. The effect of *T. crispa* extracts on the snail was monitored for three days and the snail mortality was recorded every 24 h. The stem extract prepared in methanol at 10,000 ppm showed the highest molluscicidal effect with a mortality of 80% at 72 h. Phytochemicals identified in the stem extracts included alkaloids, flavonoids, saponin, tannin, and terpenoids. Based on the probit analysis, stem extracts of *T. crispa* prepared in methanol showed the lowest LC50 value of 3,428 ppm for molluscicidal potential and followed by extracts prepared in chloroform, hexane, and distilled water at 5,888, 14,771, and 14,993 ppm, respectively.

Keywords: Molluscicidal activity, mortality, phytochemicals, *Pomacea canaliculata*, *Tinospora crispa*

Abstrak

Kajian ini menilai kesan moluskisida ekstrak batang *Tinospora crispa* dalam mengawal siput gondang emas (*Pomacea canaliculata*). Ekstrak disediakan dengan empat jenis pelarut, iaitu heksana, kloroform, metanol dan air suling dalam tiga kepekatan untuk setiap pelarut (1,000, 5,000 dan 10,000 ppm). Kandungan fitokimia ekstrak ditentukan secara kualitatif. Kepekatan LC50 untuk potensi moluskisida ekstrak dianggarkan berdasarkan kaedah analisa probit. Kesan ekstrak terhadap siput gondang emas dipantau selama tiga hari dan kematian direkodkan setiap 24 jam. Ekstrak batang disediakan dengan metanol pada kepekatan 10,000 ppm memperlihatkan kematian tertinggi, iaitu 80% pada jam 72. Fitokimia yang dikenal pasti dalam batang ekstrak termasuk alkalioid, flavonoid, saponin, tanin dan terpenoid. Berdasarkan analisa probit, ekstrak batang *T. crispa* yang disediakan daripada metanol menunjukkan nilai LC50 yang paling rendah untuk moluskisida, iaitu 3,428 ppm dan dilihat oleh ekstrak kloroform, hexan, dan air suling pada 5,888, 14,771, dan 14,993 ppm masing-masing.

Kata kunci: Aktiviti moluskisida, kematian, fitokimia, *Pomacea canaliculata*, *Tinospora crispa*

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1.0 INTRODUCTION

The cultivation of paddy in Malaysia suffers from the attack of a major pest, i.e., the golden apple snail, Pomacea canaliculata. The snail was brought from Argentina to Taiwan in the 1980s as food for locals and exportation. However, the demand for the snail was low and thus cause its farming activity was discontinued and the snail manged to flee to the paddy fields [1]. Eventually, the pest spread to Malaysia, Indonesia, Thailand, the Philippines, and other Asian countries, where it would destroy 7 to 24 rice seedlings per day [2]. It usually attacks the young paddy stems and leaves. In particular, individuals of 40 mm and above are the most damaging ones; they could completely damage the paddy seedlings at the growing stage and 20% of the transplanted seedlings [3].

Various methods such as handpicking, biological control (using ducks), and the use of chemicals had been employed to control the infestation of P. canaliculata [4]. In particular, chemicals such as niclosamide and metaldehyde are widely used due to their rapid actions and high efficacy. However, the extensive use of chemicals and pesticides would pollute the waterways and the ecosystem [5]. Consequently, the use of natural plant molluscicides is on the rise as they are considered more ecologically friendly [6]. In this respect, the stem extracts of patawali Tinospora crispa (Family: Menispermacea) had been reported to be effective in controlling P. canaliculata [7, 8, 9, 10, 11, 12]. The present study investigated the molluscicidal effect of the stem extracts of T. crispa in controlling the golden apple snail. The phytochemical contents of the extracts were qualitatively identified, and the lethal concentration (LC50) of the extracts for molluscicidal potential was determined. The study would contribute towards sustainable development by reducing the dependence on chemicals and pesticides in the pest management of paddy cultivation.

2.0 METHODOLOGY

2.1 Preparation of the Stem Extract of Tinospora crispa

Stem extracts of T. crispa were prepared in four solvents, i.e., hexane, chloroform, methanol, and the distilled water. Extractions in organic solvents generally followed the method of Aiza et al. [13], in which 200 g dried powder of T. crispa stem was soaked in 500 ml of each hexane, chloroform, and methanol for three weeks. The maceration of each sample (three extracts) was then filtered with Whatman No. 1 filter paper then evaporated using a rotary evaporator to yield crude extracts. They were stored at 4 °C until analysis. Meanwhile, the aqueous extraction of T. crispa stem was generally based on the method of Safanah et al. [14], in which the dried powder of T. crispa stem (200 g) was impregnated with water for 24 h, filtered, centrifuged (6,000 rpm, room temperature, 10 min), and dried in an oven (20 °C) for 2 h. The crude aqueous extract was stored at 4 °C until analysis.

2.2 Sampling of P. canaliculata

A total of 200 adult snails (18 – 25 mm) were collected from the paddy field in Pekan, Pahang, Malaysia. They were acclimatized to the laboratory environment (25°C) for three days and fed with papaya leaves [15, 16], after which snails were randomly selected for the molluscicidal study.

2.3 Molluscicidal Study

The molluscicidal study via immersion generally followed the method of Zhang & Zou [17]. Suspensions comprising the extract of T. crispa stem and solvent were prepared at three concentrations, i.e., 1,000, 5,000, and 10,000 ppm for each solvent with a total of 12 samples or experiments (three concentrations x four extracting solvents). For each experiment, ten adult snails (18 – 25 mm) were placed into a plastic tank (20 cm X 10 cm X 20 cm) containing 1L paddy-field water for 30 minutes, and then 100 ml suspension (extract of T. crispa stem with different concentrations of solvents) was added into the corresponding tank.

Also, a negative control was prepared for each solvent; each of these four tanks was similarly filled with 1 L paddy-field water and 100 ml control content to treat the same number of snails. Specifically, the 100-ml control content for the aqueous extract of T. crispa stem consisted of distilled water, whereas a mixture of dimethyl sulfoxide (DMSO) and methanol at a ratio of 1:1 served as the control content for the T. crispa stem extracted from hexane and chloroform, and a 50% methanol was used in the control for the T. crispa stem extracted from methanol.

The snail mortality of each tank (16 tanks in total) was recorded every 24 h (3 times). The bodies came out of the shell with the secretion of mucus and a change in the shell colour from light yellowish to discolored is the sign of the snails’ death [5, 18]. Dead snails were removed from the tanks to prevent contamination. Meanwhile, the lethal concentration (LC50), was evaluated using the probit analysis and the lethal concentration was calculated using the method of Noorshilawati et al. [19].

2.4 Qualitative Phytochemical Testing for the Stem Extract of T. crispa

Phytochemicals such as tannins, saponins, flavonoids, alkaloids, terpenoids and glycosides were qualitatively screened using modified methods from Bhandary et al., Farhat et al., Rauf et al., and
Wadood et al. [20, 21, 22, 23]. Tannins were detected by boiling 0.2 g extract in 10 ml of distilled water and filtered. The filtrate then added with ferric chloride solution (a few drops). The formation of a dark green solution would indicate the presence of tannins. For the determination of saponins, for each extract, 0.2 g extract was added into a test tube containing 2 ml distilled water and then vigorously shaken. The test tube was allowed to react for 20 min (room temperature). The formation of foam would indicate the presence of saponins.

Also, the flavonoid was tested by adding 10 ml distilled water to each crude extract, followed by 5 ml diluted ammonia solution and 1 mL concentrated H2SO4. The presence of flavonoids would be indicated by the appearance of a yellow coloration. Alkaloids were tested by adding Mayer’s reagent (a few drops) into the filtrate derived from the filtration of 0.2 g extract (each of all the four solvents) diluted in 10 ml of 2% H2SO4. Reddish-brown precipitation would indicate the presence of alkaloids.

On the other hand, terpenoid was identified by adding 0.2 g extract to 1 ml chloroform followed by the gentle addition of 1.5 ml concentrated H2SO4 to form a layer. The formation of a reddish-brown colouration on the surface would show the presence of terpenoids. Finally, the content of glycosides in extracts was examined by hydrolysing 0.2 g crude extracts with hydrochloric acid (HCl) followed by neutralisation with sodium hydroxide (NaOH) and the addition of a few drops of Fehling’s A and B solutions with gentle boiling. The formation of reddish-brown precipitation would indicate the presence of glycoside compounds.

2.5 Statistical Analysis

Differences in the relative mortality among various extracts were analysed by the analysis of variance (ANOVA) at the significance level (alpha) of 0.05 using the software Statistical Package for the Social Sciences (version 23, IBM). Mean comparisons were performed using Duncan New Multiple Range Test (DNMRT) at p < 0.05. The Pearson correlation analysis was used to examine the relationship among the variables.

3.0 RESULTS AND DISCUSSION

Table 1 shows the relative mortality of the snails treated with T. crispa extracted from hexane, in which 10% of snails died after 24 h for each treatment. For the control test, no snails died after 24 h through the end of the molluscidical study. After 72 h, no significant differences recorded between P. canaliculata mortality for the experiments of 1,000 ppm, 5,000 ppm, and 10,000 ppm. However, all the concentration showed significant differences when compared with the negative control. All treatments showed a molluscidicidal effect on the snails. However, because the mortality for all treatments was less than 50% even at 72 h, the molluscidical effect was considered weak.

Table 1 The relative mortality of P. canaliculata exposed to T. crispa extracted from hexane

| Concentration | Relative mortality (%) |
|---------------|------------------------|
|               | 24 h | 48 h | 72 h |
| 1,000 ppm     | 10\(^\circ\) | 10\(^\circ\) | 30\(^\circ\) |
| 5,000 ppm     | 10\(^\circ\) | 20\(^{ab}\) | 30\(^{ab}\) |
| 10,000 ppm    | 10\(^\circ\) | 30\(^{a}\) | 40\(^{a}\) |
| Negative control | 10\(^\circ\) | 10\(^\circ\) | 10\(^\circ\) |

*Within the same column, different letters in superscripts show significant differences at P < 0.05.

Table 2 shows the relative mortality of the snails treated with T. crispa extracted from chloroform. For the first 24 h, only 10% mortality was recorded for 1,000 ppm and 10,000 ppm, and no snails died in 5,000 ppm and the negative control (Table 2). The highest mortality of snails was recorded in 10,000 ppm T. crispa stem extract at 72 h, i.e., 70% and this mortality differed substantially from that of the negative control (10%). The molluscidical effect of T. crispa stem extracts might be caused by the presence of saponin, tannin, and alkaloid, which were soluble in chloroform and hence extracted. In particular, saponin would disrupt the cell membrane and thus highly toxic to cold-blooded animals [24, 25].

Table 2 The relative mortality of P. canaliculata exposed to T. crispa extracted from chloroform

| Concentration | Relative mortality (%) |
|---------------|------------------------|
|               | 24 h | 48 h | 72 h |
| 1,000 ppm     | 10\(^\circ\) | 20\(^{ab}\) | 30\(^{ab}\) |
| 5,000 ppm     | 0\(^\circ\) | 10\(^{a}\) | 40\(^{a}\) |
| 10,000 ppm    | 10\(^\circ\) | 30\(^{ab}\) | 70\(^{a}\) |
| Negative control | 0\(^\circ\) | 10\(^\circ\) | 10\(^\circ\) |

*Within the same column, different letters in superscripts show significant differences at P < 0.05.

Table 3 shows the relative mortality of the snails treated with T. crispa extracted from methanol. All treatments showed molluscidical activity. In particular, in 10,000 ppm the T. crispa extracted from methanol showed the most potent molluscidical effect at 72 h, i.e., 80% mortality, among other solvent extracts. This result agreed with that of other studies [26, 27, 28]. Phytochemicals extracted with methanol generally contain chemical compounds that possess pesticide effects. Due to the presence of saponin and flavonoids, methanol extracts would be toxic to snails [26]. Besides, T. crispa extracted from methanol was once used as an insecticidal agent against the diamondback moth by reducing its feeding activity [27]. For 10,000 ppm, at 24 h after treatment, 20% of the snails died, but the mortality drastically increased to 80% at 72 h. A similar finding was also reported by Comia et al. [28], and they found that the presence of saponins, tannins, and

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anthraquinones contributed to the molluscidal activity.

Table 3 The relative mortality of P. canaliculata exposed to T. crispa extracted from methanol

| Concentration | Relative mortality (%) |
|---------------|------------------------|
|               | 24 h | 48 h | 72 h |
| 1,000 ppm     | 20^a | 40^ab| 40^ab|
| 5,000 ppm     | 10^a | 20^a | 50^ab|
| 10,000 ppm    | 20^a | 40^ab| 80^a |
| Negative control | 10^a | 10^a | 20^a |

*Within the same column, different letters in superscripts show significant differences at P < 0.05.

Table 4 shows the relative mortality of the snails treated with T. crispa aqueous extract, in which the negative control recorded the lowest mortality (10%) at 72 h, and the highest mortality (50%) was attained at 10,000 ppm. This result was consistent with the findings of Somsak et al. [29] and Zulkhairi et al. [12], and they found that a higher concentration of T. crispa aqueous extract (500 mg/kg) decreased the parasitemia in infected mice. This study also showed that extensive exposure to T. crispa treatment in high concentration (5,000 and 10,000 ppm) increased the mortality of the snails, and this result agreed with the finding of Abdullahi et al. [30], Li & Zou [16] and Musri [24], which concluded that the molluscidal activity of phytochemical would depend on the treatment’s concentration and the duration of the treatment.

Table 4 The relative mortality of P. canaliculata exposed to aqueous extracts of T. crispa stem

| Concentration | Relative mortality (%) |
|---------------|------------------------|
|               | 24 h | 48 h | 72 h |
| 1,000 ppm     | 10^a | 20^ab| 20^ab|
| 5,000 ppm     | 20^a | 20^a | 30^ab|
| 10,000 ppm    | 10^a | 30^ab| 50^a |
| Negative control | 0^a | 10^a | 10^a |

*Within the same column, different letters in superscripts show significant differences at P < 0.05.

Table 5 shows the phytochemicals identified in various extracts of T. crispa stem via qualitative tests. For methanol extracts, five phytochemicals were identified in this study, i.e., alkaloid, flavonoids, saponins, tannins, and terpenoids. These active compounds would likely play an important role in the pest management of snails in the cultivation of paddy as they could fatally disrupt the nervous system of snails [34]. Incidentally, Shaki et al. [35] also detected tannin, alkaloid, flavonoid, and polyphenol substances from the T. crispa stem extracted from methanol, while Noor & Ashcroft [36] found saponin and alkaloid in the T. crispa stem extracted from methanol.

Table 5 Phytochemicals identified in the extracts of T. crispa stem via qualitative tests

| Compound | Hexane | Chloroform | Methanol | Aqueous |
|----------|--------|------------|----------|---------|
| Tannins  | -      | +          | +        | -       |
| Saponin  | -      | +          | +        | -       |
| Flavonoid| +      | -          | +        | +       |
| Alkaloid | +      | +          | +        | -       |
| Terpenoid| -      | -          | +        | -       |
| Glycoside| -      | -          | -        | -       |

** present - absent

The varying outcome of molluscidal activity appeared to be related to different quantities and types of compounds in various extracts. In general, polar solvent (e.g., methanol) would extract a great number, as well as a higher concentration, of flavonoids and phenolic compounds [31] than nonpolar solvents (e.g., chloroform and hexane). In this respect, chloroform, due to its relatively higher polarity, performed better than hexane in terms of exerting effectiveness of molluscidal activity when used to prepare extracts of T. crispa stem (Tables 2 & 3). Overall, negative controls in all the solvents showed 10 to 20% mortality of P. canaliculata at 72 h, and the deaths were likely due to environmental stress that resulted from warm and stagnant water [4, 32]. Together, T. crispa appeared to be a good molluscicide [33].

For chloroform extract, three phytochemicals were identified in this study, i.e., alkaloid, saponins, and tannins. This finding was in general agreement with that of Shakirah et al. [35], who also detected alkaloid and triterpenoid types of saponins in the T. crispa stem extracted from chloroform. For hexane extracts, only two compounds were detected, i.e., flavonoids and alkaloids. Fewer compounds in the T. crispa stem were extracted from hexane might have contributed to their weak molluscidal activities (Table 1). However, this finding differed somewhat from that of Shakirah et al. [35], who also detected polyphenol in the T. crispa stem extracted besides alkaloid and flavonoid substances. The reason for such a difference remained unclear. Also, only two compounds were detected in the aqueous extract of T. crispa stem, namely; flavonoids and saponin.

Different active compounds may interact with each other to enhance the molluscidal activity [37]. Musman [5] reported that extracts containing both flavonoid and saponins exerted a high mortality effect on P. canaliculata as compared to extracts containing only flavonoids. Prabhakaran et al. [38] found that the combinations of neem, tobacco and Nerium (1:1:1) and tobacco, piper, and nerium (1:1:1) and were effective in controlling Pomacea maculata with LC50 191.52 mg/L and 180.35 mg/L. Keshav et al. [39] in their study found that the toxicity of the mixture of Azadirachta indica oils, Embelia ribens and Cedrus deodara against Lymnaea
acuminata and Indoplanorbis exustus is 10 times more active than the toxicity of only two, Azadirachta indica oils and Cedrus deodara. Meanwhile, they found Embelia ribens itself did not show any sign of toxicity. The combination of custard apple seed powder (Annona squamosa), neem oil (Azadirachta indica) and cedar oil (Cedrus deodara) have high molluscicidal activity in controlling Lymnaea acuminata than the individual plants [40]. Molusicides from the combination of Allium sativum and Cedrus deodara and the combination of Cedrus deodara and Azadirachta indica oil can disturb the life cycle of African snail (Achatina fulica) [41].

Table 6 shows the LC50 values of the T. crispa stem extracts calculated from the probit analysis. Extracts from methanol had the most potent molluscicidal effect with an LC50 value of 3,428 ppm. Thus, the suggested concentrations for T. crispa stem extracted from hexane, chloroform, and aqueous extracts to control 50% of the P. canaliculata population were 14,771 ppm, 5,888 ppm, and 14,993 ppm, respectively (Table 6).

Table 6 The lethal concentration (LC50) of T. crispa stem extracts for molluscicidal potential, which was estimated based on the probit analysis

| Extract   | Suggested concentration |
|-----------|-------------------------|
| Hexane    | 14,771 ppm              |
| Chloroform| 5,888 ppm               |
| Methanol  | 3,428 ppm               |
| Aqueous   | 14,993 ppm              |

4.0 CONCLUSION

Extracts of T. crispa stem were found to have molluscicidal potential. Based on the probit analysis, stem extracts of T. crispa prepared in methanol showed the lowest LC50 value of 3,428 ppm for molluscicidal potential followed by extracts prepared in chloroform, hexane, and distilled water at 5,888, 14,771, and 14,993 ppm, respectively. Further studies would be essential to screen new biopesticide with high efficiency and low toxicity for the pest management of paddy cultivation.

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