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MOLLUSCICIDAL ACTIVITY OF *Entada rheedii* STEM BARK METHANOLIC EXTRACT AGAINST PADDY PEST *Pomacea canaliculata* (GOLDEN APPLE SNAIL)

(Aktiviti Moluskisida oleh Ekstrak Metanol Kulit Batang *Entada rheedii* Terhadap Perosak Padi *Pomacea canaliculata* (Siput Gondang Emas))

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Abstract

The study was conducted to evaluate the molluscicidal activity of *E. rheedii* methanol bark extract against *P. canaliculata* and to screen for phytochemical compounds of *E. rheedii* bark extracts. The golden apple snails with size range of 20 – 40 mm were treated with four different concentrations of *E. rheedii* (1000, 5000, 10000 and 20000 ppm) and paddy-field water mix with 50% methanol serving as the control treatment. The molluscicidal effects of the extract were evaluated after 24, 48 and 72 hours. The results of the study showed that high treatment concentrations (10000 and 20000 ppm) recorded the highest mortality rate (100%) while low concentrations (1000 ppm) showed the lowest mortality rate (27%). However, no mortality was recorded in the control treatment. The molluscicides activity with LC_{50} is 1,611 ppm and LC_{90} is 4,266 ppm and could be attributed to the presence of saponin in the bark extracts. *E. rheedii* bark extract provides a great potential for developing green pesticides to control *P. canaliculata*. Nevertheless, further research is needed to determine its biochemical mechanism.

Keywords: golden apple snail, *Entada rheedii*, molluscicidal activity, phytochemical content

Abstrak

Kajian ini dijalankan untuk menilai aktiviti moluskisida oleh ekstrak metanol kulit kayu *E. rheedii* terhadap *P. canaliculata* dan untuk menyaring sebatian fitokimia yang terkandung di dalam ekstrak kulit kayu *E. rheedii.* Siput gondang emas bersaiz antara 20 – 40 mm telah dirawat dengan empat kepekatan *E. rheedii* yang berbeza (1000, 5000, 10000 dan 20000 ppm) dan campuran air sawah padi dengan 50% metanol menjadi rawatan kawalan. Kesan moluskisida oleh ekstrak dinilai selepas 24, 48 dan 72 jam. Keputusan kajian menunjukkan bahawa kepekatan rawatan tinggi (10000 dan 20000 ppm) mencatatkan kadar kematian tertinggi (100%) manakala kepekatan yang rendah (1000 ppm) menunjukkan kadar kematian yang paling rendah (27%). Walau bagaimanapun, tiada kematian direkodkan dalam rawatan kawalan. Aktiviti moluskisida dengan LC_{50} adalah 1611 ppm dan LC_{90} adalah 4266 ppm boleh dikaitkan dengan kehadiran saponin dalam ekstrak kulit kayu. Ekstrak kulit kayu *E. rheedii* mempunyai potensi yang besar dalam membangunkan racun perosak yang selamat terhadap alam sekitar untuk mengawal *P. canaliculata.* Walau bagaimanapun, penyelidikan lanjut perlu dilakukan untuk menentukan mekanisme biokimianya.

Kata kunci: siput gondang emas, *Entada rheedi*i, aktiviti moluskisida, kandungan fitokimia

Introduction

The golden apple snail, *Pomacea canaliculata* (*P. canaliculata*) is native to South America [1] and has been introduced from Argentina to Taiwan in the 1980's for the purpose of food commercialization. Nevertheless, due to the low response of consumers towards snails as food, it has affected the marketing and farming activities of golden apple snail and caused producers to close their farms. Hence, it was contributed to the establishment and distribution of the snail populations that became a major invasive rice pest in South-East Asia [2]. In Malaysia, *P. canaliculata* was first found in abandoned mines; Puchong, Selangor in 1991 [3] and then was introduced in Keningau, Sabah in 1992. Then, it was spread throughout Peninsular Malaysia and caused extensive damage to paddy field areas in the Northern parts of Malaysia especially in the state of Perlis and Kedah. The problem became more serious due to the climatic change, which influences the rapid distribution and spread of snails throughout irrigation areas [4].

P. canaliculata was known as the most destructive invasive species that attacked and destroyed the young stems and paddy leaves. It has been reported that the snails could eat about 7-24 rice seedlings per day. Although farmers can control the snail populations in their paddy field plots through the application of various types of chemical fertilizers, but the problem is ever increasing because of the continuous supply and snail distribution through the water irrigation system [4]. Many approaches including chemical, mechanical or biological methods have been applied to control the widespread of golden apple snails in the field plantation area but the practice may have positive and negative impacts to humans, the environment and the ecosystem. Previous studies reported that the usage of chemical fertilizers such as metaldehyde and niclosamide can cause water pollution and thus affect the ecosystem or water sources [5]. The uses of mechanical techniques such as rice-duck mutualism [2] and attractant bite using jackfruit, papaya, spinach, cassava leaves and banana leaves are also effective. Badrulhadza and Yahya [6] found that snails are more attracted to jackfruit and papayas, thus this method is also important as it could contribute to the integrated management of golden apple snails in field plantation areas. Besides that, the application of biological control and pesticides derived from extracts of seed, leaves, fruit or other plant parts also give significant impacts in the management of the snails. Leaves extracts of *Solanum* species [7], *Barringtonia racemosa* L. kernel extract [5], and extracts of *Agave filifera*, *Ammi majua* and *Canna indica* leaves and flowers [8] have been reported as effective in controlling snails in their plantation areas. The effectiveness of the plant extracts in controlling snails may be due to the presence of bio-active compounds such as saponins, flavanoids, and terpenoids that react with pest body system.

Entada rheedii (*E. rheedii*) Spreng or known as African Dream Herb is a woody climber of the legume family. This plant is found in Africa and South-East Asia [9,10]. Some chemical contents of *E. rheedii* are antigenic acids, fatty acids, entadamide A, B, and C, phaseoloidine, echynosystic type sapogenin, saponin, saponin III, triglycerides, triterpenes and triterpenoids can be found in all parts of *E. rheedii.* Saponins and heterosides can be found in the bark and seed parts. Besides that, *E. rheedii* gives antitumor effects and is toxic to molluscs. This may be due to the presence of saponin that could break the red blood cells and disrupt the haemolysis system in molluscs [11]. Hence, the present study was carried out to evaluate the potential use of *E. rheedii* stem bark extracts on *P. canaliculata.* The information generated from this research could be beneficial for further studies on the specific compounds that lead to the mortality of *P. canaliculata.*

Materials and Methods

Plant material

The selection of *E. rheedii* as tested plant in this study was based on the presence of phytochemical constituents such as saponin, which reportedly could lower the surface tension of water and restrict the breathing process of *P. canaliculata*. The plant species *E. rheedii* was collected from Kota Bharu, Kelantan. The effectiveness of the stem bark extract against *P. canaliculata* was assessed to determine the toxicity effects.

Preparation of *Entada rheedii* stem bark extracts

About 500 mg of *E. rheedii* stem bark was grounded into fine powder and then macerated with 2.3 L of methanol (MeOH) in a 3000-mL beaker at room temperature for 24 hours. The extract was filtered with filter paper, and then concentrated to dryness under reduced pressure in a rotary evaporator. The crude extract was stored in a labelled specimen jar.

Preparation of treatment concentrations
Four treatment concentrations consisting of 1000, 5000, 10000 and 20000 ppm were prepared from the crude extract of *E. rheedii* using methanol 50% (v/v) to determine the lethal death at 50% (LD_{50}) and lethal death at 90% (LD_{90}) values. Paddy-field water with 50% methanol served as the control treatment.

Sampling of tested golden apple snail
The golden apple snails with size range of 20 – 40 mm were collected from Pahang Tua Paddy Field in the Integrated Agriculture Development Area (IADA) Pekan, Pahang. The shell height was used for size determination in order to get a uniform size for the toxicity test in laboratory conditions [12]. Tested snails were acclimatized in the laboratory in four different plastic aquariums and fed with papaya leaves for seven days.

Molluscicidal activity
The molluscicidal activity was performed using the methods described by Reish and Oshida [13] with some modifications. Each treatment was triplicated. Paddy-field water with pH 6.78 taken from the location of the collected snails was filled in four plastic containers to a depth of seven centimetres measured from the bottom of the container. Ten test snails were placed in the plastic containers and were then covered with netting cloth to prevent them from crawling out during experiments. The test snails were allowed to move freely for about 30 minutes and 100 ml of the treatment concentration were then poured into plastic containers. The mortality of the golden apple snails was assessed every 24 hours of exposure for 3 days. The death of the snails and the toxicity effects were determined by mucus secretion through the operculum gap [5] and the lack of mobility or if the body is retracted well into or hanging out of the shell [14].

Phytochemical screening
Phytochemical screening was performed using the methods described by Rauf et al. [15] to determine the presence of glycoside, alkaloids, saponins, tannins, flavanoids and terpenoids.

Test for glycosides
The extract was hydrolyzed with hydrochloric acid (HCl) and neutralized with sodium hydroxide (NaOH) solution. A few drops of Fehling's A and B solutions were added and the formation of a red precipitate indicates the presence of glycoside compounds.

Test for alkaloids
An amount 0.2g of extract was added with 2% H_2SO_4 and warmed for two minutes. The solution was filtered and then added with few drops of Dragendorff's reagent. The appearance of orange red precipitation indicates the presence of alkaloids.

Test for saponins
An amount 0.2g of extract was added with 5ml distilled water and heated to boiling point. The appearance of creamy mist with small bubbles indicates the presence of saponins.

Test for tannins
A small amount of extract was mixed with distilled water, heated to boiling point on water bath and then filtered. A few drops of ferric chloride were added and the appearance of a dark green colour indicates the presence of tannins.

Test for flavonoids
An amount 0.2g of extract was dissolved in diluted NaOH, and added with few drops of HCl. The turn of a yellowish colour to colourless indicated the presence of flavanoids.

Test for terpenoids
An amount 0.2g of extract was mixed with 2 mL of chloroform ($CHCl_3$) then 3 mL of concentrated H_2SO_4 was added to form a layer. The layer with reddish brown colour shows the presence of terpenoids in extract.

Statistical analysis

The mean and standard errors were analyzed using analysis of variance (ANOVA) and Least Significant Difference (LSD) for pairwise comparison to determine the significant differences among each treatment. The concentration that could kill 50% (LC_{50}) and 90% (LC_{90}) of tested snails were determined from Finney's Table [16].

Results and Discussion

Mortality of *P.canaliculata* against *E.rheedii stem* bark methanolic extracts

The results of the study revealed that no mortality was found in the control treatment of the tested *P. canaliculata* after 72 hours of exposure. (Figure 1). The 100% mortality rate of *P. canaliculata* was observed in 20, 000 ppm MeOH extracts after 48 hours exposure to such conditions. A 100% mortality rate was also recorded in high concentrations of 10, 000 and 20, 000 ppm respectively after 72 hours exposure (Table 1). The response of the snails to the treatments was determined through mucus production (Figure 2). Brain et al. [17] stated that *P. canaliculata* response to the pesticides through secretion of mucus by means to reduce their contact to the molluscicides. In addition, the presence of saponins cause toxicity to snails and affect cell membrane and lower the surface tension.

Table 1. Response of *P. canaliculata* against different concentrations of *E. rheedii* stem bark methanolic extract after 24, 48 and 72 hours

Treatment Concentration (ppm)	Percentage of Mortality (%)		
	24 hours	48 hours	72 hours
0(Control)	0	0	0
1000	7	17	27
5000	27	87	93
10000	40	97	100
20000	47	100	100

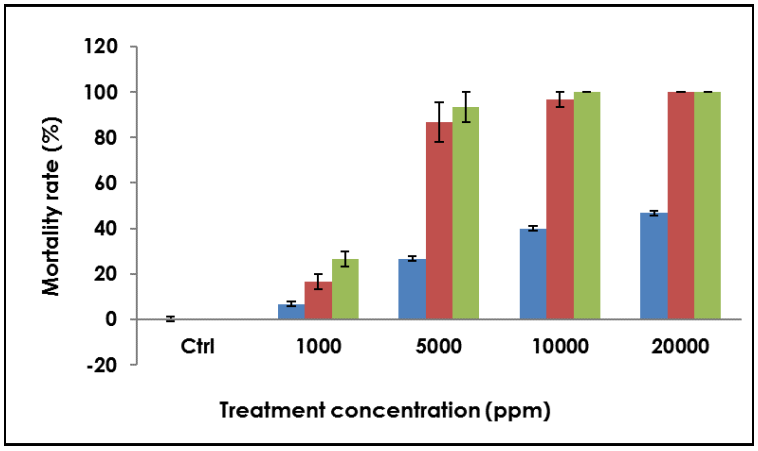

Figure 1. Mortality rate of *P. canaliculata* in control treatment and different concentrations of MeOH

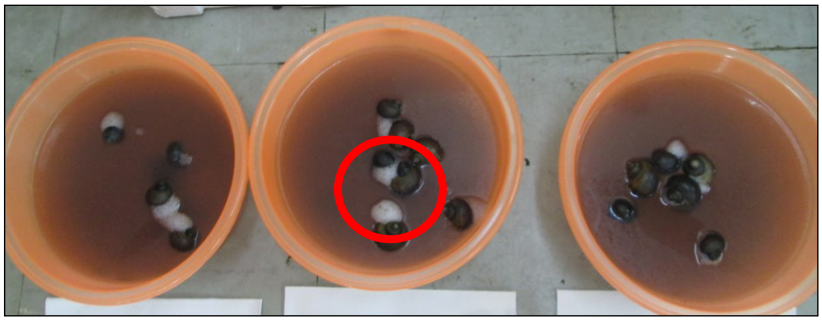

Figure 2. Mucus secretions in *P. canaliculata* during application of *E. rheedii* stem barks extract treatments

Phytochemical screening test (Table 2) showed the presence of bioactive compounds such as alkaloids, flavanoids, glycosides, saponins, tannins and terpenoids in the bark extract of *E. rheedii*. Previous studies reported that flavanoids and saponins both could block the process of breathing probably due to diffusion of oxygen through the gills of the golden apple snails which is then obstructed by the secretion of mucus [5]. Several plants with molluscicidal activity had been identified such as *Chenopodium ambrosioides* and *Ruta chalepensis* with secondary metabolites of flavanoids, triterpenes, saponins, and alkaloids [18]. Based on the probit analysis of LD_{50} and LD_{90}, in order to control 50% and 90% of the golden apple snail population, about 1611 ppm and 4266 ppm of *E. rheedii* bark crude extract concentration could be applied respectively.

Table 2. Phytochemical screening test of *E. rheedii* stem bark extract

Phytochemical Content	**Presence of Bioactives Compound in Methanolic Extract**
Alkaloids	-
Flavonoids	+
Glycosides	+
Saponins	+
Tannins	+
Terpenoids	+

Conclusion

The results of the study revealed that high concentrations of methanolic bark extracts of *E. rheedii* (10000 ppm and 20000 ppm) are able to kill 100% of tested *P. canaliculata* after 72 hours exposure. Phytochemical screening test also showed the presence of bioactive compounds such as saponins, tannins and terpenoids in the bark extract, which demonstrated the potential use of *E. rheedii* as a new source of green pesticides in controlling the population of golden apple snail in paddy field plantation areas. In addition, it is suggested to test the effectiveness of *E. rheedii* bark extract against juvenile stages of the golden apple snail. However, further studies are needed to develop its potential as green molluscicides.

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