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MOLLUSCICIDAL ACTIVITY OF THE PLANT Acacia mangium (Willd.) AGAINST THE SNAIL Pomaceae canaliculata (Lam.)

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ABSTRACT

The Golden Apple Snail (GAS) or *Pomacea canaliculata* as it is scientifically known, is a major pest of paddy. It can be managed by using cultural techniques, biological control and synthetic molluscicides. While bio-pesticide is an effective and safer alternative, economical mass production can be a constraint. In Sabah, the planting of Acacia mangium is an agroforestry initiative in the paper pulp industry, and efforts to recycle its industrial byproduct are suggested. Therefore this study aims to screen the beneficial phytochemical compounds of *Acacia mangium*, to test its molluscicidal activity, and to determine the effective dosage that can cause mortality on GAS. Phytochemical screenings of Acacia mangium methanol extract from the leaves, flower, and bark detected the presence of saponin, a class of chemical compound reported to be toxic to snails. The bark was particularly chosen due to its abundant availability as a processing byproduct. The molluscicidal activity was tested using dip bio-assay experiment. The test solution was prepared by mixing ground Acacia mangium bark with distilled water and the concentration was adjusted to 25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml respectively. Furcraea selloa var. marginata, a plant that has been reported to be toxic to GAS was used as positive control and prepared with similar concentrations while distilled water was used as negative control. The Acacia mangium aqueous extract recorded 100% snail mortality starting from the concentration of 50 mg/ml within 24 hours, similar to the Furcraea selloa var. marginata aqueous extract. Meanwhile, no mortality was observed in the negative control solution. The lethal concentration (LC₅₀) of Acacia mangium and Furcraea selloa var. marginata was identified at 25 mg/ml and 24 mg/ml respectively. The results from this study have confirmed the molluscicide effectiveness of *Acacia mangium* on GAS.

Keywords: Acacia mangium; Pomacea canaliculata; saponins; dip bio-assay

ABSTRAK

Siput gondang emas (SGE) atau nama saintifiknya *Pomacea canaliculata* adalah musuh utama tanaman padi. Siput ini boleh dikawal dengan kaedah kawalan kultura, kawalan biologi, dan racun siput sintetik. Pengunaan racun perosak biologi adalah pilihan yang selamat dan berkesan. Walaubagaimanapun, penghasilan racun perosak biologi secara besar-besaran belum dapat dilaksanakan. *Acacia mangium* adalah inisiatif perladangan hutani untuk industri pulpa dan kertas di Sabah. Usaha untuk mengitar semula sisa perindustrian telah dicadangkan, maka kajian ini dijalankan untuk mengesan kehadiran bahan kimia baik dari pokok *Acacia mangium*, menguji aktiviti racun siput, dan mendapatkan dos yang paling berkesan untuk membunuh SGE. Ujian fitokimia telah mengesan kehadiran saponin iaitu sejenis bahan kimia tanaman yang telah dilaporkan toksik terhadap siput. Kulit kayu *Acacia mangium* telah dipilih sebagai bahan kajian kerana ianya boleh diperolehi dengan banyak hasil dari sisa perindustrian. Kehadiran aktiviti racun siput diuji dengan kaedah rendaman. Bahan rawatan disediakan dengan mencampurkan kulit kayu *Acacia mangium* yang telah dihancurkan dengan air suling dan disediakan pada kepekatan 25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml. *Furcraea selloa* var. *marginata*, sejenis tumbuhan yang telah dibuktikan toksik kepada SGE digunakan sebagai kawalan positif

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dan disediakan pada kepekatan yang sama manakala kawalan negatif adalah menggunakan air suling. Ekstrak basah *Acacia mangium* didapati mencatatkan peratusan kematian siput sebanyak 100% pada kepekatan 50 mg/ml dalam masa 24 jam dan *Furcraea selloa* var. *marginata* juga memberikan keputusan yang sama. Tidak ada siput yang mati pada rawatan kawalan negatif. Kepekatan maut (LC₅₀) *Acacia mangium* dan *Furcraea selloa* var. *marginata* dicatatkan pada kepekatan 25 mg/ml dan 24 mg/ml. Hasil dari kajian ini telah mengesahkan keberkesanan *Acacia mangium* sebagai racun perosak biologi kepada SGE.

Kata kunci: Acacia mangium; Pomacea canaliculata; saponin; kaedah rendaman

1. Introduction

The major problem faced by farmers in paddy cultivation is pests and one of them is the Golden Apple Snail (GAS) or *Pomacea canaliculata* (Lam.). It is native to South America and was initially introduced to developing countries for commercial production (Anderson, 1993; Matienzo, 1984). The many unsuccessful events to commercialise the snail has led to its uncontrolled release into the open environment via floods and irrigation canals, proliferated to such an extent that they are now considered as one of the 100 worst invasive alien species in the world (ISSG, 2006). GAS was first reported in Malaysia in 1991 in the state of Kedah (Anon., 1992). Meanwhile, in Sabah it was first sighted in Keningau in 1992. Currently the total infested area in Sabah stands at 8000 ha (Teo, 2012). Among the suggested control measures for GAS are the cultural technique by handpicking and managing the water level (Teo, 1999), biological control by introducing ducks into the paddy field (Teo, 2001), and using synthetic molluscicides for example CuSO₄, metaldehyde and niclosamide (Halwart, 1994).

Bio-pesticides are natural products from plants that give a toxicity effect on pests and are gaining more attention because they are considered safer to humans, animals, and the environment. Some plants have been found to exhibit molluscicidal activities and among them are *Nicotiana tobaccum* (Tangkoonboribun & Sassanarakkit, 2009), *Furcraea selloa* var. *marginata* (Teo, 2012), *Azadirachta indica* (Rosdiyani & Siti Noor Hajar, 2012), *Moringa oleifera* (Coelho da Silva et al., 2013), *Camellia oleifera* (Kijprayyoon et al., 2014), and many others. The chemical compound that is responsible for the molluscicidal activities is saponin as reported by Huang et al. (2003), Sparg et al. (2004), San Martín et al. (2008), and Kijprayyoon et al. (2014). Saponin can cause apoptosis, an incident of uncontrolled cell death, and inflammatory responses that eventually lead to death of the snails (Hostettmann & Marston, 2005; Oleszek, 2002).

Acacia mangium (Willd.) belongs to the genus Acacia and is characterised by a large amount of substances in the wood structures. It was introduced in Malaysia as source of timber for the wood industry (Matsumura, 2011). The tree part of Acacia mangium, particularly the bark, is usually unwanted and the leave, unused. Since calls have been made for greater sustainability and an ecologically friendly environment, recycling of industrial byproducts is a good initiative. A previous study has reported the presence of triterpenoid saponin in Acacia auriculiform (Uniyal et al., 1992). Therefore, in this study we aim to screen the phytochemical constituents from the tree parts of Acacia mangium to assess its molluscicidal activity and to determine the effective dosage that can cause mortality on GAS.

2. Materials and Methods

2.1. Source of GAS

GAS were collected from the paddy fields in Kota Belud, authenticated and brought to the Science and Agrotechnololgy Laboratory Complex, *Universiti Teknologi MARA* (UiTM) Sabah, Kota Kinabalu Campus.

2.2. Acclimatisation of GAS

A number of healthy adult snails were allowed to acclimatise for 7 days in a plastic aquarium containing a 10 cm layer of soil which was taken from the paddy field. Thereafter, each aquarium was filled with 15-20 cm of distilled water and this was changed daily. During acclimatisation, the snails were supplied with untreated newly sprouted rice seedlings as their food source.

2.3. Plant parts collection

Disease-free and healthy leaves, flowers, and the bark of the *Acacia mangium* were collected from the Sabah Forest Development Authority (SAFODA) Research and Development Centre in Kinarut, Papar. The samples were cleaned, dried, and cut into small pieces. *Furcraea selloa* var. *marginata* were collected at *Unit Ladang*, UiTM Sabah, and used as positive control.

2.4. Plant parts extraction

The plant parts were cleaned and left to dry in an open shaded area. The dry samples were grounded into powder form with an electrical blender in order to provide a greater surface area (Redfern et al., 2014) To obtain a solvent extract, 20 g of the finely powdered dry sample was homogenised in 200 ml of methanol (0.1 w/v), was left overnight, and filtered with Whatman No.1 sterilised filter paper. The filtrate was then evaporated using Rotary Vacuum Evaporator (EYALA OSB-2400) with a temperature of 50°C (Zhang et al., 2010) at 150 rpm. The whole process was repeated to obtain 0.2 w/v and 0.3 w/v of plant crude extracts.

2.5. Phytochemical screening

A range of phytochemicals was tested, specifically saponin, phenol, protein (Tiwari et al., 2011), tannins (Obi et al., 2011), flavonoids (Aliyu et al., 2012), steroids (Harborne, 1973), terpenoids (Yadav & Agarwala, 2011), glycosides (Jigna & Chanda, 2007), and alkaloids (Rasool et al., 2010).

2.6. Aqueous plant extraction

All plant samples were cleansed by washing them with tap water, dried in an oven for 48 hours, cut into small pieces, and ground into powder form. The powder was weighed separately to 37.5 g, 75.0 g, 112.5 g, and 150.0 g respectively with a digital electronic balance and then mixed with 1500 ml of distilled water to get the concentration of 25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml. Distilled water was used as a negative control. The solutions were allowed to mix well for 48 hours before they were filtered through a sieve for removal of suspended particles. The solutions were kept at 4°C until the dip bio-assay was conducted (Siti Noor Hajjar et al., 2012).

2.7. Dip bio-assay

The aqueous extraction of Acacia mangium and Furcreae selloa var. marginata were tested against adult Pomacea canaliculata according to the method recommended by the World Health Organization (Molla et al., 2013; WHO, 1983). Four different concentrations of the aqueous extraction were tested, each with three replicates of 10 snails. The snails were kept in containers with 300 ml of testing solutions for 24 hours and were statistically arranged according to a completely randomised design (CRD). Tests were carried out at room temperature (28 ± 2 °C). After 24 hours of exposure, the suspension was decanted, the snails were rinsed thrice with distilled water and transferred into fresh distilled water and maintained for another 24 hours for recovery. All groups were observed carefully after 24 hours and the number and the percentage of mortality in each group was calculated. The snails were considered dead if they could not move or retract well into or hang out of the shell, with the body and shell discoloured (Singh & Singh, 2009; Vijay, 2010). The LC₅₀ was estimated using probit analysis (Finney, 1971).

2.8. Data analysis

The data was analysed using the two-way ANOVA with 95% confidence interval followed by Fishers least significant different (LSD) to separate the means using the IBM SPSS Statistical Version 21.0 software.

3. Results and Discussion

3.1. Phytochemical screening

All the phytochemical secondary metabolites that were screened in this study were detected. Saponin was detected in all parts of the *Acacia mangium*, from the bark, leaves, and flower (Table 1). Saponin compounds are beneficial and possess insecticidal and molluscicidal activities since it has a broad and fast-working effect against insect pests (De Geyter et al., 2007) and moluscus (Kijprayyoon et al., 2014).

Alkaloids were detected in all parts of the *Acacia mangium* which were the bark, leaf, and flower (Table 1). The presence of alkaloid compounds in the plant indicates toxic properties (Wink, 2004). However, Schiff (2002) reported that it could also be used as a pain reliever.

Colour changes to reddish-black indicated the presence of terpenoids in the plants. Terpenoids are responsible for antibacterial and antiviral actions (Cock and Kukkonen, 2011). According to Giardi et al. (2010) terpenoids can be used as a substance to reduce cancer cells.

	Extracts of Acacia mangium		angium			
Plant constituents	Leaf Bark F		Flower	Colour changes		
Saponin	Detected	Detected	Detected	The formation of layers after indicates the presence of saponin		
Alkoloids	Detected	Detected	Detected	The formation of white precipitate indicates that alkaloids were detected		
Phenol	Detected	Detected	Detected	The colour reddish-blue determines that the phenolic compound is present		
Flavonoids	Detected	Detected	Detected	The yellowish colour shows that flavonoids were detected		
Terpenoids	Detected	Detected	Detected	The colour changed to reddish-black and this indicates that the presence of terpenoids		
Tannins	Detected	Detected	Detected	The colour of the plant extract changed to greenish- black and this indicates that tannins were detected		
Quinoine	Detected	Detected	Detected	Reddish-black colour formed indicates the presence of quinoine		
Glycoside	Detected	Detected	Detected	Greenish-blue colour formed indicates the presence of glycoside		
Steroids	Detected	Detected	Detected	The colour of the plant extract turned to blue-black and this indicates that steroids were detected		
Proteins	Detected	Detected	Detected	Blue colour determines that the plant contains proteins compounds		

Table 1: Phytochemical screening of the leaf, bark and flower extract of Acacia mangium

The presence of phenolic compunds is consistent in all parts of the plants. According to a study by Zhang et al. (2010) the most concentrated phenolic compound can be found in the bark. The results from Table 1 show that the compound can also be found in the leaf and flower. These compounds are strong antioxidants that can be beneficial to living things (Zhang et al., 2010). Santi et al. (2010) reported that this phenolic compound is essential in plant-microbes interaction, and it can also be used as an antimicrobial agent against pathogens (Lattanzio et al., 2006).

Flavonoid compounds also appear as one of the compounds detected in the Acacia mangium extract. Umi Kalsom et al. (2001) stated that flavonoid compounds from Acacia mangium have

the potential to be the substances that support plant growth since they act as antioxidants that react against free radicals during photosynthesis.

Tannin compounds that were present in the plant appeared constantly in each of the plant parts of *Acacia mangium*. It has been know to give a pesticide effect to insects (Freeman & Beattie, 2008). Dykes and Rooney (2007) reported that plants that are rich in tannins are not preferred by birds and insects.

Quinoline in the plant extract has a wide range of benefits including as an antimalarial, antifungal and antibacterial agent (Mohammad et al., 2013). Glycoside components also appeared in the plant extracts. Glycoside has a wide range of characteristics that include antimicrobial, anti-insect, and allelopathic activities which can be used in plant protection (Asri, 2013).

Steroids and proteins were also detected in the screening. Proteins are a very important food source. According to Freeman and Beattie (2008), defensive protein and enzymes are able to suppress the growth of fungi, bacteria, insects, and nematodes. The presence of the bioactive agent in the plants can act as a defence compound against microbes (Wink, 2004) and can be useful in therapeutic treatments.

3.2. Molluscicidal activity of Acacia mangium

Snail mortality at 100% was observed starting from the concentration of 50 g/ml for Acacia mangium and Furcraea selloa var. Marginata (the positive control) extracts. There is no significant different (P < 0.05) of snail mortality rate after 24 hours between Furcraea selloa var. Marginata and Acacia mangium (Table 2), which means Acacia mangium could be a potential substitute to Furcraea selloa var. Marginata as molluscicide against GAS.

	Aqueous extrac	ct concentration	
25 mg/ml	50 mg/ml	75 mg/ml	100 mg/ml
25 112 111	Means of		100 115/111

100.00 a

100.00.2

100.00 a

100.00 2

60.00 a

53 330

Table 2: The means of mortality of GAS in Acacia mangium and Furcraea selloa var. marginata aqueous extract.

eatn	neueru sp.	55.55a	100.00 a	100.00 a	100.00 u	
μË	Control	0.00b	0.00 b	0.00 b	0.00 b	
* Mean	s within column followed by the	ame letters are not	significantly diffe	rent as determined	l by least significa	nt

* Means within column followed by the same letters are not significantly different as determined by least significant difference at P<0.05.

3.3. Effective dosage of plant's aqueous extract

Furcraea sp.

Acaria en

nent

The Probit Analysis Programme was used to determine the lethal concentration (LC₅₀) of *Acacia* mangium and *Furcraea selloa* var. marginata. The current practical bio-molluscicide is the plant extract from *Furcraea selloa var. Marginata* (which is used as the positive control in this study) and this recorded the lowest LC₅₀ at 24 mg/ml. Meanwhile for *Acacia mangium* LC₅₀, it is slightly higher at 25 mg/ml. Again, the data from the Probit analysis also suggests that *Acacia mangium* is a potential molluscicide against GAS.

4. Conclusion and Recommendation

Acacia mangium has the potential to act as a bio-pesticide against GAS. Its molluscicidal activities are as potent as that of the *Furcraea selloa* var. marginata, a garden plant that was reported earlier to have good mollucicidal activities on GAS. Both Acacia mangium and *Furcraea selloa* var. marginata gave 100% snail mortality at the concentration of 50 mg/ml. The LC_{50} for Acacia mangium is 25 mg/ml compared to 24 mg/ml for Furcraea selloa var.

100.00 a

100.00 a

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marginata. In order to get a more stable data, a smaller dose of concentration within the range of 25 mg/ml to 75 mg/ml should be tested in future studies. This will reflect a specific rate of mortality on specific concentrations of *Acacia mangium* aqueous extracts. In addition, *in-vivo* studies need to be carried out to observe its effectiveness in the field, as GAS are known to be fitter in their natural environment. Different extraction methods are also suggested because recent studies have shown that a lower LC_{50} was achieved using the alcohol extraction method.

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