Chapter 94
Potential of *Carica papaya* and *Artocarpus integer* Extracts as Botanical Pesticides for Controlling, Golden Apple Snail, *Pomacea canaliculata*

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Abstract Golden apple snail (GAS), *Pomacea canaliculata*, is a major pest of paddy in Southeast Asia. Due to serious effects of GAS in rice cultivation, farmers have resorted to use synthetic molluscicides for controlling damages caused by GAS leading to negative impact toward the environment and human health. Accordingly, it is essential to study and develop new botanical pesticides for controlling GAS. The objective of this study is to expose the potential of selected plant extracts using bioassay test and antifeedant activity are that responsible for controlling GAS. Selective of potent extracts from *Carica papaya* leaves and peels of *Artocarpus integer* were tested on GAS in laboratory conditions. Five different concentrations of crude extracts were extracted using 95% of methanol and ethanol. Mortality of GAS was observed within 96 h with 24 h nterval. The obtained results from bioassay test show that methanol extracts of *C. papaya* (LC_{50} = 18. 5 g/L) gave a higher mortality of GAS compared to *A. integer* extract (LC_{50} = 39.8 g/L). High antifeedant activity of GAS imposed by ethanol extracts of *A. integer* (LC_{50} = 25.7 g/L) is compared with *C. papaya* extract (LC_{50} = 20.6 g/L). Thus, this study showed that extracts of *C. papaya* are more effective for sustainable control of GAS and extracts of *A. integer* have a potential use as an antifeedant activity toward GAS.

Keywords Pomacea canaliculata · Botanical pesticides · Carica papaya
Artocarpus integer

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1 Introduction

Pomacea canaliculata, golden apple snail (GAS) is known as major pervasive rice pest in Asia. This invasive freshwater snail was native to South America and Northern Argentina (Du et al. 2007; Joshi 2005). In the early 1980s, GAS was accidentally widespread in Asia when snail-farming projects for a dietary high-protein supplement in Asia were being rejected and low in market value as there were less consumptions of GAS among Asians (Massaguni and Latip 2012; Liu et al. 2006; Teo 2004). Due to that abandoned project, GASs were being released into irrigation ditches, natural waterways, and subsequently invaded into rice fields (Massaguni and Latip 2012). GAS was reported as a major and serious pest in paddy fields as GAS has a voracious appetite in both young rice seedlings of transplanted and direct-seeded rice (IRRI, 2011). This nocturnal GAS tends to eat the succulent base part of young paddy seedling up to 15 days of transplanting. GAS can feed by scraping on plant surfaces with rough tongue, with unique features GAS was able to consume a leaf blade of rice in just 3–5 min (Massaguni and Latip 2015; Massaguni and Latip 2012). An ability of GAS to grow and make an adaptation in high water levesl, able to hibernate up to 3 months during dry season, high in fecundity, and fast growth also influenced rapid multiplication in population and widespread of its distribution and caused mass loss in rice production (Massaguni and Latip 2012; Zhao et al. 2012). Uncontrolled infestation of GAS caused a huge economic loss in rice production, $1.47 billion per annum (Nghiem et al. 2013). Naylor (1996) reported that over 90% of young rice seedlings up to 15 days after transplanting can be damaged by only 8 individuals of GAS per m^2 with size from 10 to 40 mm within overnight (as stated by Massaguni and Latip 2012). The obvious mass destruction caused by GAS can be identified when there are floating leaf parts and missing of hills in the paddy field (Joshi et al. 2005).

Nowadays, most of the farmers relied on heavy use of synthetic molluscicides for immediate and is the fastest way to control and reduce the population of GAS within short time intervals. However, overuse of synthetic molluscicides raised negative effects on human health and increased toxicity in the environment (Joshi et al. 2008). Otherwise, improper and uncontrolled use of synthetic molluscicides among farmers also increased residue impacts in the environment and interrupt the nature of natural enemies and nontarget insects in the environment. Numerous studies investigating molluscicidal properties from potential plants on GAS have been carried out due to concern with this problem. Thus, an alternative use of botanical pesticides to reduce damage caused by GAS is safer and less toxic compared to synthetic molluscicides. Botanical pesticides are eco-friendly pest controls as they are formulated by natural active compound from potential plants. There are many types of plant chemicals known as secondary plant metabolites that act as repellents, antifeedant, and insect growth regulatory activities toward insect's infestation such as lactones, phenols, terpenes, furans, flavonoids, and saponins (Latip et al. 2015; Prakash et al. 2008; Barasa et al. 2002).

In Malaysia, there are numerous indigenous plants that have the potential to be used as botanical pesticides. Neem, *Azadirachta indica,* is able to control the infestation of GAS as its active compound, Azadirachtin, is able to cause anti-feedant activities and mortality toward GAS (Latip et al. 2012). Massaguni and Latip (2015) also reported that butanol extracts of neem leaves and seeds able to cause 100% mortality of GAS. Moreover, *Nerium indicium* Mill, is one of the most poisonous plants which cause toxicity toward freshwater snails such as *Lymnaea acuminate, Oncomelania hupensis,* and *Indoplanorbis excustus.* Glycosidase of fresh leaves of *N. indicium* also had a positive effect in controlling *O. hupensis* (Dai et al. 2011).

Leaves extracts of *C. papaya* are reported to contain alkaloids, saponins, and flavonoids which highly possess antitumor and have pesticidal effects against pest and disease infestation to act as a defensive mechanism (Baskaran et al. 2012; Ayoola and Adeyeye 2010). Otherwise, saponin extracts of soap nut also have potential to be used botanical pesticides as extractions of soap nut able to influence activities of GAS (Huang et al. 2005). Saponin contents in some plants were reported to be highly toxic and can be used as agriculture spray as saponin exhibit hemolytic properties which are able to acts as poison or pesticidal activity (Taguiling 2010). The extraction of *Artocarpus* also has the ability as an anti-inflammatory activity, the potential for antifungal activity and antibacterial activity against the pest. Therefore, this study is conducted to quantify the phyto-chemical content from *C. papaya* and *A. integer* that are responsible for controlling GAS and to identify the mode of action of GAS; either mortality or antifeedant activity toward the extracts of *C. papaya* and *A. integer*.

2 Materials and Methods

2.1 Test Organism

Adults of GAS were collected at paddy field, Sungai Besar, Selangor, Malaysia. The average size of GAS being used in the experiment was 25 mm of shell height and weight 3.0 g each. Uniform size of GAS was selected using the height of shell (Massaguni and Latip 2012).

2.2 Plant Materials

Fresh leaves of *C. papaya* and peels of *A. integer* were collected from Sungai Besar, Selangor, Malaysia. The leaves of *C. papaya* and peels of *A. integer* were dried and stored at 40 °C for 48 h for further used in plant extractions.

2.3 Extraction of Plant Extracts

Plant extracts were prepared by percolation method described by Handa et al. (2008) and Sujatha et al. (2012) with slight modification. Dried leaves of *C. papaya* and peels of *A. integer* were grounded to a powder using a grinder. The powder (20 g) was extracted using 95% of methanol and ethanol using Soxhlet extractor with slight modification. Crude extracts were evaporated under vacuum at 40 °C for 60 min. The plant extracts were stored in a dark container to avoid light intensity at −8 °C.

2.4 Phytochemical Screening Test

The chemical compound in selected indigenous plants was screened using standard methods with modification (Sujatha et al. 2012). Flavonoid compounds in selected indigenous plants were screened by using alkaline reagent test. A few drops of sodium hydroxide solution were added into plant extracts. Formation of decolorizes of intense yellow color into colorless after addition of dilute acid indicates the presence of flavonoids compound (Tiwari et al. 2011). Saponins compound in selected indigenous plants was determine through frothing test. Formation of 1 cm of foam indicated the presence of saponins (Somkuwar and Kamble 2013).

2.5 Toxicity Test

Toxicity effects of leaves extracts of *C. papaya* and peels extracts of *A. integer* on GAS were carried out in the laboratory according to the methods of Talukder and Howse (1994) with some modifications. 20 g of paddy seedlings were sprayed with 10 ml of (range 10–50 g/L) extracts of *C. papaya* and *A. integer* was tested on 10 adults of GAS, sequentially and placed in the aquarium. As control treatments, paddy seedling was treated with methanol, ethanol, Tween 80, and distilled water. All of the treatments were air dried for 30 min to evaporate the solvent (Khani et al. 2011). The experiments were done with five replicates. The mortality of GAS was recorded after 24, 48, 72, and 96 h. The LC_{50} values of both plant extracts were calculated by probit analysis (Finney 1997) using Polo-Plus Software (LeOra 2003). The obtained data were corrected by Abbott's (1925) formula, transformed into percentage and square root values and then variance analysis (ANOVA) was done using SAS V.9.4 programme. Mean values were adjusted by Duncan's Multiple Range Test.

2.6 Evaluation of Antifeedant Activities

Antifeedant activities of GAS were determined using non-choices test as described by Keita et al. (2001) and Mahdi and Rahman (2008) with some modifications. 10 ml of diluted extracts of *C. papaya* and *A. integer* with five different concentrations were sprayed on paddy seedlings respectively. Ten adults of GAS were weighed and placed in the aquarium and allowed to feed for 7 days. After the feeding period, paddy seedlings were weighed and weight loss was measured by Eq. (1):

$$\text{weight loss}(\%\text{WL}) = (\text{IW} - \text{FW}) \times 100/\text{IW} \tag{1}$$

where the IW is the initial weight and FW is the final weight.

Results of mortality were adjusted for mortality in the control using Abbott's formula and expressed as percentages (Abbot 1925). The significance of the mean difference between treatments and control was analyzed using variance procedure at 5% probability level with individual pairwise comparisons with Duncan's test using SAS V.9.4 software package in Microsoft Windows 7 (SAS 2016).

3 Results and Discussion

3.1 Phytochemical Screening Content in Plant Extracts

The phytochemical analysis in Table 1 showed the extracts of *C. papaya* and *A. integer* contained flavonoids, papain, saponins, unsaturated sterols, alkaloids, and triterpenes and phenolic compounds. From the obtained results, active compounds of flavonoids and saponins extracted from leaves of *C. papaya* and peels of *A. integer* showed highly positive (+++) potential toxic effect towards GAS. While alkaloids and terpenoid compounds were moderately (++) found from both plants extracts. Meanwhile, papain was negative (-) in peels extracts of *A. integer*. Leaves extract of *C. papaya* showed larvicidal and pupicidal effects on target insects within 24 h of exposure (Koyendan et al. 2012). The peel and seed of *Artocarpus* were found to be effective as antioxidant agents and displayed higher phytochemical contents as compared with the flesh (Abu Bakar et al. 2015).

3.2 Toxicity Test of Plant Extracts Against GAS

Probit analysis at Table 2 showed higher toxicity for LC_{50} of the methanol extracts of *C. papaya* (18.5 g/L) compared with all treatments. Otherwise, ethanol extracts of *A. integer* (25.7 g/L) imposed high toxicity compared with methanol

Table 1 Phytochemical screening analysis of *Carica papaya* and *Artocarpus integer*

Test for physiologically active constituents	Carica papaya	Artocarpus integer
1. Flavonoids – Alkaline Reagent Test	++	+++
2. Saponins – Frothing Test	+++	++
3. Papain	++	−
4. Alkaloids	++	++
5. Terpenoids – Salkowski Test	++	+

Note (+) Positive, (−) Negative, + low, ++ moderate, +++ high

extracts. Results from Table 3 showed mortality of GAS were significantly increase ($p < 0.05$) as the time of exposure increased for both extracts of *C. papaya* and *A. integer*. Mortality of GAS (Table 3) was gradually increased from 24 to 72 h of exposure with treatments but after 96 h the mortality rate of GAS was declined. Toxicities of tested plant extracts indicate that concentration of crude extracts and the exposure time was dependent as there was a significant correlation between the mortality rate of GAS with exposure time and concentrations. High concentration of plant extracts (50 g/L) caused high in mortality of GAS compared with a lower concentration (10 g/L) of plant extracts that indicates less in mortality of GAS. Moreover, obtained results from Table 4, showed the use of solvents in plant extracts also have a correlation with mortality of GAS. At 96 h of exposure, methanol extracts of *C. papaya* indicate higher mortality of GAS (mean = 5.640) compared with ethanol extracts of *C. papaya* (mean = 4.560). Meanwhile, ethanol extracts of *A. integer* imposed high in mortality of GAS (mean = 4.000) compared with methanol extract of *A. integer* (mean = 2.960).

From the obtained results of phytochemical screening test, a compound that screens from leaves extracts of *C. papaya* and *A. integer* were highly positive (+++) found flavonoids and saponins. Bioactive compounds of flavonoids are able to affect on insect's endocrine systems, diet behaviors, insect development, and reproduction by directly or indirectly interacting with hormone system (Latip et al. 2015; Narciso et al. 2011). According to a study conducted by Musman et al. (2013), seed extracts of *Barringtonia racemosa* has molluscicidal effects toward GAS due to the presence

Table 2 Probit analysis for toxicities of *Carica papaya* and *A. integer* extracts toward GAS

Plant	Solvent	LC50 (Min–Max)	Slope ± SEM	X2	df
Carica papaya	Methanol	18.5 (11.2–24.4)	3.274 ± 0.43	4.669	3
	Ethanol	20.6 (16.7–24.2)	2.356 ± 0.36	1.313	3
Artocarpus integer	Methanol	39.8 (31.7–55.4)	1.917 ± 0.41	1.880	3
	Ethanol	25.7 (16.4–38.6)	2.229 ± 0.36	4.463	3

Units LC_{50} = g/L, applied for 24, 48, 72 and 96 h at 25 °C ± 2 °C. Values were based on 5 concentrations, 5 replications of 10 adults of GAS

Table 3 Mean (± SEM) percentage in mortality of GAS versus time and plant extracts

Treatment	Mortality of GAS			
	24 h	48 h	72 h	96 h
Carica papaya	1.080 ± 01.51[a]	2.440 ± 0.200[a]	3.760 ± 0.237[a]	5.100 ± 0.279[a]
Artocarpus integer	0.800 ± 01.03[a]	1.780 ± 0.122[b]	2.640 ± 0.139[b]	3.480 ± 0.181[b]

Each data represents the mean of five replicates. [a]Means within the same column followed by different letters are significantly different ($P < 0.05$) by two-way ANOVA, Duncan's multiple range test. [b]Mean within a column followed by the same letter is not significantly different: (Duncan's multiple range test, $p < 0.05$)

Table 4 Mean values in mortality of GAS versus time and solvent used in plant extracts

Treatment	Solvent	Mortality of GAS			
		24 h	48 h	72 h	96 h
C.papaya	Methanol	0.800	2.360	3.880	5.640
C.papaya	Ethanol	1.360	2.520	3.640	4.560
A.integer	Methanol	0.840	1.680	2.400	2.960
A.integer	Ethanol	0.760	1.880	2.880	4.000

Means ± SEM mortality of GAS treated with the treatment of *Carica papaya* and *Artocarpus integer*, two-way ANOVA, LSD test, ($P < 0.05$)

of saponins and flavonoids which significantly caused mortality on GAS. Both bioactive compounds of flavonoids and saponins could reduce air supply to the embryos in eggs of GAS, thus altering the normal development embryo of GAS and exhibits ovicidal effects on older egg mass of GAS (Demetillo et al. 2015; Wu et al. 2005). As cited in Joshi et al. (2008), saponins are also able to alter the behavior of GAS at different growth stages which are related to relative use for respiration of their lung and gills as smaller GAS frequently ventilates its lungs by extending the siphon compared to larger or adults of GAS. Saponins are able to cause cell disruption and affect gills of GAS through hydrophobic interactions. Complex forms of saponins with steroids, proteins, and membrane phospholipids are responsible for the action on the cell membrane and causing the destruction of cells for pests (Souza et al. 2013).

3.3 Antifeedant Activities Toward GAS

The results shown in Table 5 revealed the ethanol extracts of *A. integer* and *C. papaya* showed high antifeedant activities compared with methanol extracts for both plants. All concentrations of ethanol extracts for *A. integer* and *C. papaya* showed 80 and 70% of repellency, after 6 h against adults of GAS respectively. However, after 24 h, only ethanol extracts of *A. integer* strongly repelled the adults of GAS (80%). Furthermore, this study demonstrated that the plant extracts tested are effective as a repellent for adults of GAS.

Table 5 Antifeedant activity of extracts of *Carica papaya* and *Artocarpus integer* toward adults of GAS

Treatments	Solvent	Concentration of plant extracts (g/L)	Weight loss, g	
			GAS	Paddy seedling
C. papaya	Methanol	10	0.194	13.172
		20	0.232	12.964
		30	0.360	12.605
		40	0.410	10.835
		50	0.516	8.422
	Ethanol	10	0.160	15.116
		20	0.154	13.694
		30	0.200	13.453
		40	0.300	12.105
		50	0.332	10.340
A. integer	Methanol	10	0.178	16.364
		20	0.242	15.390
		30	0.263	15.402
		40	0.280	14.257
		50	0.326	13.276
	Ethanol	10	0.286	15.282
		20	0.290	14.760
		30	0.267	13.960
		40	0.320	13.382
		50	0.396	12.058

Each data represents the mean of five replicates with five different concentrations (ranging from 10 to 50 g/L) per plant extracts, two-way ANOVA, (Duncan's multiple range test, $P < 0.05$)

There was also a significant weight loss on paddy seedling treated with plant extracts and weight of the GAS. The weight loss in paddy seedling is higher in low dosage (10 g/L) for methanol extracts of *C. papaya* (mean = 13.172) and *A. integer* (mean = 16.364). Meanwhile, high dosage (50 g/L) for ethanol extracts of *A. integer* (mean = 12.058) and *C. papaya* (mean = 10.340) showed a low number in weight loss of paddy seedling. Whereas, highest weight losses in adults of GAS were imposed with a high dosage of ethanol extracts of *A. integer* (mean = 0.396) compared with ethanol extracts of *C. papaya* (mean = 0.332). The similar finding by Khani et al. (2011) on *Jatropha curcas* seed oil showed a repellency of 47.5% of *Callosobruchus maculatus* and *Dinarmus basalis* at the lowest dose (0.5 ml), whereas the highest dose (2 ml) repelled 95% for both *C.maculatus* and *D.basalis*.

Thus, weight loss in adults of GAS was dependant on the concentration of plant extracts. The results showed ethanol extracts of *A. integer* is effective as anti-feedants against adults of GAS. This indicated that plant materials can be considered as alternative controls for GAS. Results of the growth and development study of the antifeedant test showed that weight gained of larvae treated with *Citrus hystrix* essential oil were lower as compared to control treatment (Loh et al. 2011).

4 Conclusions

The study was conducted to evaluate the toxicity and repellency of crude extracts of *C. papaya* and *A. integer* on mortality of GAS and weight losses of GAS and paddy seedlings. The mortality increased with increasing concentration and exposure time. Methanol extracts of *C. papaya* showed highest toxicities compared to methanol extracts of *A. integer* followed with ethanol extracts of *C. papaya* and *A. integer*. In addition, *A. integer* extracted with ethanol showed a higher repellent effect on GAS compared with ethanol extracts of *C. papaya* and methanol extracts of *C. papaya* and *A. integer* sequentially. Repellency of ethanol extracts of *A. integer* it is possible due to flavonoids compounds and toxicity of methanol extracts of *C. papaya* might be due to saponins compounds. Therefore, it is suggested that further study should consider the comparison of the repellent activity and toxicity of *C. papaya* and *A. integer* with an analytical grade of flavonoids and saponins in investigating the bioactive compounds. As a conclusion, extracts of *C. papaya* and *A. integer* is potent to be used as a supplement for other control methods in sustainable agriculture practices in controlling of GAS.

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