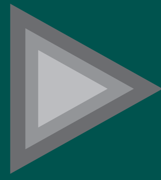

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Research article

Active compounds and biological activities of geranium aralia (*Polyscias guilfoylei* (W. Bull) L.H. Bailey) leaf extract against larval stage of melon fly (*Zeugodacus cucurbitae* Coquillett)

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Secondary metabolites,
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Abstract

Importance of the work: *Polyscias guilfoylei* is a shrub that is known as a medicinal plant whose leaf extracts contain active compounds, such as saponins.

Objectives: To determine the active compounds and biological activities of *P. guilfoylei* leaf extracts against *Zeugodacus cucurbitae*.

Materials & Methods: The research was conducted in the Plant Pest Laboratory using a randomized block design arranged in a factorial manner. The solvents used were ethanol and n-hexane at concentrations of 0%, 1.25%, 2.50% and 5.00%. All treatments were replicated four times. The parameters observed were: active compound content, larval feeding and growth activities.

Results: Ultraviolet-visible spectrophotometry and thin layer chromatography indicated that the ethanol *P. guilfoylei* leaf extracts contained phenols, tannins, alkaloids, flavonoids and saponins, while the n-hexane *P. guilfoylei* leaf extracts contained terpenoids, steroids, phenols, tannins, alkaloids, flavonoids and saponins. Application of 5.00% ethanol or n-hexane *P. guilfoylei* leaf extracts caused lower values for feeding activity and the feeding deterrent index. In addition, the extracts affected larval and pupal periods, the number of larvae reaching pupation and the pupal weight of *Z. cucurbitae*. Application of higher concentrations of extracts resulted in longer larval and pupal periods, a reduced pupal weight and fewer larvae reaching the pupal stage.

Main finding: The active compounds in *P. guilfoylei* leaf extracts were phenols, tannins, alkaloids, flavonoids and saponins. Application of leaf extract at up to 5.00% could deter larval feeding and the growth activities of *Z. cucurbitae*.

† Equal contribution.

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Introduction

Polyscias guilfoylei (W. Bull) L.H. Bailey is an Araliaceous shrub known as a medicinal plant. *P. guilfoylei* can grow rapidly and within a year can reach a height of up to 1.5 m, so that pruning is necessary; this plant species can be used in windbreaks, for noise ablation, as an odor insulator or as an ornamental plant (Duvauchelle, 2010; Teves, 2014). Locally, it known by the name pudding leaf (Elya and Kusmana, 2002). *P. guilfoylei* contains active compounds such as oleanane saponin (Cioffi et al., 2008; Tuyet et al., 2009; Elgindi et al., 2015). Saponins are secondary metabolites found in most plants or plant products and are categorized as triterpenes and steroid glycosides and have antifungal properties, causing mortality in protozoa and mollusks, as well as protecting plants from pathogens and herbivores (Addisu and Assefa, 2016). Several research results have indicated that saponins are toxic and acts as a feeding inhibitor and a growth inhibitor (Agerbirk et al., 2003; Golawska, 2007; Saha et al., 2010; Dowd et al., 2011). In addition, beside containing saponins, *P. guilfoylei* leaf extract may also contain other active compounds depending on the type of solvent used in the extraction process (Harborne, 1987). Therefore, it is important to identify active compounds in *P. guilfoylei* leaf extract using different solvents. Other studies regarding *P. guilfoylei* extract have focused on the bioactivity associated with certain taxa such as mollusks, rats, rabbits bacteria and fungi (Bernard et al., 1998; Elya and Kusmana, 2002; Elya et al., 2010; Sundu et al., 2015; Ashmawy et al., 2019). However, bioactivity studies on insects, especially plant pests, are still limited. Studies regarding the extract from *P. guilfoylei* leaves have only investigated its possibility as an attractant for fruit flies (Jang et al., 1997; McQuate and Vargas, 2007) and other bioactivity studies have not been systematically documented. To learn more about the bioactivity of *P. guilfoylei* leaf extract, the current study was conducted using melon fly *Zeugodacus cucurbitae* Coquillett. The melon fly *Z. cucurbitae* is an important pest for fruit and vegetable commodities from the family Cucurbitaceae (Khan and Hugar, 2019). Nevertheless, information is limited regarding bioactivity testing of *P. guilfoylei* leaf extract because bioactivity testing of P. guilfoylei extract toward melon fly *Z. cucurbitae* has been conducted merely as an attractant. The purpose of the current study was to determine the content of the active compounds contained in ethanol and n-hexane *P. guilfoylei* leaf extracts and their biological activities against the melon fly *Z. cucurbitae*. The parameters observed were active compound content, feeding activity and growth activity. These data are important to determine the biological activities of *P. guilfoylei* leaf extracts against plant pests.

Materials and Methods

Plant materials

P. guilfoylei leaves were collected from the village of Bojongmenger, Cijeungjing district, Ciamis regency, West Java province, Indonesia at 7.33°S, 108.42°E at an altitude of 124 m above mean sea level. *P. guilfoylei* collection occurred in May 2017. Approximately 6.15 kg of leaves were collected and were specifically the third to seventh leaves from the tips of the shoot. The leaves were identified in the Plant Systematic Laboratory of the Faculty of Biology, Universitas Gadjah Mada (UGM), Yogyakarta, Indonesia. Wet *P guilfoylei* leaves were dried for 3 d in the Phytochemical Laboratory, Department of Pharmaceutical Biology, Faculty of Pharmacy, UGM using an oven at 50 °C and then milled using a grinder for leaves to produce a dry powder (about 800 g).

P. guilfoylei leaf extraction

The preparation of *P. guilfoylei* leaf extraction was conducted in the Phytochemical Laboratory, Department of Pharmaceutical Biology, Faculty of Pharmacy, UGM using the maceration method (Harborne, 1987). The *P. guilfoylei* leaf powder was dissolved in two solvents (ethanol 70%, and n-hexane), with a ratio of 1:10 (weight per volume) for 72 h. As much as 400 g of *P. guilfoylei* leaf powder was dissolved in 4 L ethanol and the other approximately 400 g of leaf powder was dissolved in 4 L n-hexane, both for 72 h. The solution of each solvent was strained using a Buchner funnel lined with a paper filter. The filtered results were evaporated using a water bath for the ethanol solvent until a thick extract (80.83 g) was acquired, while the n-hexane solvent was evaporated in a vacuum room until a thick extract (19.38 g) was acquired. Each thick extract was placed in a glass bottle and stored in a refrigerator at 10 °C until ready for testing.

Insect rearing for testing

The insects used for testing was the melon fly *Z. cucurbitae* Coquillett, with the pupae obtained from the Forecasting Center for Plant Pest Organism (BBPOPT) Jatisari, Karawang

41374, Indonesia. These pupae were reared in the Plant Pest Laboratory of Invertebrate Pest Division, Department of Plant Protection, Faculty of Agriculture, UGM. The rearing technique for the melon flies was carried out using an artificial diet (Chang et al., 2004) and following the standard procedure developed in the laboratory. The insects used for testing in the study were at egg stages.

Bioactive compounds of P. guilfoylei leaf extracts

To identify the most dominant active compounds, the ethanol and n-hexane *P. guilfoylei* leaf extracts were analyzed in the Integrated Research and Testing Laboratory, UGM. The active compounds analyzed included qualitative tests of terpenoids and steroids using a thin layer chromatography (TLC) method and total saponins, alkaloids, flavonoids, phenol and tannins using an ultraviolet-visible spectrophotometry method (Koomson et al., 2018; Tan, 2018). The methods were carried out according to the standard procedures in the Integrated Research and Testing Laboratory of UGM. For the terpenoid test, 0.1 mg of sample was used with the addition of 5 mL of ethanol and vortexed for 2 min, sonicated for 30 min and centrifuged for 3 min. Then, 10 µL of solution was spotted on a silica gel plate 60 F_{254} along with another variant. Then, the silica gel plate was inserted into a chamber of toluene-ethyl acetate movement phase (93:7) and eluted to the limit before being lifted and left to dry, sprayed with vanillin sulfuric acid reagent and heated at 110 °C for 2 min. For steroid testing, 0.1 mg of sample was used and added with 1 mL of ethanol, vortexed and sonicated for 60 min. Spotting used 20 µL of sample placed on a silica gel plate 60 F_{254} along with another variant. Then, the silica gel plate was inserted into a chamber of toluene-ethyl acetate movement phase (80:20) and eluted to the limit before being lifted, left to dry, sprayed with Liebermann-Burchard and then heated at 110 °C for 2 min. Density-metric measurement was performed using a 340 nm wavelength. The total flavonoid test used 100 mg of extract sample, added with 0.3 mL of 5% sodium nitrite. After 5 min, 0.6 mL of 10% aluminum chloride was added. After another 5 min, 2 mL of 1 M hydroxide and distilled water were added, until the volume reached 10 mL. Then, it was diluted 10 times, transferred into a cuvette and retained for absorption at a wavelength of 510 nm. In the total phenol test, a sample of 0.05 g was used with 0.5 mL Folin-Ciocalteu reagent and 7.5 mL aqua bidest. The mixture was left for 10 min at room temperature; then, 1.5 ml of 20% sodium carbonate and aqua bidest were added, until the volume reached 10 mL. Then, it was diluted 5 times, transferred into a cuvette and retained for absorption at a wavelength of 760 nm.

Feeding and growth activities bioassay

The feeding activity bioassay was done together with the growth activity test using a no-choice method based on a factorial randomized block design with each treatment having four replications. In a Petri dish with a diameter of 5 cm, 10 g of artificial diet was added (160 g wheat husk, 35 g sugar, 8.7 g yeast, 0.33 g sodium benzoate, 0.33 g nipagin and 180 mL distilled water). In the first treatment, the artificial feed was mixed with 2 mL of 0%, 1.25%, 2.50% or 5.00% ethanol *P. guilfoylei* leaf extracts. In the second treatment, it was mixed with 2 mL n-hexane *P. guilfoylei* extracts with the same concentration series. Furthermore, 10–20 eggs of *Z. cucurbitae* aged 12 hr were collected from the melon fly hatching cup and placed in a Petri dish. The negative control was treatments without adding eggs to the Petri dish with artificial feed. This treatment was used to calculate the artificial feed shrinkage during the test, which was used as a correction factor in calculating the weight of the feed consumed. Petri dishes that already contained eggs were placed in box measuring 10 cm × 10 cm × 10 cm with the bottom lined with sawdust. The box was covered with a black cloth and set aside for 7–8 d. On day 8, the box was opened and the pupae that had developed were harvested and weighed. In addition, the remaining diets in each Petri dish were weighed. The parameters observed were the weight of diet consumed and the number of pupae formed. Feeding activity was measured by calculating the feeding deterrent index (FDI), according to Akhtar et al. (2003) using Equation 1:

$$\text{FDI } \% = 100\ [(C\text{-}T/C+T)] \quad (1)$$

where T is the approximate amount of diet eaten during the treatment and C is the approximate amount of diet eaten during control treatment. Amount of diet eaten = (Initial amount of feed × %Feed loss without larva) – Final amount of diet.

The growth activity test was conducted by observing the parameters: larval period, percentage of larvae reaching pupation, pupal weight, pupal period, percentage of adult emergence and the sex ratio. The criteria for each parameter were according to Singh et al. (2010): 1) Larval period = time consumed after the eggs hatched into larvae and then turned into pupal, measured in in days; 2) % pupation = [(Σ pupae formed) / (Σ larvae formed)] × 100; 3)

pupal period = time consumed for pupal stage to turning into an adult, measured in days; 4) pupal weight = weight of each pupa formed, measured in milligrams; 5) % adult emergence = [(Σ adults formed) / (Σ pupae formed)] × 100; 6) sex ratio = the proportion of male-to-female adults emerging from the pupae formed.

Data analysis

Data of feeding and growth activities were subjected to statistical analysis using the SPSS software (Version 10.0 for Windows; SPSS Inc.; USA). Two-way analysis of variance was performed to identify significant differences among the treatments. If the variable was significant, Fisher's least significant difference method was performed to explain the variation within the dataset. Significances was set at 95% ($p < 0.05$).

Results and Discussion

Bioactive compounds of P. guilfoylei leaf extract

The content of the bioactive compounds in the *P. guilfoylei* leaf extract that were analyzed using TLC and ultraviolet-visible spectrophotometry are shown in Table 1. The dominant active compounds in the ethanol *P. guilfoylei* leaf extracts were phenols, tannins, alkaloids, flavonoids and saponins. The dominant active compounds in the n-hexane *P. guilfoylei* leaf extracts were terpenoids, phenols, steroids, tannins, alkaloids, flavonoids and saponins. The total amounts of each active compound varied depending on the type of solvent. In the ethanol extract, the amounts of the phenolic and alkaloid active compounds were higher than those in the n-hexane extract. The amounts of tannins, flavonoids and saponins in the ethanol extract were lower than in the n-hexane extracts. There were no detectable compounds of the terpenoids and steroids in the ethanol extract.

Feeding activity bioassay

The feeding activity was measured based on the difference between the amounts of eaten diet, untreated control and diets with the ethanol and n-hexane extracts. The difference between diet weights was used to measure the value of the FDI. The weight of the diets eaten by the *Z. cucurbitae* larvae and the FDI values in each concentration used are shown in Table 2. Concentrations of ethanol and n-hexane *P. guilfoylei* leaf extracts up to 5.00% had a negative effect on the amount of the diet eaten. A higher concentration of extract used resulted in less diet being eaten. The weights of the diet eaten by the larvae of *Z. cucurbitae* at concentrations of 2.50% and 5.00% were lower than for concentrations of 0% and 1.25%, indicating that at the former two concentrations, there was a greater decrease in feeding activity by the *Z. cucurbitae* larvae compared to the latter two concentrations, which was consistent with the value of FDI being higher for higher concentrations of the *P. guilfoylei* leaf extracts.

Table 1 Active compounds in ethanol and n-hexane *Polyscias guilfoylei* leaf extracts

No.	Parameter	Ethanol extract	n-hexane extract	Method
1.	Terpenoids	Negative	Positive	Thin layer chromatography
2.	Total phenols as gallic acid equivalent	15.07 % b/b	2.66 % b/b	Ultraviolet spectrometry
3.	Steroids as beta sitosterol equivalent	Not detected	8,750.15 mg/kg	Thin layer chromatography
4.	Total tannins as tannic acid equivalent	4.36 % b/b[a]	2,949.99 % b/b[a]	Ultraviolet spectrometry
5.	Total alkaloids as quinine equivalent	2,432.37 mg/Kg	2,417.35 mg/kg	Ultraviolet spectrometry
6.	Total flavonoids	4.83 % b/b[a]	1,482.32 % b/b[a]	Ultraviolet spectrometry
7.	Saponins from quilaja bark	4,708.68 mg/Kg	7,027.37 mg/kg	Ultraviolet spectrometry

% b/b = percentage of total phenol, tannins and flavonoids, measured in grams

Table 2 Amounts of diet eaten and feeding deterrent index of melon fly *Zeugodacus cucurbitae* treated with ethanol and n-hexane *Polyscias guilfoylei* leaf extracts

Concentration (%)	Ethanol extract			n-hexane extract		
	N	Amount of diet eaten (g)	Feeding deterrent index (%)	N	Amount of diet eaten (g)	Feeding deterrent index (%)
0	44	0.219±0.058[a]	0.00±0.00[a]	60	0.218±0.037[a]	0.00±0.00[a]
1.25	32	0.182±0.058[a]	9.57±3.09[b]	45	0.171±0.022[a]	11.92±3.53[b]
2.50	48	0.122±0.048[b]	29.26±12.24[c]	48	0.128±0.056[b]	28.96±13.76[c]
5.00	47	0.096±0.023[b]	38.41±14.64[c]	49	0.105±0.051[b]	37.41±13.28[c]

N = Total number of insects test

Mean ± SD in the same column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

The ethanol and n-hexane *P. guilfoylei* leaf extracts contained saponins, flavonoids, alkaloids and tannins. These compounds can reduce the feeding activity by *Z. cucurbitae* larvae that was supported by several research results showing that the addition of these compounds acted as an antifeedants for several plant pests. For example, the contents of oleanolic saponin compounds from *Medicago truncatula* had potential as antifeedants against caterpillar (Cai et al., 2017), while the addition of the flavonoid pinocembrin was reported to reduce and stop the feeding activity of *Spodoptera frugiperda* larvae (Napal and Palacios, 2015) and *Spodoptera litura* larvae fed diets containing tannins reduced their feeding activity, thereby reducing plant damage and the herbivore population (Nomura and Itioka, 2002). The active compound triterpene glycoside of saponin ginsenoside type had strong antifeedant activity against *Pieris rapae* larvae (Zhang et al., 2017). The addition of the saponin ginsenosides type acted as an antifeedant on *Plutella xylostella* larvae. Ginsenosides caused the activity of glutathione S-tranterase, acetylcholine esterase and carboxyl esterase to decrease, but the activity of mixed-function oxidase to increase (Yang et al., 2018). The addition of leaf extracts containing alkaloids can be beneficial as a feeding inhibitor against *Tribolium castaneum* larvae because it decreases the activity of the α-amylase enzyme (Rharrabe et al., 2020). In addition, several active compounds in plant extracts together produce a stronger more pungent aroma for insects that can affect the performance of the taste buds and affect eating activity, causing reduced consumption (Widiyaningrum et al., 2020).

The addition of the ethanol and n-hexane *P. guilfoylei* leaf extracts produced the same effects of reducing the amount (weight) of the eaten diets and of lowering the FDI values than for the weight of the diets not including the extracts. The used of the ethanol and n-hexan solvent in control treatment (0%) did not have a negative effect on feeding activity of *Z. cucurbitae* larval. It can be observed in the control treatment (0%), *Z. cucurbitae* larval continue to eat the feed provided, so there is no visible feeding inhibition.

Growth and development activity bioassay

The growth and development activity bioassay of the ethanol and n-hexane *P. guilfoylei* leaf extracts effect against *Z. cucurbitae* was carried out together with the feeding activity bioassay; the results are shown in Tables 3–5.

Table 3 Larval period and % pupation of *Zeugodacus cucurbitae* treated with ethanol and n-hexane *Polyscias guilfoylei* leaf extracts

Concentration	Ethanol extract			n-Hexane extract		
(%)	N	Larval period (d)	% Pupation	N	Larval period (d)	% Pupation
0	44	7.000±0.000[a]	88.34±5.61[a]	60	7.025±0.050[a]	89.79±7.01[a]
1.25	32	7.000±0.000[a]	81.46±5.29[b]	45	7.075±0.096[a]	80.22±3.99[b]
2.50	48	7.050±0.058[ab]	78.06±7.57[b]	48	7.100±0.082[ab]	74.13±5.56[b]
5.00	47	7.100±0.082[b]	65.05±5.93[c]	49	7.125±0.050[b]	66.89±2.85[c]

Mean±SD in the same column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

Table 4 Pupal weight and pupal period of *Zeugodacus cucurbitae* treated with ethanol and n-hexane *Polyscias guilfoylei* leaf extracts

Concentration	Ethanol extract			n-Hexane extract		
(%)	N	Pupal weight (mg)	Pupal period (d)	N	Pupal weight (mg)	Pupal period (d)
0	44	12.60±1.44[a]	7.100±0.141[a]	60	12.60±1.39[a]	7.225±0.330[a]
1.25	32	9.15±4.86[b]	7.350±0.252[b]	45	9.20±1.17[b]	8.050±0.173[b]
2.50	48	8.65±0.96[b]	8.050±0.443[c]	48	8.40±1.58[b]	8.400±0.455[c]
5.00	47	7.58±1.02[b]	8.150±0.191[c]	49	7.58±1.31[b]	8.450±0.238[c]

Mean±SD in the same column superscripted by different lowercase letters are significantly ($p < 0.05$) different.

Table 5 Adult emergence and sex ratio of *Zeugodacus cucurbitae* treated with ethanol and n-hexane *Polyscias guilfoylei* leaf extracts

Concentration	Ethanol extract			n-Hexane extract		
(%)	N	% Adult emergence	Sex ratio (male:female)	N	% Adult emergence	Sex ratio (male:female)
0.00	39	83.08±11.88[a]	3.5:4.5	56	79.51±11.52[a]	6.0:7.0
1.25	26	71.40±11.71[a]	2.0:2.8	34	68.99±6.08[a]	2.8:3.0
2.50	37	67.92±6.29[a]	3.0:3.5	39	64.51±16.10[a]	3.8:2.8
5.00	34	64.25±3.17[a]	2.8:2.3	33	64.11±11.37[a]	2.8:2.5

Mean ± SD in the same column superscripted by different lowercase letters are significantly ($p < 0.05$) different

The growth and development of *Z. cucurbitae* treated with ethanol and n-hexane *P. guilfoylei* leaf extracts were measured based on the parameters of larval period and percentage of larvae reaching pupation (Table 3) and pupal weight and pupal period (Table 4).

Concentrations of ethanol and n-hexane *P. guilfoylei* leaf extracts up to 5.00% inhibited the growth of *Z. cucurbitae*. The concentrations of ethanol and n-hexane *P. guilfoylei* leaf extracts affected the larval period, percentage of larval reaching pupa, pupal weight and pupal period. The higher the concentration of extracts, the longer the larval and pupal period, the less pupae were formed and the lower the pupal weight. The addition of 2.50% or 5.00% ethanol extracts resulted in longer larval periods compared to the addition of 0% or 1.25% ethanol extracts. On other hand, the addition of 1.25%, 2.50% or 5.00% n-hexane extracts resulted in longer larval periods compared to 0% concentration of n-hexane. The addition of the ethanol and n-hexane extracts also affected the weight of the pupae formed. At extract concentrations of 1.25%, 2.50% or 5.00%, the weight of the pupal formed was lower than for the concentration of 0%. The addition of 2.50% or 5.00% ethanol and n-hexane extracts resulted in the longest pupal periods compared to the periods with 0% or 1.25% concentration. The total number of pupae formed were also affected by the addition of the ethanol and n-hexane extracts. At a concentration of 5.00%, the total pupal formed was lowest relative to concentrations of 0%, 1.25% and 2.50%.

Provision of plant leaf extracts containing active compounds such as saponins, flavonoids, alkaloids and tannins, influenced the growth of *Z. cucurbitae*. The addition of ethanol and n-hexane *P. guilfoylei* leaf extracts inhibitted the growth of the larvae and pupae of *Z. cucurbitae*. The addition of extract extended the larval periods and lowered the number of larvae that developed into pupae. Likewise, the pupal period was extended and the formation of adults was inhibited causing reduced growth and development for the next generation and a relatively longer emergence time for the next generation.

These results were consistent with the results of other research showing that the addition of hexane-based *Lantana camara* leaf extracts containing saponin and flavonoid against *Dysdercus koenigii*, reduced reproduction and fitness, fecundity and percentage hatchability in nests. The presence of saponin and flavonoid compounds increased the activity of juvenile hormones (Kayesth et al., 2019). Routine application of compounds containing flavonoid had negative effects on the biological activity of *Spodoptera frugiperda* by prolonging the larval development time, reducing larval and pupal weights and reducing pupal viability (Silva et al., 2016). In addition, it delayed the growth and development of *S. litura* and *Helicoverpa armigera* larvae by affecting physiological processes at the time of molting (Jadhav et al., 2012). Feeding diets containing flavonoids inhibited the growth and development activity of *S. litura* larva by reducing the activity of serine protease, trypsin and esterase (Su et al., 2017). The addition of tannins also inhibited the growth of *S. litura* larval by reducing the pupal weight and larval resistance and extending the larval period (Nomura and Itioka, 2002).

Table 5 shows that the addition of ethanol and n-hexane *P. guilfoylei* leaf extracts up to a concentration of 5.00% did not affect the percentage of adults that emerged but did affect the sex ratio of emergent *Z. cucurbitae* adults.

The addition of ethanol and n-hexane *P. guilfoylei* leaf extracts did not affect the number of adults that emerged compared to the controls. In addition, the amount of extract concentration did not affect the percentage of adults that emerged. Treatment using extract concentrations up to 5.00% caused similar percentage of adult emergence. However, the addition of 5.00% extract had a negative effect because it caused some pupae to fail to become adults.

Not all pupae developed into adults, especially ones treated with the ethanol and n-hexane *P. guilfoylei* leaf extracts at high concentrations. Pupae that failed to become adults were characterized by a black color; in addition, there were adults that emerged but with the pupal skin still attached or only half the adult body appeared (Fig. 1). This was consistent with Ginasti et al., (2020) that reported the provision of clove leaf oil at 0.25% caused abnormal imagoes, characterized by curly wings and individuals that still has pupal skin attached to parts of the body. Clove leaf oil contains tannins compounds that act as stomach toxins, which can inhibit the activity of digestive enzymes so that insects lack nutrients. Papantoniou et al. (2021) stated that there were only a few healthy individuals of adult *Manduca sexta* following treatment with the arbuscular mycorrhizal fungus *Rhizophagus irregularis* and the growth-

Fig. 1 Malformations of *Zeugodacus cucurbitae* stages after treatment with ethanol and n-hexane *Polyscias guilfoylei* leaf extracts:(A) normal adult; (B) pupae that failed to become adults, with color change to black; (C): abnormal adult with pupal skin still attached; (D) abnormal adult half emerged from pupa

promoting fungus *Trichoderma harzianum* Most of the adult insects exhibiting anomalous morphology and undeveloped wings. Arbuscular mycorrhizae contain large amounts of iridoid glycosides and flavonoids. *Trichoderma* inoculation can trigger higher levels of accumulation of alkaloids, flavonoids and phenolics and the presence of these secondary metabolic compounds can affect the performance of herbivorous insects.

Treatment of ethanol and n-hexane *P. guilfoylei* leaf extracts with concentrations up to 5.00% reduced the sex ratio of the adults that emerged because the ethanol and n-hexane *P. guilfoylei* leaf extracts contained secondary metabolite compounds that negatively affected the appearance of adults. This was supported by Kaur et al. (2021) who reported that the ratios of male to female insects of melon fly treated with neem oil, cedar oil and pongamia oil were lower than for the control, as neem oil, cedar oil and pongamia oil can be antifeedants, toxins, growth regulators and growth inhibitors against most plant pests. In addition, Ginasti et al. (2020) stated that neem seed oil and clove leaf oil could reduce the number of adults that successfully matured.

Treatment of ethanol-based *P. guilfoylei* leaf extracts at concentrations up to 2.50% produced a lower number of male adults than female adults, while at 5.00% concentration the number of male adults formed was higher than the number of female adults. In addition, n-hexane-based *P. guilfoylei* leaf extracts up to 5.00% produced a higher number of male adults than female adults. It could be expected that more male adults compared to female adults could affect the next generation of pests that would likely be lower, so pest infestation in the field would also be lower.

One of the factors affecting the sex ratio was the availability of food and nutrients. If the availability of food and nutrients is high, then the sex ratio is dominated by females. However, if the availability of food and nutrients is low, then the sex ratio is dominated by males. Treatment with the ethanol-based or n-hexane-based *P. guilfoylei* extracts at a concentration of 5.00% could inhibit feeding, resulting in more male adults than female adults. This was consistent with the results of Kaur et al. (2021) that showed that treatments of neem oil, cedar oil and pongamia oil at 1,000 parts per million produced more male adult melon flies than female adults. Likewise, the results of research by Papantoniou et al. (2021) showed that the addition of *R. irregularis* and *T. harzianum* produced more male adult *M. sexta* compared to female adults.

The current study showed that ethanol *P. guilfoylei* leaf extracts contained active compounds, such as phenols, tannins, alkaloids, flavonoids and saponins, while n-hexane *P. guilfoylei* leaf extracts contained terpenoids, steroids, phenols, tannins, alkaloids, flavonoids and saponins. Treatments of ethanol and n-hexane *P. guilfoylei* leaf extracts could inhibit the feeding activity; thus, these extracts could be categorized as antifeedants. In addition, the leaf extracts could inhibit the growth and development of the melon fly (*Z. cucurbitae*). Consequently, these bioactive properties suggested that *P. guilfoylei* leaf extracts could be used to inhibit the development process of the next generation of melon flies.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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