

# Evaluation of the insecticidal activity of *Solanum mammosum* (L.) fruit extract against *Drosophila melanogaster*



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**Abstract** *Solanum mammosum* (L.) is known as a poisonous plant and has been extensively used for anti-cancer, antifungal, antibacterial properties. However, the effectiveness of *Solanum mammosum* against insects has not been fully reported yet. To assess the ability of *Solanum mammosum* fruit extract against insects, we used fruit fly (*Drosophila melanogaster*) as a model. In this study, our results showed that phytochemicals and many secondary metabolites were present in *Solanum mammosum* fruit extracts such as alkaloids, saponins, tannins, terpenoids, coumarins, cardiac glycosides, steroids-triterpenoids, flavonoids, and phenolic compounds. The total polyphenol and flavonoid content were also determined to be  $275 \pm 1.89$  mg GAE/g extract and  $676 \pm 5.14$  mg QE/g extract, respectively. Besides, *Solanum mammosum* fruit extract caused lethal to 2<sup>nd</sup> instar, reduced a pupae formation rate of *Drosophila* larvae. Interestingly, *Solanum mammosum* extract is more effective in the suppression of pupae eclosion than Ascend as a commercial pesticide. These phenomena might be associated with reduced accumulation of energy, including carbohydrates, lipids, and protein. In addition, the activities of esterases and phosphatases were inhibited in *Drosophila* by the intake of *Solanum mammosum*. The study concluded that *Solanum mammosum* has great potential as an insecticidal agent.

**Keywords:** *Solanum mammosum* (L.), fruit fly, enzyme inhibition, esterase, phosphatase, phytochemical

## 1. Introduction

The high demand for agricultural products has led to the abuse of chemical pesticides at increasing concentrations (Rodrigues et al 2021). That causes more serious toxic effects on other beneficial organisms that co-exist with pests in the agricultural system, resulting in severe effects on human health and undesirable adverse effects on the environment. To limit the use of chemical pesticides, scientists have been researching biological pesticides that are environmentally friendly with less impact on human health and safer than chemical pesticides (Attaullah et al 2020). Screening studies for plant bioactive compounds have been investigated, including polyphenols, alkaloids, flavonoids, tannins, terpenoids, essential oils, steroids, lignin, and fatty acids. These compounds are secondary plant metabolites shown to be effective against a wide range of insect pests. Moreover, these compounds may have effects on the feeding, growth, survival, and distribution of many insects in nature or may have the ability to inhibit one or more molecules, such as esterases and phosphatases (Riaz et al 2018), carbohydrates (Kissoum et al 2020), lipids (Parkash and Aggarwal 2012) and proteins (Bensafi-Gheraibia et al 2021).

*Solanum mammosum* belongs to the family Solanaceae. Many species of this family are thought to be toxic due to the properties of alkaloids (Chowański et al 2016). The steroidal alkaloids and saponins were found and considered the main components of *S. mammosum* fruit (Alzérreca and Hart 1982; Lim 2013; Seelkopf 1968; Wong 2007). *S. mammosum* can synthesize sterol compounds such as cholesterol, sitosterol, stigmasterol, and campesterol (Indrayanto and Sutarjadi 1986; Pilaquinga et al 2021). Polyphenols and alkaloids are reported to be the two main groups of compounds present in *S. mammosum* that are both highly toxic to humans and have medicinal benefits. In addition, they are also teratogenic and considered precursors to pharmaceutical production of oral contraceptives, which has also been researched on anticancer, antifungal, anti-parasitic, or antibiotic properties (Hanelt et al 2001; Lim 2016; Roddick 1996; Telek et al 1977). Almost all the parts of *S. mammosum* are poisonous; therefore, they can be used to poison cockroaches and rats (Hanelt et al 2001; Mabblerley 2017).

In this study, *S. mammosum* was analyzed to identify its chemical components with toxic abilities on the fruit fly

model, thereby providing scientific evidence for the potential of insect resistance in applying biopesticide production.

## 2. Materials and Methods

Research work was performed at the College of Natural Sciences, Cantho University and Department of Functional Chemistry, Kyoto Institute of Technology, to investigate the efficacy of the *S. mammosum* fruit against *Drosophila melanogaster* (hereafter *Drosophila*).

### 2.1. Extraction preparation

*S. mammosum* fruit was collected in Can Tho city in February 2020. The plant species were identified by Dr. Nguyen Thi Kim Hue, Department of Biology, Faculty of Natural Sciences, Can Tho University, based on the Vietnamese plant classification system. After collection, the plant sample was washed, cut into small pieces, and dried naturally until its weight remained constant. The dried material was ground into a raw powder sample. 10 g of dried powder was extracted with 100 mL of ethanol (FUJIFILM-Wako Chemicals, Osaka, Japan) at room temperature for 24 hours, then filtered by filter paper (Advantec, No.1, Japan) to collect the supernatants. The residues were extracted three more times, and supernatants were combined. The *S. mammosum* fruit extracts were concentrated under pressure by using a rotary evaporator (Rotavapor R-300, BUCHI, Switzerland), freeze-dried, and then stored at 4 °C until use. Voucher specimens of *S. mammosum* were kept in the Laboratory of Plant Biology, Department of Biology, Can Tho University, Vietnam under code number SMf02.2020-CT007.

### 2.2. Preliminary qualification of chemical composition

The biologically active compounds such as alkaloids, flavonoids, saponins, phenolics, tannins, terpenoids, coumarins, cardiac glycosides, and steroids-triterpenoids were preliminarily analyzed according to the methods described by Sofowora et al (1993).

### 2.3. Quantification of polyphenols and flavonoids

The total polyphenol content of *S. mammosum* was determined using the modified Folin–Ciocalteu's assay (Singleton et al 1999). First, the reaction mixture consisted of 250 µL of extract, 250 µL of water, and 250 µL of Folin–Ciocalteu ethanol (FUJIFILM-Wako Chemicals) reagent was well shaken. Then, 250 µL of sodium carbonate solution (Na<sub>2</sub>CO<sub>3</sub>; 10%; w/v, FUJIFILM-Wako Chemicals) was added to the solution. The sample was mixed by vortex and incubated at 40 °C for 30 min. The absorbance at 756 nm was measured with a SH-1200 microplate reader (CORONA electric, Ibaraki, Japan). Gallic acid (FUJIFILM-Wako Chemicals) was employed as a positive control to make the standard curve equation. The total polyphenol content of *S. mammosum* was calculated using the gallic acid standard curve equation, and the results were expressed in milligrams of gallic acid equivalent (GAE) per gram weight of dried extract (mg GAE/g extract).

The total flavonoid content of *S. mammosum* was analyzed, following the aluminum chloride (AlCl<sub>3</sub>) colorimetric method with a minor modification (Bhaigyabati et al 2014). The reaction mixture, including 200 µL of extract or standard at the studied concentration and 200 µL of distilled water, was subjected to react with 40 µL of sodium nitrite (NaNO<sub>2</sub>; 5%; w/v; FUJIFILM-Wako Chemicals). The reaction mixture was mixed well and then allowed to stand for 5 min. After 5 min, continued to add 40 µL of the aluminum chloride (AlCl<sub>3</sub>; 10%; w/v; FUJIFILM-Wako Chemicals) to the mixture and mixed well. The reaction was mixed and incubated at room temperature for 6 min before 400 µL of 1 M sodium hydroxide (NaOH, FUJIFILM-Wako Chemicals) and distilled water were added to make up a volume of 1 mL. The absorbance at 510 nm was measured with a SH-1200 microplate reader. Quercetin (FUJIFILM-Wako Chemicals) was used as the positive control. Total flavonoid content was determined as mg/g quercetin equivalence (QE) of dried extract.

### 2.4. Feeding assay

The wild-type strain used was Canton-S (CS). CS strain was used in the study obtained from the Bloomington Stock Center at Indiana University (Bloomington, IN, USA). The fly stocks were reared at 25 °C on standard food (4% dry yeast, 9% cornmeal, 10% glucose, 0.8% agar, 0.5% propionic acid, and 0.05% ethyl parahydroxybenzoate) (Binh et al 2019). Thirty male and ten female (1:3) CS flies were mated and kept for 1 d at 25 °C, after which they were transferred to a new tube containing standard food for two hours to deposit eggs, to obtain a synchronized larval age (Binh et al 2019). During the desired larval growth period, 2<sup>nd</sup> instar larvae were collected for the assays.

The 2<sup>nd</sup> instar larvae were fed with standard food contained *S. mammosum* extracts (0 mg/mL and 50 mg/mL) and 2.5% (w/v) brilliant Blue R for 24 hours. Ten larvae were homogenized with 200 µL of ddH<sub>2</sub>O and then added 800 µL of ddH<sub>2</sub>O. The supernatant was collected by filter paper. The absorbance was measured with a SH-1200 microplate reader at 630 nm.

### 2.5. Mortality assay

The 2<sup>nd</sup> instar larvae of *Drosophila* were selected for experiments. Twenty 2<sup>nd</sup> instar larvae were fed with a standard medium containing *S. mammosum* extract at different concentrations (0, 25, 50, 75, 100, and 125 mg/mL) at three days. Ascend, the commercial insecticide was used as positive control us. Each experiment was repeated five times. The mortality rate was calculated following the formula as described previously (Riaz et al 2018).

$$\text{Mortality rate (\%)} = \frac{\text{number of dead larvae}}{\text{number of larvae tested}} \times 100 \quad (1)$$

### 2.6. Investigation of *S. mammosum* extract on the growth and development of fruit flies

Fifty male and thirty virgin female flies of CS strain were mated on standard food and kept for 1 d at 25 °C, after which they were transferred to a new tube containing standard food and *S. mammosum* extract (80 mg/mL) standard food without *S. mammosum* extract for 24 hours to deposit eggs. Ascend was employed as a positive control at a concentration of 30 µg/mL. The total number of pupae and mortality rate was counted and analyzed by formula (2) to evaluate the mortality rate of fruit flies (Riaz et al 2018).

$$\text{Mortality rate (\%)} = \frac{\text{number of dead flies}}{\text{number of pupae formed}} \times 100 \quad (2)$$

## 2.7. Energy storage components assays

The adult flies at 14-day-old fed with *S. mammosum* and controls were collected to determine the total content of the basic energy storage components such as carbohydrates, proteins, and total lipids.

For total carbohydrate contents, fifteen adult flies were homogenized with 1 mL of sterile distilled water. Samples were centrifuged at 10,000 rpm for 15 min. The supernatant was collected for total protein; the pellet was washed several times with 1 mL of sterile distilled water. And then, 3.2 mL sulfuric acid (H<sub>2</sub>SO<sub>4</sub>, 98.0%, FUJIFILM-Wako Chemicals) and 50 µL of phenol (C<sub>6</sub>H<sub>5</sub>OH, 99.0%, Sigma-Aldrich, MA, USA) were added in turn. The mixture was considered to shake well and allowed to stand for 30 min. Its spectral absorbance was estimated at 486 nm. The total carbohydrate content in the sample was determined based on the standard curve equation of glucose (Nielsen 2017).

For total protein, the supernatants obtained from carbohydrate assay were used. The reaction mixture consisted of 1 mL of sample solution and 2 mL of Bradford reagent. Its spectral absorbance was measured at 595 nm. Albumin (FUJIFILM-Wako Chemicals) was used as a positive control to form the equation of the standard curve (Bradford 1976)

For total lipid, fifteen adult flies were weighted ( $m_1$ ) and placed in the test vials and dried at 60 °C for 48 h. After drying, those flies were weighed ( $m_2$ ). Then, 1.5 mL diethyl ether (Sigma-Aldrich) was added and shaken at 200 rpm for 24 h at room temperature. The solvent was removed, and the fruit flies were again dried at 60 °C for 24 h. The final weight was determined ( $m_3$ ). The relative lipid content was calculated according to the following formula (Parkash and Aggarwal 2012).

$$\text{Lipid (\%)} = \frac{(m_2 - m_0) - (m_3 - m_0)}{(m_1 - m_0)} \times 100 \quad (3)$$

where:  $m_0$  prefers to the weight of the test vial;  $m_1$  represents the initial weight;  $m_2$  represents the weight after 48 h of drying;  $m_3$  represents the final weight.

## 2.8. Enzyme assays

The adult flies at 14-day-old fed with *S. mammosum* and controls were collected to evaluate the esterase and phosphatase inhibitory activities. The fruit flies were

simultaneously washed and homogenized in 0.5 mL of 20 mM (pH 7.0) cold sodium phosphate buffer (PBS) and then centrifuged at 8,000 rpm at 4 °C for 20 min. The supernatant was collected to analyze the activities of esterases (acetylcholinesterase, carboxylesterase,  $\alpha$ -carboxylesterase) and phosphatases (phosphatase acid and alkaline phosphatase).

For acetylcholinesterase (AChE) activity: AChE activity was determined according to the method of Riaz et al (2018). The reaction mixture consisted of 50 µL of sample solution, 50 µL of 2.6 mM acetylcholine iodide (ATCI, FUJIFILM-Wako Chemicals), and 1 mL of 20 mM (pH 7.0) PBS was incubated at 25 °C for 5 min. Then added 400 µL of Fast Blue B salt (C<sub>14</sub>H<sub>12</sub>N<sub>4</sub>O<sub>2</sub>Cl<sub>2</sub>·ZnCl<sub>2</sub>, 0.3% w/v, FUJIFILM-Wako Chemicals) to the mixture to stop the reaction. The spectral absorbance of the reaction mixture was measured at 405 nm.

For carboxylesterase activity:  $\alpha$ -carboxylesterase and  $\beta$ -carboxylesterase activities were determined as described previously (Riaz et al 2018). The reaction mixture, including 50 µL of sample solution, 1 mL of 20 mM (pH 7.0) PBS, 50 µL of  $\alpha$ -naphthyl acetate or  $\beta$ -naphthyl acetate as substrates (Sisco Research Laboratories Ltd., Telangana, India), was added separately to determine the amount of  $\alpha$ -carboxylesterase and  $\beta$ -carboxylesterase, respectively. The reaction mixture was incubated at 30 °C for 20 min. After incubation, the reaction mixture was added 400 µL of Fast Blue B (0.3% w/v, FUJIFILM-Wako Chemicals) mixed in sodium dodecyl sulfate (SDS, 3.3% w/v) to stop the enzymatic reaction. Then the mixture was kept standing for 15 min at 20 °C. Its spectral absorbance was determined at 430 or 590 nm for  $\alpha$ -carboxylesterase or  $\beta$ -carboxylesterase, respectively.

For acid phosphatase and alkaline phosphatase activities: Acid phosphatase (AcP) and alkaline phosphatase (AkP) activities were determined according to the method of Riaz et al. (Riaz et al 2018). For the estimation of AcP activity, 50 µL of sample solution was mixed with 50 µL of 50 mM (pH 7.0) sodium phosphate buffer (PBS) and 100 µL of 20 mM *p*-nitrophenyl phosphate as substrate (Sisco Research Laboratories Ltd.). For the estimation of AkP activity, 50 µL of sample solution was mixed with 50 µL of 50 mM (pH 9.0) Tris-HCl buffer and 100 µL of 20 mM *p*-nitrophenyl phosphate. Both AcP and AkP solutions were then incubated at 37 °C for 15 min. The enzymatic reaction was stopped by adding 0.5 N sodium hydroxide (NaOH, FUJIFILM-Wako Chemicals). The absorbance at 440 nm ( $A_{440}$  nm) was measured using a microplate reader (Model SH-1200 Lab, Corona Electric, Japan). The percent inhibition (PI) of enzyme activity was calculated using the following equation:

$$\% \text{ inhibition} = \frac{(A_{440} \text{ nm of control} - A_{440} \text{ nm of test sample})}{(A_{440} \text{ nm of control} - A_{440} \text{ nm of blank})} \times 100 \quad (4)$$

## 2.9. Statistical Analysis

The experiments were repeated at least three times. Excel 2016 and GraphPad Prism version 9.2.0 (332) software were used for statistical analysis and figure preparation.

Results are expressed as mean  $\pm$  standard deviation (SD). Differences among means were tested for statistical significance using one-way analysis of variance (ANOVA) followed by Tukey's multiple tests. Statistical significance was set at  $P < 0.05$

### 3. Results and Discussion

#### 3.1. Phytochemical components of *S. mammosum* fruit ethanol extract

Preliminary qualification result of chemical composition in *S. mammosum* extract indicated the presence of alkaloids, flavonoids, saponins, phenolics, tannins, terpenoids, coumarins, cardiac glycosides, and steroids-triterpenoids. These above compounds have also been reported to have a wide range of biological activities, including the toxic activity on the *Drosophila* fruit fly model (Luna et al 2021; Quijano et al 2014; Riaz et al 2018; Valéria Soares de Araújo Pinho et al 2014).

The total phenolic and flavonoids in the ethanol fruit extract from *S. mammosum* were observed to be  $275 \pm 1.89$  mg GAE/g dried extract and  $676 \pm 5.14$  mg QE/g dried extract, respectively. These results indicated that the extract from fruit consists of a high amount of phenolic and flavonoids, which is consistent with the total content of phenolic and flavonoids in the leaf extract of *S. mammosum* and another species of the *Solanaceae* family (Kudale et al 2016; Pilaquinga et al 2021). Interestingly, a previous study demonstrated that *Solanum lycopersicum* roots contained a high amount of phenolic compounds that might be prohibited the growth of insects such as *Helicoverpa armigera* and *Spodoptera litura* (Singh et al 2014). In addition, flavonoids could inhibit enzyme activity and prevent larval development of various insects (Structures 2000). Therefore, new types of pesticides are currently being developed from natural sources that use flavonoids as the key ingredients to alternative synthetic pesticides. In this study, we found that the fruit extract of *S. mammosum*

possessed an enormous amount of phenolic and flavonoids, suggesting that this extract may have the potential for insect resistance.

#### 3.2. Effect of *S. mammosum* extract on mortality of 2<sup>nd</sup> instar larvae of *Drosophila*

The potential of *S. mammosum* against insect development was assessed using *Drosophila* as a model. To test whether 2<sup>nd</sup> instar larvae can consume the food containing *S. mammosum* extract, the feeding ability was indirectly evaluated through absorbance of brilliant Blue R by feeding assay. These results indicated that the intake of the standard food contained *S. mammosum* extract had no significant difference to standard food without *S. mammosum* extract, implying that *S. mammosum* extract was insensitive to the *Drosophila* larvae (data not shown).

The toxicity of *S. mammosum* extract was examined by calculating the mortality rate of *Drosophila* larvae that were fed with standard food containing various *S. mammosum* concentrations. Our results showed that *S. mammosum* extract act on the duration of larval development and larval mortality in a dose-dependent manner. The three concentrations (75 – 125 mg/mL) showed high larvicidal activity at the end of mortality monitoring (3 days after treatment). Notably, the mortality rate of *Drosophila* 2<sup>nd</sup> instar larvae was increased by 78.9 % at a *S. mammosum* extract concentration of 125 mg/mL (Figure 1A). As shown in table 1, a lethal dose (LD<sub>50</sub>) of the extract is required to kill about 50% of *Drosophila* larvae was 80 mg/mL (equivalent to 27.1  $\mu$ g/mL Ascend). A previous study demonstrated that the fruit extract of *S. mammosum* has toxicity to *Aedes aegypti* mosquito larvae (Pilaquinga et al 2021). Besides, Bagu et al (2020) reported that *Ximenia americana* extract could kill the fruit fly at 327.7 mg/mL, which is extremely higher than *S. mammosum* fruit extract. These results imply that *S. mammosum* is a good candidate for use as insect resistance.

**Table 1** LD<sub>50</sub> of *S. mammosum* fruit extract and Ascend insecticide.

Treatments	Linear regression equation	LD <sub>50</sub> value
Ascend ( $\mu$ g/mL)	$y = 1.5719x - 1.2125$	27.1
	$R^2 = 0.9964$	
<i>S. mammosum</i> (mg/mL)	$y = 610.79x + 3.8624$	80
	$R^2 = 0.9899$	

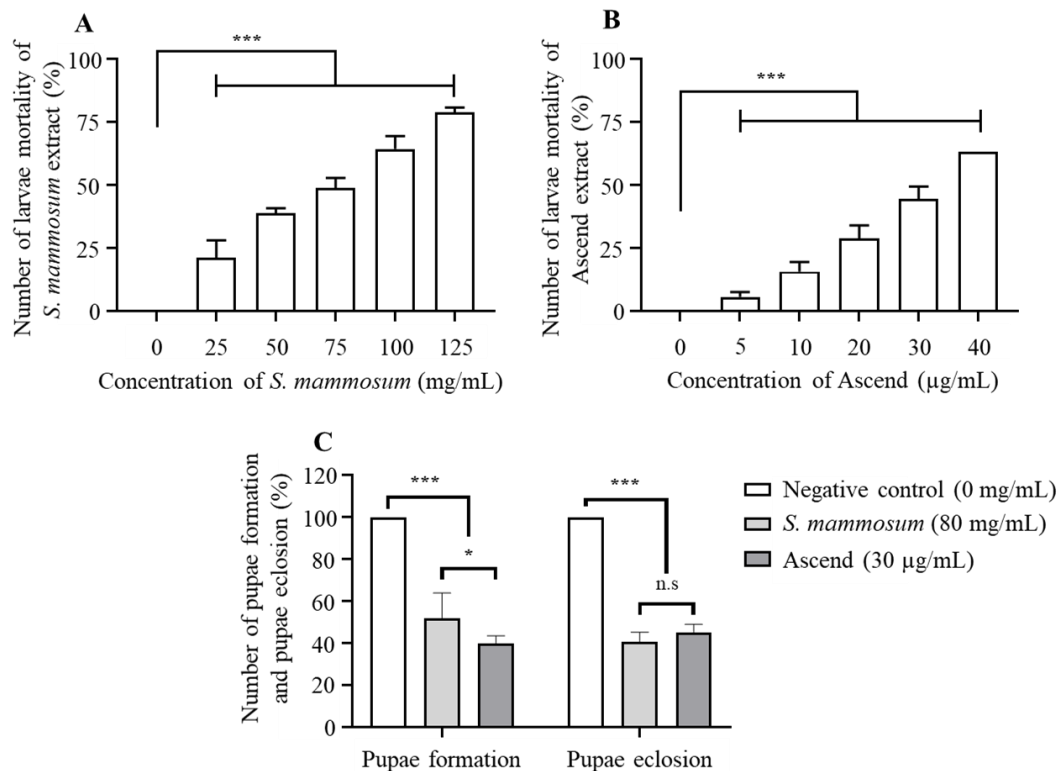
#### 3.3. Effect of *S. mammosum* extract on the *Drosophila* development

To access the ability of *S. mammosum* fruit extract on the growth of *Drosophila*, the formation and eclosion of pupae were analyzed. The results indicated that *S. mammosum* fruit extract significant reduced the development of *Drosophila* after 14 days of treatment. As shown in Figure 1C, the pupae formation of *Drosophila* was

significantly suppressed up to 48.3% at an 80 mg/mL concentration of *S. mammosum* extract, compared to the negative control. At an Ascend concentration of 30 mg/mL, the formation of pupae was inhibited by 60.1%. The decrease in pupae formation induced by intake of *S. mammosum* extract may relate to the accumulation of energy in the larvae stage of *Drosophila*. In addition, *S. mammosum* extract was more effective than Ascend for preventing pupae eclosion of

*Drosophila*. The mortality rate of *Drosophila* pupae was 59.5% in *S. mammosum* extract and 54.7% in Ascend (Figure 1C). These findings suggest that the *S. mammosum* extract

may inhibit energy storage and contain harmful compounds to the growth of insects.



**Figure 1** *S. mammosum* fruit extract suppresses the development of fruit fly. The 2<sup>nd</sup> instar larvae were fed standard food added varying concentrations of *S. mammosum* extract. Ascend was used as a positive control. The number of larvae was calculated after three days to confirm the mortality of fruit fly larvae (A, B). The number of larvae was analyzed after seven days to evaluate the pupae formation, while the pupae eclosion was evaluated after ten days (C). Data are expressed as means  $\pm$  SD. Statistical significance was calculated by one-way ANOVA. n.s, not significant; \*  $P < 0.05$ ; \*\*\*  $P < 0.001$ .

### 3.4. Effect of *S. mammosum* extract on the accumulation of energy *Drosophila* larvae.

Previous studies reported that polyphenols and flavonoid compounds extracted from plants might affect the food intake of several insects (Franzetti et al 2015; Peres et al 2017). To clarify these points, we analyzed the basic energy storage components, including carbohydrates, lipids, and proteins. The energy storage of adult *Drosophila* was significantly decreased by feeding with *S. mammosum* extract at 80 mg/mL after 14 days. As shown in Figure 2 indicated that carbohydrate, lipid, and protein content in flies fed with *S. mammosum* extract were 208.0  $\mu\text{g/mL}$ , 4.0  $\mu\text{g/mL}$  and 49.1  $\mu\text{g/mL}$ , respectively, compared to Ascend (524.0  $\mu\text{g/mL}$ , 5.6  $\mu\text{g/mL}$  and 62.8  $\mu\text{g/mL}$ , respectively) and negative control (723.0  $\mu\text{g/mL}$ , 13.0  $\mu\text{g/mL}$  and 96.6  $\mu\text{g/mL}$ , respectively). Hamida et al (2021) reported that the commercial Oberon insecticide inhibited the lipid synthesis and affected the sexual behavior and fertility of *Drosophila*. The energy storage content in the *Drosophila* ovary was also decreased by feeding with this insecticide (Kissoum et al 2020). It seems reasonable to assume that *S. mammosum* fruit extract not only contains harmful compounds and but suppresses the accumulation of carbohydrate, lipid, and

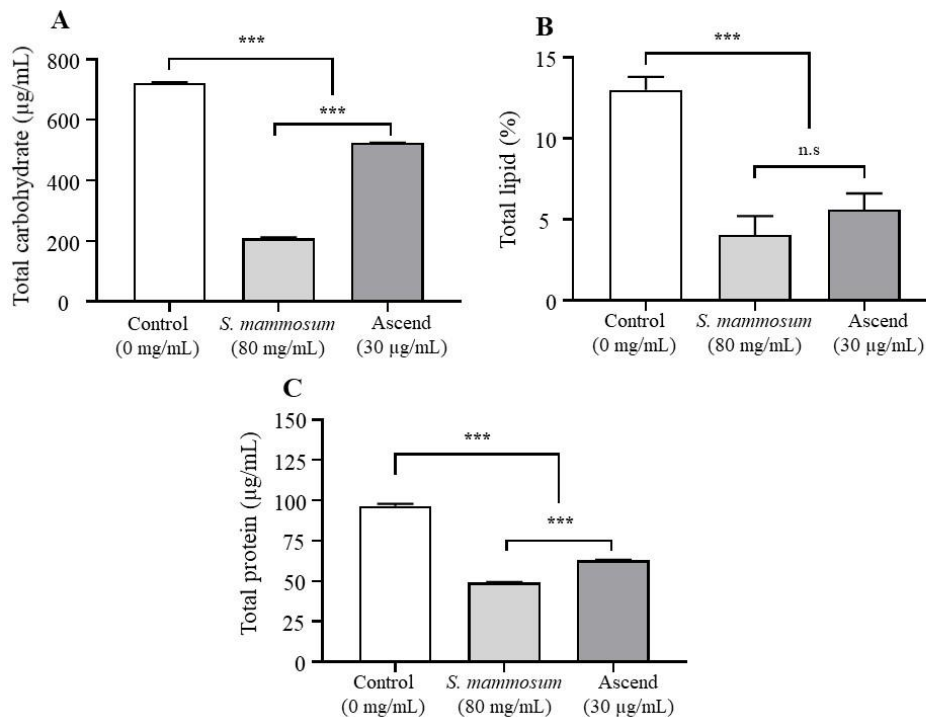
protein content that is essential for producing pheromones of insects, thereby preventing insect growth. This evidence enforces our hypothesis that *S. mammosum* fruit extract has the ability to be used as an insecticide.

### 3.5. Effect of *S. mammosum* extract on the activity of esterase and phosphatase

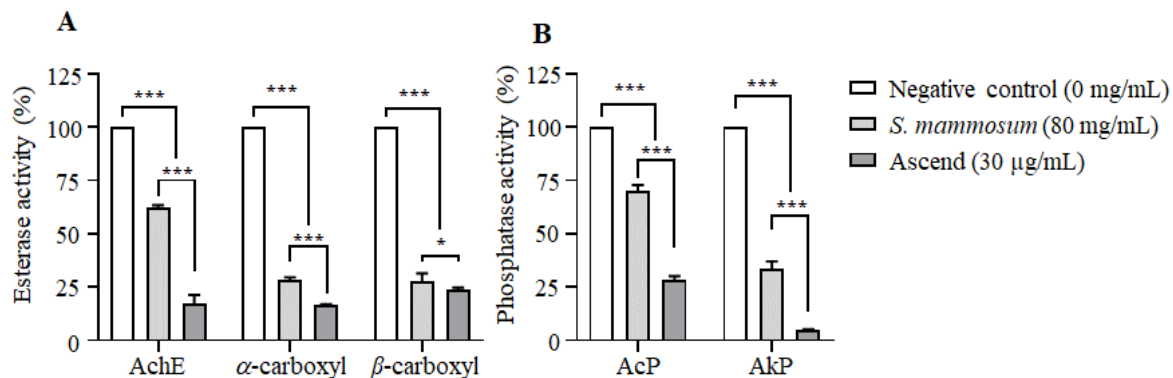
The current studies reported that insecticides that are origin from plant extracts suppress the growth of insects by inhibiting enzyme activity (Attaullah et al 2020; Menozzi et al 2004; Riaz et al 2018; Senthil Nathan et al 2008). To further evaluate the effect of *S. mammosum* extract on the enzyme activity, the adult flies at 14-day-old that fed with *S. mammosum* (80 mg/mL) were collected and analyzed the activity of AChE,  $\alpha$ - and  $\beta$ -carboxyl, AcP, and AkP. The results demonstrated that *S. mammosum* extract had the ability to inhibit the activity of these enzymes at a concentration of 80 mg/mL. Figure 3 indicated that the fruit extract of *S. mammosum* reduced the activity of AChE,  $\alpha$ - and  $\beta$ -carboxyl AcP, and AkP by 62.3%, 28.5%, and 27.57%, 70.1%, and 33.5%, respectively. Attaullah et al (2020) demonstrated that reduced activity of enzymes AChE, AcP, AkP,  $\alpha$ -carboxyl, and  $\beta$ -carboxyl could suppress the development of *Musca*

domestica larvae. To control the population of *Drosophila*, Menozzi et al (2004) suppressed the activation AchE by deleting Ace gene encoding AchE in 30 strains of *Drosophila*. Moreover, Younes et al (2011) indicated that the plant oils from several culinary and medicinal plants could be against *Trogoderma granarium* development by inhibiting the

activity of AcP and AkP. These data implied that *S. mammosum* extract induced mortality 2nd instar larvae inhibited the formation of pupae, reduced pupae eclosion might associate with suppressing the activity of these enzymes.



**Figure 2** *S. mammosum* fruit extract reduces the accumulation of energy on *Drosophila*. The total carbohydrate (A), lipid (B) and protein (C) were analyzed ( $n = 3$ , 100 flies per replicate). Data are expressed as means  $\pm$  SD. Statistical significance was calculated by one-way ANOVA. n.s, not significant, \*\*\*  $P < 0.001$ .



**Figure 3** Intake of *S. mammosum* fruit extract inhibits the activity of esterase and phosphatase on *Drosophila*. The esterase activity (A) and phosphatase activity were analyzed ( $n = 3$ , 100 flies per replicate). Data are expressed as means  $\pm$  SD. Statistical significance was calculated by one-way ANOVA. n.s, not significant, \*  $P < 0.05$ ; \*\*\*  $P < 0.001$ .

**4. Conclusions**

Our study indicated the phytochemical components of *S. mammosum* fruit extract, such as alkaloids, flavonoids, saponins, phenolics, tannins, terpenoids, coumarins, cardiac glycosides, and steroids-triterpenoids. Besides, total polyphenol and flavonoid content were determined to be  $275 \pm 1.89$  mg GAE/g extract and  $676 \pm 5.14$  mg QE/g extract,

respectively. The *S. mammosum* fruit extract might suppress the development of the fruit fly by preventing the accumulation of energy and inhibiting the activity of esterase and phosphatase. These results suggest that *S. mammosum* extract possesses potential properties to use as an insect pesticide and management. Thus, further research is needed to isolate active compounds and evaluate their effects on insects.

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## Conflict of Interest

The authors declare that they have no competing interests.

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## References

- Alzérreca A, Hart G (1982) Molluscicidal steroid glycoalkaloids possessing stereoisomeric spirosolane structures. *Toxicol. Lett.* 12:151–155.
- Attaullah, Zahoor MK, Zahoor MA, Mubarak MS, Rizvi H, Majeed HN, Zulhussnain M, Ranian K, Sultana K, Imran M, Qamer S (2020) Insecticidal, biological and biochemical response of *Musca domestica* (Diptera: Muscidae) to some indigenous weed plant extracts. *Saudi J. Biol. Sci.* 27:106–116.
- Bagu GD, Omale S, Iorjiim WM, Uguru MO, Gyang SS (2020) Determination of LD<sub>50</sub>, fecundity and locomotor effects of methanol root extract of *Ximenia americana* (L.), in *Drosophila melanogaster*. *Asian J. Biochem. Genet. Mol. Biol.* 5:1–9.
- Bensafi-Gheraibia H, Kissoum N, Hamida ZC, Farine JP, Soltani N (2021) Topical bioassay of Oberon® on *Drosophila melanogaster* pupae: delayed effects on ovarian proteins, cuticular hydrocarbons and sexual behavior. *Invertebr. Reprod. Dev.* 65:35–47.
- Bhaigyabati T, Devi PG, Bag GC (2014) Total flavonoid content and antioxidant activity of aqueous rhizome extract of three *Hedychium* species of Manipur valley. *Res. J. Pharm. Biol. Chem. Sci.* 5:970–976.
- Binh TD, Pham TLA, Men TT, Dang TTP, Kamei K (2019) LSD-2 dysfunction induces dFoxO-dependent cell death in the wing of *Drosophila melanogaster*. *Biochem. Biophys. Res. Commun.* 509:491–497.
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72:248–254.
- Chowrański S, Adamski Z, Marciniak P, Rosiński G, Büyükgüzel E, Büyükgüzel K, Falabella P, Scranò L, Ventrella E, Lelario F, Bufo SA (2016) A review of bioinsecticidal activity of Solanaceae alkaloids. *Toxins (Basel)*. 8:1–28.
- Binh TD, Pham TLA, Nishihara T, Men TT, Kamei K (2019) The function of Lipin in the wing development of *Drosophila melanogaster*. *Int. J. Mol. Sci.* 20:3288.
- Franzetti E, Romanelli D, Caccia S, Cappellozza S, Congiu T, Rajagopalan M, Grimaldi A, de Eguileor M, Casartelli M, Tettamanti G (2015) The midgut of the silkworm *Bombyx mori* is able to recycle molecules derived from degeneration of the larval midgut epithelium. *Cell Tissue Res.* 361:509–528.
- Hamida ZC, Farine JP, Ferveur JF, Soltani N (2021) Pre-imaginal exposure to Oberon® disrupts fatty acid composition, cuticular hydrocarbon profile and sexual behavior in *Drosophila melanogaster* adults. *Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol.* 243:108981.
- Hanelt P, Büttner R, Mansfeld R (2001) *Mansfeld's encyclopedia of agricultural and horticultural crops*. Springer Berlin, Heidelberg.
- Indrayanto G, Sutarjadi I (1986) Sterols in callus cultures of *Solanum mammosum*. *Planta Med.* 5:413.
- Kissoum N, Bensafi-Gheraibia H, Hamida ZC, Soltani N (2020) Evaluation of the pesticide Oberon on a model organism *Drosophila melanogaster* via topical toxicity test on biochemical and reproductive parameters. *Comp. Biochem. Physiol. Part - C Toxicol. Pharmacol.* 228:108666
- Kudale S, Ghatge S, Shivekar A, Sule C, Desai N (2016) Comparative study of antioxidant potential in hairy roots and field grown roots of *Solanum nigrum* L. *Int. J. Curr. Microbiol. Appl. Sci.* 5:42–54.
- Lim TK (2016) *Edible medicinal and non-medicinal plants, edible medicinal and non-medicinal plants*. Springer, Dordrecht.
- Lim TK (2013) *Edible medicinal and non-medicinal plants: Volume 6, fruits*. *Edible Med. Non-Medicinal Plants.* 6:1–606.
- Luna EM, Freitas TS, Campina FF, Costa MS, Rocha JE, Cruz RP, Sena Júnior DL, Silveira ZS, Macedo NS, Pinheiro JCA, Pereira-Júnior FN, Lisboa MAN, Cruz GV, Calixto Júnior JT, Teixeira AMR, Coutinho HDM (2021) Evaluation of phytochemical composition, toxicity in *Drosophila melanogaster* and effects on antibiotics modulation of *Plathymenia reticulata* (B.) extract. *Toxicol. Reports* 8:732–739.
- Mabberley DJ (2017) *Mabberley's Plant-book: A portable dictionary of plants, their classification and uses*, 4th ed. Cambridge University Press, Cambridge.
- Menozi P, Shi MA, Lougarre A, Tang ZH, Fournier D (2004) Mutations of acetylcholinesterase which confer insecticide resistance in *Drosophila melanogaster* populations. *BMC Evol. Biol.* 4:4.
- Nielsen SS (2017) Total carbohydrate by phenol-sulfuric acid method. In: *Food analysis laboratory manual*. Food Science Text Series. Springer, Cham.
- Parkash R, Aggarwal DD (2012) Trade-off of energy metabolites as well as body color phenotypes for starvation and desiccation resistance in montane populations of *Drosophila melanogaster*. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* 161:102–113.
- Peres LLS, Sobreiro AI, Couto IFS, Silva RM, Pereira FF, Heredia-Vieira SC, Cardoso CAL, Mauad M, Scalón SPQ, Verza SS, Mussury RM (2017) Chemical compounds and bioactivity of aqueous extracts of *Alibertia* spp. in the control of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). *Insects* 8:125.
- Pilaquinga F, Morey J, Fernandez L, Espinoza-Montero P, Moncada-Basualto M, Pozo-Martinez J, Olea-Azar C, Bosch R, Meneses L, Debut A, Piña MN (2021) Determination of antioxidant activity by oxygen radical absorbance capacity, cellular antioxidant activity, electrochemical and microbiological analyses of silver nanoparticles using the aqueous leaf extract of *Solanum mammosum* (L.). *Int. J. Nanomedicine* 16:5879–5894.
- Quijano M, Riera-Ruiz C, Barragán A, Miranda M, Orellana T, Manzano P (2014) Molluscicidal activity of the aqueous extracts from *Solanum mammosum* (L.), *Sapindus saponaria* (L.) and *Jatropha curcas* (L.), against *Pomacea canaliculata*. *Emirates J. Food Agric.* 26:871–877.
- Riaz B, Zahoor M, Majeed HN, Javed I, Ahmad A, Jabeen F, Zulhussnain M, Sultana K (2018) Toxicity, phytochemical composition, and enzyme inhibitory activities of some indigenous weed plant extracts in fruit fly, *Drosophila melanogaster*. *Evid. Based. Complement. Alternat. Med.* 2018:11
- Roddick JG (1996) Steroidal glycoalkaloids: nature and consequences of bioactivity-saponins used in traditional and modern medicine, In: Waller, G.R., Yamasaki, K. (Eds.), Springer US, Boston, MA, 277–295.
- Rodrigues GCS, Dos Santos Maia M, Silva Cavalcanti AB, de Sousa NF, Scotti MT, Scotti, L (2021) In silico studies of *lamiaceae diterpenes* with bioinsecticide potential against *Aphis gossypii* and *Drosophila melanogaster*. *Molecules.* 26:766
- Seelkopf C (1968) Alkaloid glycosides of the fruit from *Solanum mammosum* (L.). *Arch. Pharm. Ber. Dtsch. Pharm. Ges.* 301:111–114.
- Senthil NS, Young CM, Yul SH, Hoon PC, Kalaivani K, Duk KJ (2008) Effect of azadirachtin on acetylcholinesterase (AChE) activity and histology of the brown planthopper *Nilaparvata lugens*. *Ecotoxicol. Environ. Saf.* 70:244–250.
- Singh H, Dixit S, Verma PC, Singh PK (2014) Evaluation of total phenolic compounds and insecticidal and antioxidant activities of tomato hairy root extract. *J. Agric. Food Chem.* 62:2588–2594.
- Singleton VL, Orthofer R, Lamuela-Raventós RMBTME (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent, In: *Oxidants and Antioxidants Part A*. Academic Press. 152–178.
- Sofowora A (1993) Recent trends in research into African medicinal plants. *J. Ethnopharmacol.* 38:197–208.
- Structures MB (2000) Inhibition of  $\alpha$ -glucosidase and amylase by luteolin, a flavonoid. *Biosci. Biotechnol. Biochem.* 64:2458–2461.
- Telek L, Delpin H, Cabanillas E (1977) *Solanum mammosum* as a source of solasodine in the lowland tropics. *Econ. Bot.* 31:120–128.

Valéria Soares de Araújo Pinho F, Felipe da Silva G, Echeverria Macedo G, Raquel Muller K, Kemmerich Martins I, Lausmann Ternes AP, Martins da Costa JG, Athayde ML, Boligon AA, Kamdem JP, Franco JL, Rose Alencar de Menezes I, Posser T (2014) Phytochemical constituents and toxicity of *Duguetia furfuracea* hydroalcoholic extract in *Drosophila melanogaster* (2014) Evid. Based. Complement. Alternat. Med. 2014:838101.

Wong CC (2007) Isolation of saponins from *Solanum mammosum* and characterization of their anticancer activity by proteomics. Dissertation.

Hong Kong University.

Younes MWF, El Othman ME, Youssef NS, Omar GA, Salah A (2011) Effect of seven plant oils on some biochemical parameters in *Khapra beetle*, *Trogoderma granarium* (E.) Coleoptera: Dermestidae. J. Exp. Biol. 7:53-61.