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Short Communication

Exploring the antidiabetic potential of Sulawesi ethnomedicines: A study of *Cordia myxa* and *Syzygium malaccense* in a *Drosophila* model of hyperglycemia

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Abstract

The escalating prevalence of diabetes represents a critical challenge to global health and quality of life. Indonesia, particularly the Sulawesi region, is home to a diverse array of endemic plants with potential as sources of novel antidiabetic compounds. However, traditional preclinical models for evaluating these candidates are limited by high costs and lengthy timelines. The aim of this study was to explore the antidiabetic potential of *Cordia myxa* and *Syzygium malaccense* extracts using *Drosophila melanogaster* as a novel, cost-effective and efficient in vivo model. Hyperglycemia was induced in *D. melanogaster* larvae through a high-sugar diet, and the plant extracts were incorporated into the larval diets at concentrations ranging from 0.3125% to 2.5%. Phenotypic parameters, including body size, body weight, crawling activity, and hemolymph glucose levels, were evaluated, and the expression of metabolism-related genes (*dilp2, dilp5*, and *srl*) was analyzed using RT-qPCR. This study found that *C. myxa* and *S. malaccense* extracts improved crawling activity and body size in hyperglycemic larvae. Notably, *C. myxa* extract significantly reduced hemolymph glucose levels ($p<0.01$), increased body weight ($p<0.01$), and upregulated the expression of metabolic genes such as *dilp2* ($p<0.001$), *dilp5* ($p<0.001$), and *srl* ($p<0.0001$). In contrast, *S. malaccense* extract showed less pronounced effects, highlighting the efficacy of *C. myxa* extract in alleviating hyperglycemia and restoring metabolic homeostasis. The study highlights that *C. myxa* extract demonstrated promising antidiabetic properties in the *Drosophila* model, underscoring the utility of this model for early-stage antidiabetic drug screening and supporting further preclinical investigation into the therapeutic potential of *C. myxa* for managing hyperglycemia.

Keywords: Diabetes, *Cordia myxa*, *Syzygium malaccense*, *Drosophila*, larvae

Introduction

Diabetes, a chronic disease, is recognized as one of the top ten leading causes of death worldwide, garnering considerable attention in global health initiatives [1]. According to the International Diabetes Federation, the prevalence of diabetes has been steadily increasing, with

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Southeast Asia reporting the second-highest incidence globally as of 2021 [2]. In Indonesia, the prevalence of diabetes is rising significantly, with cases projected to reach 28.57 million by 2045 [2]. Antidiabetic drugs are essential for regulating blood glucose levels [3], mitigating microvascular and macrovascular complications [4], and reducing healthcare costs [5]. Although synthetic drugs are a cornerstone of diabetes management, their long-term use is frequently associated with side effects, including hypoglycemia, gastrointestinal disturbances, edema, and weight gain [6]. Consequently, natural product-based therapies are emerging as promising alternatives, offering the potential for safer and more sustainable long-term use.

Several endemic plants from Sulawesi have been traditionally recognized for their medicinal properties, supported by empirical evidence [7]. Among these, extracts of *Cordia myxa* and *Syzygium malaccense* have been reported to exhibit antihyperglycemic effects [8,9]. However, rigorous scientific evaluation, particularly regarding safety and efficacy, is essential to facilitate their application in therapeutic contexts. In vitro testing, while valuable for preliminary screening, often fails to replicate the intricate metabolic regulation that occurs in vivo across multiple tissues and organs [10]. As a result, many promising candidates identified through in vitro studies demonstrate limited efficacy in vivo, leading to substantial investments of time and resources and perpetuating reliance on mammalian models [11,12]. To overcome these limitations, the development of robust and cost-effective in vivo screening platforms is crucial [12].

Drosophila melanogaster has emerged as a powerful alternative for in vivo drug screening, particularly for evaluating antidiabetic drug candidates [13-15]. This model offers several advantages, including shorter testing durations and the absence of ethical approval requirements typically associated with vertebrate models [16-18]. Previous studies have demonstrated the utility of *D. melanogaster* for pharmacological assessments of both natural and synthetic compounds, including extracts from rosella [19,20], caffeine [21], vitamin D3 [22], aspirin, and curcumin [23]. Moreover, *D. melanogaster* has been widely employed to investigate antidiabetic activity, including studies on traditional Chinese medicine [13,24].

The aim of this study was to evaluate the antidiabetic effects of *C. myxa* and *S. malaccense* extracts using *D. melanogaster* as a model for diet-induced diabetes. The primary objective was to assess the impact of these extracts on key phenotypic parameters, including body size, body weight, locomotor activity, hemolymph glucose levels, and the expression of metabolism-related genes. By focusing on natural compounds, this study seeks to contribute to the identification and development of potential therapeutic candidates for diabetes management. Promising extracts identified through this model can then proceed to mammalian in vivo testing to confirm efficacy and safety, conserving resources in early-stage research.

Methods

Study design and setting

This experimental pre-clinical study employed a post-test only control design and utilized *D. melanogaster* w^{1118}, kindly provided by Prof. Yoshinobu Nakanishi from the Laboratory of Host Defense and Responses, Kanazawa University, Kanazawa, Japan. The research was conducted from August to December 2024 at the Laboratory of Pharmacology-Toxicology and the Laboratory of Biofarmaka, Faculty of Pharmacy, Universitas Hasanuddin, Makassar, Indonesia. Most of the experiments were carried out using third-instar larvae, while developmental toxicity assay was specifically conducted on second-instar larvae. The experiments were carried out in *Drosophila* culture vials containing either a standard or high-sugar diet (HSD). Larvae were randomly allocated to experimental groups: one group served as the control and was fed a standard diet, while the other groups received HSD in the presence or absence of *C. myxa* or *S. malaccense* extracts. HSD was prepared by increasing the sucrose concentration to 30% above the standard levels. The hyperglycemia state was determined by monitoring developmental growth, larval size and weight, crawling activity, hemolymph glucose concentrations, and the expression of key metabolic genes (*dilp2, dilp5*, and *srl*). Developmental growth was assessed by determining the percentage of larvae that progressed to the pupal stage, and the percentage of pupae that advanced to adulthood after treatment with *C. myxa* or *S. malaccense* extracts. Larval

growth was evaluated by measuring size, weight, length, and width. Locomotor performance was measured using a crawling assay, while hyperglycemia was assessed by measuring the glucose levels in hemolymph samples. Additionally, gene expression analysis was performed via reverse transcription quantitative polymerase chain reaction (RT-qPCR) to examine the expression of genes involved in metabolic processes (*dilp2, dilp5*, and *srl*). The measurement of metabolism-related gene expression was performed only for the groups where glucose levels were significantly affected. A summary of the experimental design is presented in **Figure 1**.

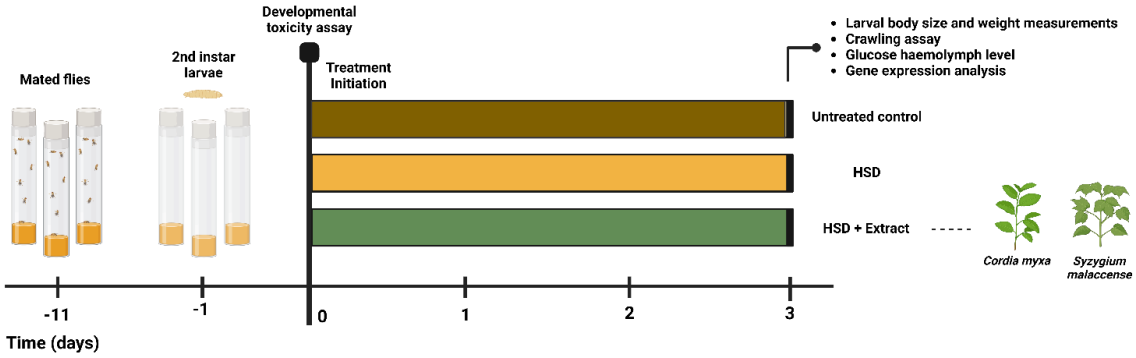

Figure 1. Experimental design to assess the antidiabetic potential of *Cordia myxa* and *Syzygium malaccense* extracts using *Drosophila melanogaster* larvae. A total of 15 males and 15 females were mated in 15 vials per group for approximately ten days to produce larvae. Each vial contained a diet supplemented with varying concentrations of *C. myxa* and *S. malaccense* extracts. Control groups included an untreated control and a high-sugar diet (HSD) group. Larvae were then analyzed for various phenotypic and metabolic outcomes to evaluate the antidiabetic effects of the extracts.

C. myxa and *S. malaccense* extraction

The leaves of *C. myxa* L. were collected from Rante Padang-Rante Mario village, Malua district, Enrekang regency, South Sulawesi, Indonesia (3°22'26"S; 119°53'15"E), while the leaves of *S. malaccense* were sourced from Kalebarembeng village, Bontonompo district, Gowa regency, South Sulawesi, Indonesia (5°18'21"S; 119°23'48"E). Taxonomic identification of the plants was performed by the Botanical Division of the Pharmacognosy-Phytochemistry Laboratory, Faculty of Pharmacy, Universitas Muslim Indonesia, Makassar, Indonesia. Fresh leaf samples were thoroughly cleaned and air-dried at room temperature for one week. After drying, 300 g of the plant material was ground and extracted with 2 liters of 95% ethanol, with the extraction process repeated three times. The resulting liquid extracts were then evaporated to yield a gummy extract with a yield of 1.93% for *C. myxa* and 2.97% for *S. malaccense*. To prepare the extract-containing fly food, each extract was weighed and dissolved in 70% ethanol before being incorporated into the fly food to achieve final concentrations of 0.3125%, 0.625%, 1.25%, and 2.5%.

Animal husbandry

D. melanogaster w^{1118} was utilized in all experiments. The flies were maintained on standard fly food and kept in vials under controlled conditions (25°C, 60–70% humidity, and a 12-hour light/dark cycle) for over 20 inbred generations prior to use in this study. The standard fly food was prepared by combining cornmeal (7.5%), yeast (2.5%), sucrose (4.5%), and agar (0.9%), supplemented with propionic acid (400 µL) and methyl hydroxybenzoate (450 µL) as preservatives, to produce a total volume of 100 mL. The food was freshly prepared and replaced every three days to maintain its quality. Adult flies were allowed to mate for 3–5 days to produce larvae to be used in the experiments.

Sample size, sampling method, randomization, allocation, and blinding

A total of 10–30 larvae per group were used, with a minimum of three biological replicates in each experiment to ensure statistical power. The larvae were derived from the mating of 15 male and 15 female parental flies. Larvae were randomly assigned to one of the following groups: untreated control (fed a standard diet), high-sugar diet (HSD), HSD supplemented with varying concentrations of *C. myxa* (0.3125% to 2.5%) or *S. malaccense* (0.3125% to 2.5%) extract, or HSD

with ethanol, which served as the solvent control group. The same procedure was applied for both extracts. Randomization was achieved using a random number generator (https://www.randomizer.org/) to minimize bias in group allocation. While blinding was not applied during outcome assessments, the results were observed by three independent researchers, ensuring an unbiased evaluation of phenotypic parameters such as size, weight, locomotor activity, glucose levels, and gene expression.

Eligibility criteria

The inclusion criteria were larvae derived from age-matched flies (15 males and 15 females per group) and healthy second-instar larvae, with no visible deformities or signs of illness. Exclusion criteria included larvae that were physically malformed or exhibited abnormal behavior prior to the start of the experiment. Dropout criteria, aside from those related to the developmental toxicity assay, included larvae that deceased during the course of the experiment or those that did not progress to the next developmental stage (e.g., pupation or adulthood). Daily evaluations were performed to monitor larval health, developmental progress, and any signs of abnormality. Larvae showing signs of mortality or developmental arrest were promptly removed from the experiment, and the corresponding data were excluded from the final analysis to ensure the accuracy of the results.

Hyperglycemia induction

A hyperglycemic state in *D. melanogaster* larvae was induced by feeding them a high-sugar diet, prepared by adding 30% sucrose (CAS No: 57–50–1, Smart Lab, Jakarta, Indonesia) to the standard fly food. To confirm that the larvae were in a hyperglycemic state, hemolymph glucose levels were assessed using a glucose oxidase-peroxidase aminoantipyrine (GOD-PAP) reagent (Glory Diagnostics, Barcelona, Spain). The glucose concentrations were measured using a spectrophotometer (Shimadzu UV-Vis 1800, Kyoto, Japan) at a wavelength of 500 nm, according to the manufacturer's protocol.

Developmental toxicity assay

The developmental toxicity assay was conducted based on a previously reported method [21], with minor modifications. To select F1 larvae for the assay, larvae from the mating process were carefully observed and selected for health and developmental consistency. Healthy second-instar larvae were identified and removed from the vials using a fine brush, ensuring no contamination or damage. Seven experimental groups were included in the study, with each group comprising three vials containing 10 larvae per vial, resulting in a total of 30 larvae per group. Each group received a specific treatment: an untreated control (fed standard diet), HSD (fed food supplemented with 30% sucrose), solvent control (food containing 3.5% ethanol without extract), and varying concentrations of the extract (0.3125%, 0.625%, 1.25%, and 2.5%). The larvae were transferred to the vials and maintained under controlled environmental conditions (25°C, 60–70% humidity, and a 12-hour light/dark cycle) and monitored daily for developmental progression. Developmental stages were assessed by monitoring the survival rates and the duration of each stage (larvae-to-pupae stage and pupae-to-adult stage) under exposure to different extract concentrations. The time from larval hatching to pupation and from pupation to adult emergence were recorded. The monitoring continued until the adults emerged, and all data were collected over a period of 10 days (six days for the larval-to-pupal stage and nine days for pupal-to-adult-stage).

Larval body size and weight measurements

Larval body size was measured using vernier calipers (Taffware, Jakarta, Indonesia) by selecting three third-instar larvae per group and recording the length and width. For body weight measurements, ten third-instar larvae were placed into pre-tared microtubes and weighed using an analytical balance (Ohaus®, Nänikon, Switzerland). The average body weight for each group was then calculated. Both measurements were conducted in three biological replicates. To ensure accuracy and minimize bias, the experiments were performed by two trained researchers independently.

Crawling assay

The crawling assay was conducted based on a previously reported method [25], with slight modifications. Briefly, larvae were placed on petri dishes containing a thin layer of agarose overlaid on graph paper with millimeter blocks. Larval crawling was recorded for 60 seconds, and the number of blocks crossed within one minute was manually counted. The assay was repeated three times for each experimental group, with at least three biological replicates. Due to developmental delays observed in different groups, the assay was conducted using larvae at the third instar stage, ensuring comparable developmental stages across all groups.

Glucose hemolymph level

Glucose levels were assessed using a GOD-PAP kit (Glory Diagnostic, Barcelona, Spain), following the manufacturer's instructions. For hemolymph extraction, approximately 70–100 larvae were homogenized with a micropestle, adapting a previously reported method [26], with slight modifications. After centrifugation at 13,000 rpm for 2 minutes, 10 µL of the supernatant was collected and transferred into a microtube using a micropipette. Then, 1000 µL of GOD-PAP reagent was added and incubated at room temperature for 10 minutes. Absorbance was measured at 500 nm using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). Glucose concentration was calculated from a standard curve generated with the manufacturer's glucose standards, prepared, and analyzed according to the protocol.

Gene expression analysis

Ten larvae of *D. melanogaster* w^{1118}, previously treated with *C. myxa*, were subjected to RNA isolation using the PureLink RNA Mini Kit (Invitrogen, Thermo Fisher Scientific Inc., Massachusetts, USA), following the manufacturer's instructions. The larvae were collected separately and transferred to designated tubes. The expression levels of targeted genes were analyzed using the RT-qPCR method. The RT-qPCR assay was conducted in a 10 µL reaction volume with the Universal One-Step RT-qPCR Kit (Luna, New England Biolabs, Inc., Massachusetts, USA), in accordance with the manufacturer's protocols. The RT-qPCR reactions were performed using specific primers for the target genes listed in **Table 1**. The cycling conditions were as follows: an initial cycle at 37°C for 15 minutes, followed by 95°C for 10 minutes, and then 40 cycles of 95°C for 10 seconds, 63°C for 30 seconds, and 72°C for 30 seconds. To ensure the amplification of only the expected products, a standard melt curve analysis was conducted during each RT-qPCR run. RNA levels of the host ribosomal protein *rp49*, which served as an internal control, were quantified using *rp49*-specific primers under the same reaction conditions as the target genes.

Table 1. List of primers used in RT-qPCR

Genes	Forward primer	Reverse primer
dilp2	5' – TCTGCAGTGAAAAGCTCAACGA – 3'	5' – CAAACTGCAGGGGATTGAGG – 3'
dilp5	5' – CCCCGCCTTGATGGACATG – 3'	5' – CATGTGGTGAGATTCGGAGCTA – 3'
srl	5' – CTCTTGGAGTCCGAGATCCGCAA – 3'	5' – GGGACCGCGAGCTGATGGTT – 3'
rp49	5' – CGCTTCAAGGGACAGTATCTG – 3'	5' – AAACGCGGTTCTGCATGAG – 3'

Statistical analysis

Data from the developmental toxicity assay, larval weight and body size, crawling assay, hemolymph glucose levels, and estimated gene expression levels for all experimental groups were presented as bar graphs and analyzed using one-way analysis of variance (ANOVA), followed by post-hoc test using Dunnett's test. Data were expressed as mean ± standard deviation (SD), with *p*-values less than 0.05 considered statistically significant. All data were processed and visualized using Prism 9 (GraphPad Software, Boston, USA).

Results

Evaluation of developmental safety of *C. myxa* extract and *S. malaccense* in *Drosophila* larvae

This study investigates the antidiabetic potential of two ethnomedicines from Sulawesi using an in vivo *D. melanogaster* model. As an initial assessment, toxicity assays were conducted to

determine safe extract concentrations that support *D. melanogaster* development through the larval, pupal, and adult stages. *C. myxa* extract demonstrated no significant impact on the developmental stages of *D. melanogaster* w^{1118} larvae ($p>0.05$) (**Table 2**), while the *S. malaccense* extract exhibited a dose-dependent toxicity effect, with adverse effects observed at higher concentrations (**Table 3**). The results indicated that all concentrations of *C. myxa* extract tested in this study were safe. For *S. malaccense*, more than 70% of larvae survived to become adult flies when treated with the 0.3125% and 0.625% concentrations, making these concentrations suitable for the subsequent experiment. In contrast, the 1.25% and 2.5% concentrations were toxic, causing dose-dependent mortality and developmental delays.

Table 2. Developmental safety assessment following *Cordia myxa* treatment

Experimental group	Larval to pupae (%)	*p*-value*	Pupae to adult (%)	*p*-value*
Untreated control	100.0	-	96.6	-
Solvent control	96.6	0.971	93.3	0.980
C. myxa 0.3125%	100.0	>0.999	90.0	0.773
C. myxa 0.625%	96.6	0.971	86.6	0.457
C. myxa 1.25%	93.3	0.715	86.6	0.457
C. myxa 2.5%	90.0	0.477	75.0	0.050

*Compared to untreated control group

Table 3. Developmental safety assessment following *Syzygium malaccense* treatment

Experimental group	Larval to pupae (%)	*p*-value*	Pupae to adult (%)	*p*-value*
Untreated control	100.0	-	96.6	-
Solvent control	100.0	>0.999	86.6	0.795
S. malaccense 0.3125%	96.6	0.969	83.3	0.589
S. malaccense 0.625%	80.0	0.026	73.3	0.152
S. malaccense 1.25%	45.0	<0.0001	36.5	<0.001
S. malaccense 2.5%	50.0	<0.0001	20.0	<0.001

*Compared to untreated control group

Improvement of phenotypical features of hyperglycemic *Drosophila* larvae by *C. myxa* and *S. malaccense* extract

A high-sucrose diet (30%) resulted in a significant reduction in larval length ($p<0.01$), width ($p<0.0001$), and weight ($p<0.0001$). Treatment with *C. myxa* (**Figure 2A**) at concentrations of 0.625% ($p<0.05$) and 1.25% ($p<0.01$) significantly improved larval body length compared to the control group. Furthermore, an increase in larval body width (non-significant for 0.625% and $p<0.05$ for 1.25%) and weight ($p<0.05$ for 0.625% and $p<0.01$ for 1.25%) was observed exclusively in the *C. myxa* treatment group (**Figure 2C** and **2E**). In contrast, *S. malaccense* extract at a concentration of 0.625% also improved larval body length ($p<0.0001$) (**Figure 2B**), but it did not significantly affect body width or weight ($p>0.05$) (**Figure 2D** and **2F**). These findings demonstrate that while both extracts influenced larval body length, only *C. myxa* treatment resulted in significant changes in body width and weight under the high-sucrose diet.

Enhancement of crawling ability in *Drosophila* larvae by *C. myxa* and *S. malaccense* extracts

A high-sucrose diet resulted in a significant reduction in the crawling ability of *D. melanogaster* larvae ($p<0.0001$), indicating a negative impact on locomotor function. This reduction in movement was evident when larvae from the HSD group were compared to the control group. In contrast, treatment with both *C. myxa* (**Figure 3A**) and *S. malaccense* (**Figure 3B**) extracts resulted in significant improvements ($p<0.0001$) in the crawling ability of the larvae. The larvae treated with *C. myxa* at concentrations of 0.625% and 1.25% showed enhanced mobility, demonstrating a remarkable recovery from the locomotor impairment caused by the high-sucrose diet. Similarly, larvae treated with 0.625% of *S. malaccense* extract exhibited a significant increase in crawling ability ($p<0.0001$), although the extent of improvement was less pronounced compared to *C. myxa*.

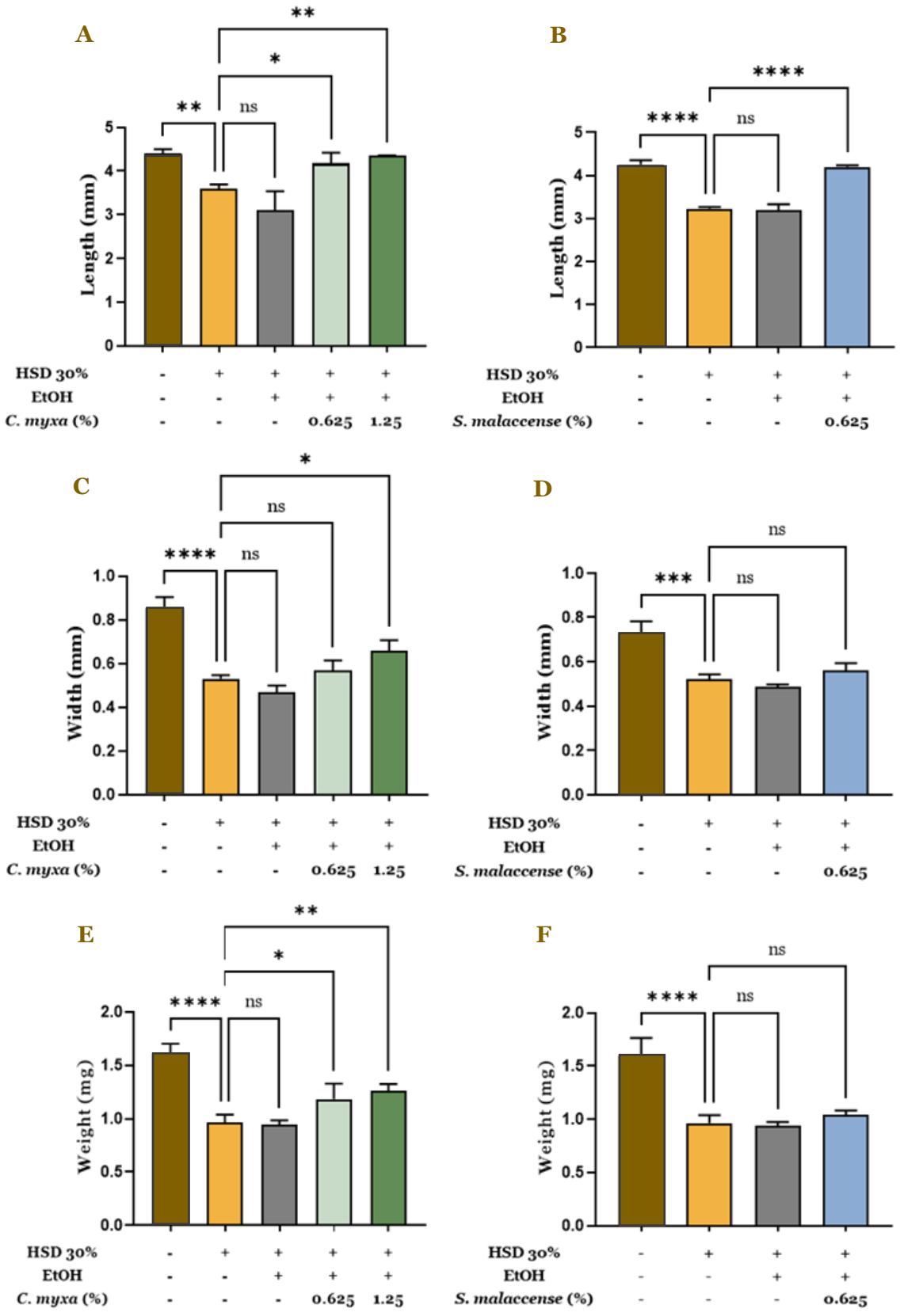

Figure 2. Effect of *Cordia myxa* and *Syzygium malaccense* extract treatment on the phenotypic profile of larval body size. Larval body length, width, and weight were reduced due to high sucrose exposure. Nevertheless, body length was restored in groups treated with *C. myxa* (A) and *S. malaccense* (B) extracts, approaching normal larval size. An improvement in body width (C) and weight (E) was observed in the *C. myxa* extract-treated group, but not in the body width (D) and weight (F) of larvae treated with *S. malaccense*. EtOH: ethanol; HSD: high-sugar diet; ns: non-significant; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; $^{****}p<0.0001$.

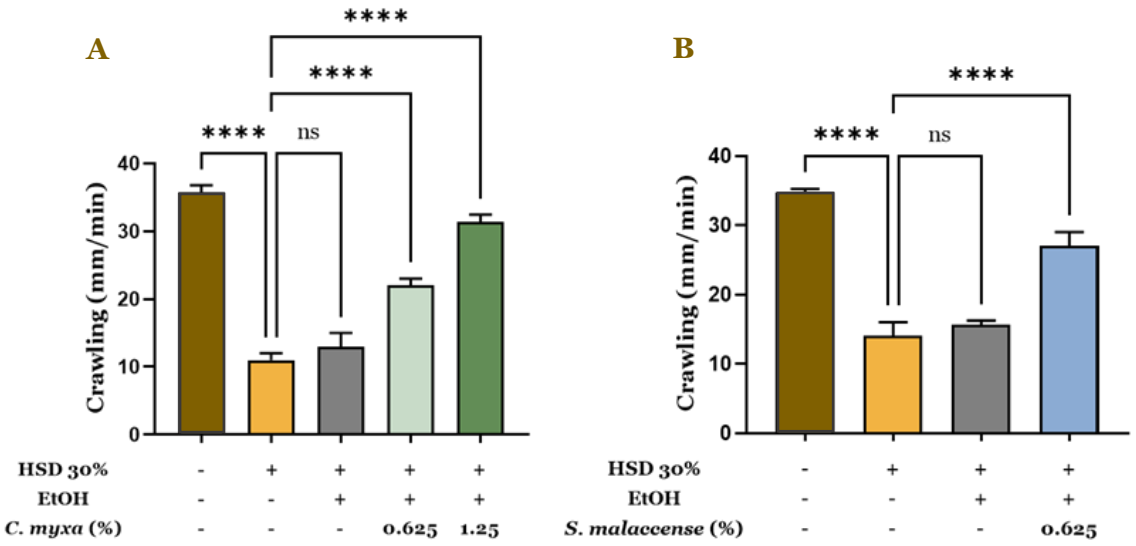

Figure 3. Effect of extract administration on larval locomotion. Enhanced locomotor activity was observed in larvae treated with both *Cordia myxa* (A) and *Syzygium malaccense* (B) extracts. EtOH: ethanol; HSD: high-sugar diet; ns: non-significant; $^{****}p<0.0001$.

Reduction of hemolymph glucose levels observed with *C. myxa* but not *S. malaccense* extracts

A high-sucrose diet led to a significant increase in glucose levels in the hemolymph of *D. melanogaster* larvae ($p<0.01$), as expected due to the 30% sucrose consumption (**Figure 4A** and **4B**). This increase in glucose levels was clearly evident in the HSD group, reflecting a state of hyperglycemia. Interestingly, the group treated with *C. myxa* extract (**Figure 4A**), exhibited a significant reduction in glucose levels ($p<0.01$ for both concentrations), bringing glucose levels close to the baseline observed in the untreated control group. This finding indicates that both 0.625% and 1.25% concentrations were equally effective in lowering glucose levels. In contrast, treatment with *S. malaccense* extract (**Figure 4B**) did not result in any significant change in glucose levels compared to the HSD group ($p>0.05$).

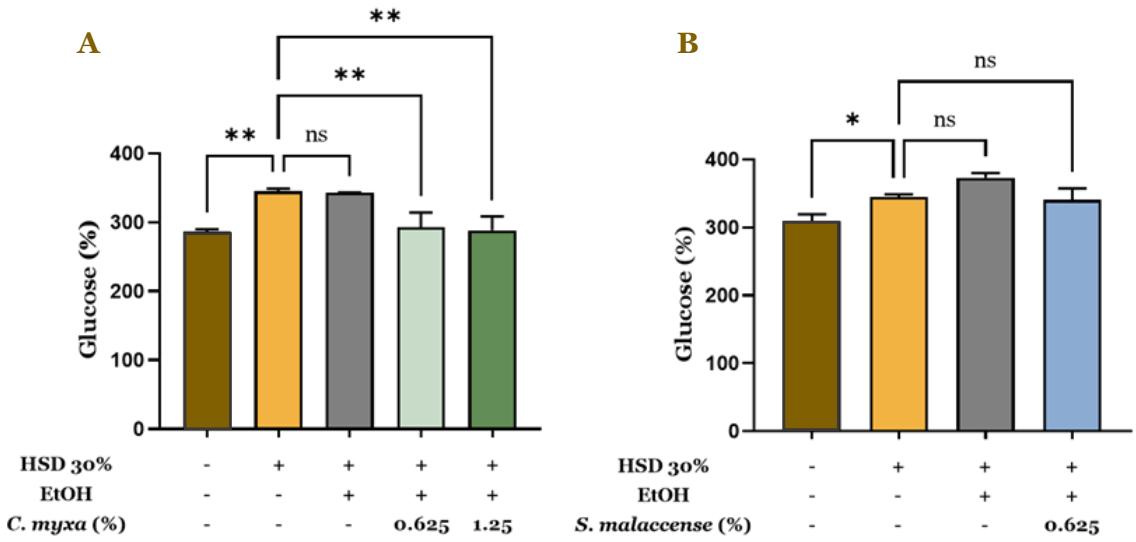

Figure 4. Evaluation of extract efficacy in lowering glucose levels in larval hemolymph. Hemolymph glucose levels decreased with the administration of *Cordia myxa* extract (A), whereas *Syzygium malaccense* extract did not show a significant effect (B). EtOH: ethanol; HSD: high-sugar diet; ns: non-significant; $^{*}p<0.05$; $^{**}p<0.01$.

Elevated expression of metabolism-related genes following *C. myxa* extract treatment

The results indicate that *C. myxa* extract at concentrations of 0.625% and 1.25% enhances larval metabolism following HSD exposure, restoring metabolic function closer to normal levels. This improvement is supported by the upregulation of insulin-producing genes, *dilp2* ($p<0.001$ for both concentrations of 0.625% and 1.25%) and *dilp5* ($p<0.001$ for concentration of 0.625% and $p<0.01$ for concentration of 1.25%) (**Figure 5A** and **5B**), as well as the increased expression of *srl*, a gene involved in insulin regulation (**Figure 5C**). These findings highlight the potential of *C. myxa* extract as an antidiabetic agent, potentially acting through pathways associated with insulin regulation.

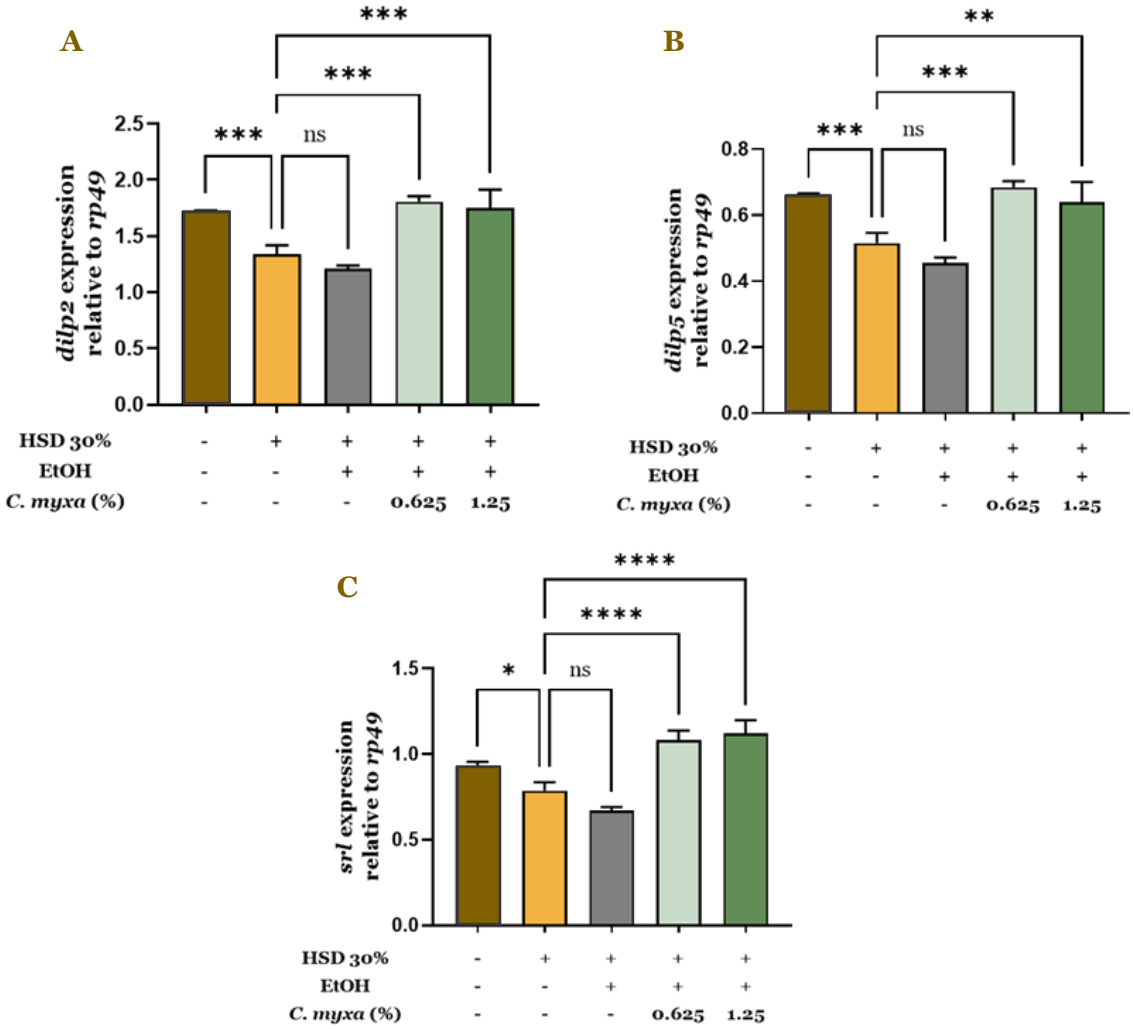

Figure 5. Gene expression related to metabolism following *Cordia myxa* extract administration. Increased levels of expression of the genes *dilp2* (A), *dilp5* (B), and *srl* (C) were observed in the groups treated with *C. myxa*. EtOH: ethanol; HSD: high-sugar diet; ns: non-significant; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; $^{****}p<0.0001$.

Discussion

In the present study, the effects of *C. myxa* and *S. malaccense* extracts on *D. melanogaster* model of hyperglycemia were evaluated across several parameters, including developmental toxicity, larval body size, hemolymph glucose levels, and gene expression. The findings indicated that all concentrations of *C. myxa* extract were safe, with no significant effects on the developmental progression of *Drosophila*. In contrast, *S. malaccense* extract exhibited dose-dependent toxicity, with mild toxicity observed at 0.625%, which did not cause major developmental issues, while the 1.25% and 2.5% concentrations led to increased mortality and developmental delays. Further analysis showed significant differences in body size and weight between the treated and control

groups, with *C. myxa* treatment resulting in improvements in body length, width, and weight. Hemolymph glucose levels were also significantly reduced in the *C. myxa*-treated groups, but not in the *S. malaccense*-treated groups, compared to the high-sugar diet control. Subsequent gene expression analysis revealed alterations in the mRNA levels of key metabolic-related genes of *Drosophila* (*dilp2*, *dilp5*, and *srl*) following treatment with *C. myxa* extract, suggesting that *C. myxa* may exert its antihyperglycemic effects through modulation of insulin and spargel-related metabolic pathways.

To assess the potential toxicity of the extracts, an initial toxicity evaluation was conducted using larvae to determine whether the extracts induced significant adverse effects on the developmental stages of the model organism. The results revealed that *C. myxa* extract was relatively safe for larval development, while *S. malaccense* extract exhibited a dose-dependent increase in toxicity at higher concentrations. These findings suggest that *C. myxa* extract has a broader safety margin, making it a more promising candidate for further investigation. The observed safety of *C. myxa* extract, compared to the dose-dependent toxicity of *S. malaccense*, can likely be attributed to differences in their bioactive compound profiles. While the *Syzygium* genus, including *S. malaccense*, is characterized by the presence of eugenol—a compound absent in *C. myxa* [27]. *C. myxa* has been reported to exhibit antioxidant and anti-inflammatory properties [8] with low cytotoxicity [28]. These characteristics may explain its relatively mild effects on *Drosophila* development, even at higher concentrations. In contrast, the eugenol present in *S. malaccense* has been documented to exhibit insecticidal activity in *D. melanogaster* at elevated concentrations [29,30], potentially contributing to the developmental delays and increased mortality rates observed at the 1.25% and 2.5% concentrations.

C. myxa and *S. malaccense* extracts demonstrated the ability to mitigate the adverse effects of a high-sucrose diet, which typically induces a reduction in larval size and weight due to hyperglycemic metabolic stress [13]. Under such conditions, the body compensates by breaking down fat and muscle as alternative energy sources when glucose utilization is impaired [31]. *C. myxa* extract at concentrations of 0.625% and 1.25% exhibited a robust effect, increasing larval length, width, and weight, suggesting its potential to enhance nutrient metabolism and promote phenotypic recovery in hyperglycemic states. In contrast, the *S. malaccense* extract at a concentration of 0.625% influenced only larval length but had no significant effect on width or weight, indicating a more limited biological activity compared to *C. myxa*. These findings highlight the potential of *C. myxa* in counteracting growth disturbances caused by metabolic stress.

The present study also observed that *C. myxa* and *S. malaccense* extracts improved larval crawling ability. The decline in locomotor function induced by a high-sucrose diet indicates metabolic dysfunction and oxidative stress in muscle tissues, resulting from insufficient adenosine triphosphate (ATP) production [32]. The significant improvement in locomotor ability suggests that both *C. myxa* and *S. malaccense* contribute to enhancing cellular energy resilience under hyperglycemic conditions. This effect is likely due to their bioactive compounds, such as flavonoids and phenolics, which are widely recognized for their potent antioxidant properties [8,29]. Hyperglycemia is known to cause oxidative stress by promoting the generation of reactive oxygen species, which can damage essential cellular components, including proteins, lipids, and DNA [33]. By mitigating oxidative stress, these extracts may protect cellular structures and enzymes critical for energy production, particularly in the mitochondria, thereby supporting locomotor function. The antioxidant activity of these compounds likely preserves cellular integrity and function, promoting enhanced energy resilience [34]. This observation supports the hypothesis that both extracts may facilitate energy production and alleviate metabolic stress, thereby counteracting the adverse effects of hyperglycemia on muscle activity.

Regarding glucose regulation, *C. myxa* demonstrated greater efficacy than *S. malaccense* in significantly reducing hemolymph glucose levels. Further molecular analysis revealed that the administration of *C. myxa* can increase the expression of *D. melanogaster* insulin-like peptides (encoded by *dilp2* and *dilp5*) and Spargel (*srl*), which are homologous to insulin and peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC-1α) in mammals, a key regulator of mitochondrial biogenesis and energy metabolism [35,36]. The increased expression of *dilp2* and *dilp5*, which are involved in insulin synthesis [37], suggests that *C. myxa* may stimulate

mechanisms that enhance insulin production. Additionally, the upregulation of *srl*, which is associated with increased mitochondrial activity, supports cell growth and insulin signaling responses [38]. By upregulating these genes, cells can produce more energy to sustain various cellular functions, including glucose metabolism, which is crucial for diabetes management [37,38]. These findings further substantiate the hypothesis that *C. myxa* holds promise as an antidiabetic candidate capable of restoring glucose homeostasis to near-normal conditions through pathways associated with energy metabolism and insulin regulation.

The present study has several limitations, including the use of *D. melanogaster*, which, despite being cost-effective, does not fully replicate human physiology. Additionally, the bioactive compounds in *C. myxa* and *S. malaccense* extracts were not identified, and the study was limited to a single time point, preventing the assessment of long-term effects. A broader dose range could also help in better understanding the therapeutic potential and safety of the extracts. Despite these limitations, *D. melanogaster* proves to be a valuable platform for drug discovery, especially in resource-limited settings like Indonesia. However, further validation in mammalian models is essential to confirm the safety and efficacy of these extracts for clinical use. Future research should focus on identifying bioactive compounds, exploring mechanisms, and assessing long-term effects in more complex models.

Conclusion

The *S. malaccense* extract exhibited dose-dependent toxicity in *D. melanogaster* larvae, with significant effects at 1.25% and 2.5% concentrations, while *C. myxa* extract had no significant impact on larval development. *C. myxa* improved larval size, weight, and crawling activity, whereas *S. malaccense* only enhanced length and crawling ability. At concentrations of 0.625% and 1.25%, *C. myxa* significantly reduced hemolymph glucose levels and upregulated key metabolism-related genes (*dilp2, dilp5*, and *srl*), suggesting its potential to enhance insulin production and mitochondrial function. These findings highlight *C. myxa* as a safer and more effective candidate for further investigation as a prospective antidiabetic agent.

Ethics approval

Not required.

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Competing interests

All the authors declare that there are no conflicts of interest.

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Underlying data

All data underlying the results are available from the corresponding author upon reasonable request.

Declaration of artificial intelligence use

This study used artificial intelligence (AI) tools and methodologies in the following capacities of which AI-based language models ChatGPT was employed in the language refinement (improving

grammar, sentence structure, and readability of the manuscript). We confirm that all AI-assisted processes were critically reviewed by the authors to ensure the integrity and reliability of the results. The final decisions and interpretations presented in this article were solely made by the authors.

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