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Seagrass *Enhalus acoroides* extract mitigates obesity and diabetes via GLP-1, PPAR γ , SREBP-1c modulation and gut microbiome restoration in diabetic zebrafish

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Abstract

Background The global rise in obesity and type 2 diabetes highlights the need for safe and effective therapeutic interventions. *Enhalus acoroides* is a tropical seagrass rich in carotenoids and other bioactives. Its potential for metabolic regulation has been suggested in vitro, but in vivo efficacy and molecular mechanisms remain unexplored. This study aimed to evaluate the anti-obesity and anti-diabetic effects of *Enhalus acoroides* extract (SEAE) in a zebrafish model of diet- and glucose-induced metabolic dysfunction.

Methods Adult zebrafish were subjected to overfeeding and glucose immersion, after overfeeding and 14 days of glucose immersion to induce diabetes, adult zebrafish were randomized into three groups: untreated diabetic, SEAE-treated (5 mg/L), and metformin-treated (3.3 mg/L) for 20 days. Body weight, fasting blood glucose, lipid profile, gene expression (GLP-1, PPAR γ , SREBP-1c), and gut microbiota profiles via 16 S rRNA sequencing were assessed.

Results SEAE significantly reduced body weight and blood glucose in diabetic zebrafish ($p < 0.05$), with efficacy comparable to or exceeding Metformin. It upregulated GLP-1 and downregulated PPAR γ and SREBP-1c. SEAE also reduced total cholesterol, triglycerides, and LDL levels, while increasing HDL levels. Moreover, SEAE restored the Firmicutes/Bacteroidetes ratio, increased alpha diversity, and shifted beta diversity toward healthy controls. SEAE-treated fish showed microbial profiles closer to normal than those treated with Metformin.

Conclusions SEAE exhibits strong anti-obesity and anti-hyperglycemic effects by modulating key metabolic pathways and restoring gut microbial homeostasis. These findings highlight SEAE as a promising marine-derived therapeutic candidate for metabolic syndrome and warrant further investigation as a functional food or nutraceutical.

Clinical trial Not applicable.

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Keywords Metabolic syndrome, Functional food, Seagrass, Gut microbiome, *In vivo*, Marine product, Hyperglycemia, Overweight, Metabolic dysfunction

Introduction

The global rise in diabetes mellitus and obesity represents a significant public health concern. In 2024, over 589 million people worldwide were affected by diabetes, a number projected to reach 852.5 million by 2050 [1]. This increase parallels the growing prevalence of obesity, one of the primary risk factors for type 2 diabetes, which impacted 988 million individuals in 2020 and is estimated to affect 1.91 billion by 2035 [2]. Addressing obesity is, therefore, crucial in mitigating the diabetes epidemic.

At the molecular level, these metabolic disorders involve complex dysregulation of pathways controlling energy balance, glucose metabolism, and lipid storage. Several key regulators include glucagon-like peptide-1 (GLP-1), peroxisome proliferator-activated receptor gamma (PPAR γ), and sterol regulatory element-binding protein-1c (SREBP-1c). GLP-1 promotes insulin secretion and satiety, PPAR γ enhances insulin sensitivity and lipid storage, and SREBP-1c stimulates lipogenesis in the liver and adipose tissue [3–5]. In metabolic disorders such as obesity and type 2 diabetes, these pathways are often disrupted, leading to insulin resistance, hyperglycemia, dyslipidemia, and increased adiposity.

While existing therapies target some of these mechanisms, their limitations—such as poor compliance and the lack of multi-targeted mechanism of action—have prompted the search for safer, more comprehensive alternatives. One promising candidate is *Enhalus acoroides* (*E. acoroides*), a tropical seagrass native to coastal waters of the Indian and Western Pacific oceans, which is rich in bioactive compounds with reported antioxidant, anti-diabetic, and anti-obesity properties [6, 7]. *E. acoroides* seeds have been used especially by coastal populations as a flour substitute, and showed nutritional properties similar to wheat, cassava, and rice. On the other hand, marine plants like *E. acoroides* have shown the potential to modulate metabolic pathways and gut microbiota, offering a novel therapeutic approach. Our prior *in silico* and *in vitro* study employed ultrasound-assisted extraction (UAE) to isolate several potent carotenoids from *E. acoroides*, including fucoxanthin (C1), lutein (C2), astaxanthin (C3), canthaxanthin (C4), zeaxanthin (C5), β -cryptoxanthin (C6), and β -carotene (C7), all of which are associated with key metabolic and regulatory functions [7].

This study investigates the effects of *E. acoroides* extract in a zebrafish model of diet- and glucose-induced diabetes. The focus has been set on its regulatory effects on GLP-1, PPAR γ , and SREBP-1c pathways, along with its influence on gut microbial composition, to explore

its potential for future functional food development targeting metabolic dysfunction. To the best of our knowledge, this is the first *in vivo* study to demonstrate the dual therapeutic effects of *E. acoroides* extract on both glucose and lipid metabolism through modulation of GLP-1, PPAR γ , and SREBP-1c expression, as well as restoration of gut microbiota composition and diversity in a zebrafish model of overfeeding- and glucose-induced diabetes. This research integrates molecular, metabolic, and microbiome-based mechanisms, highlighting *E. acoroides* as a promising candidate for marine-derived functional foods targeting metabolic syndrome.

Materials and methods

Sample collection and preparation of *enhalus acoroides* extract

Enhalus acoroides specimens were collected with formal permission from local authorities and community representatives. The seagrass was harvested from the coastal waters of Indonesia. Botanical identification and taxonomic validation were previously performed in a preliminary study by the National Center for Biotechnology Information (NCBI) taxonomy guidelines (Taxonomy ID: NCBI: txid55455). Taxonomic classification includes Eukaryota, Viridiplantae, Streptophyta, Streptophytina, Embryophyta, Tracheophyta, Euphyllophyta, Spermatophyta, Magnoliopsida, Mesangiospermae, Liliopsida, Alismatales, Hydrocharitaceae, and *Enhalus*. The collected specimens were documented and archived for future reference.

All procedures adhered to standardized protocols for marine plant collection and *in vivo* experimental research. Upon collection, the seagrass samples underwent multiple washes with distilled water to eliminate residual soil and particulate matter. Cleaned samples were subsequently dried in a Memmert IN55 incubator (Schwabach, Germany) at a constant temperature of 60 °C for 72 h. The dried material was then cut into smaller fragments and ground into coarse simplicia powder using a high-speed blender (Cosmos 2 L ReBlend, Tangerang, Indonesia). The characterization of compounds, along with *in silico* and *in vitro* analyses, has been previously conducted and reported in a published study [7].

Extraction of *E. acoroides*

The powdered *E. acoroides* material was subjected to ultrasound-assisted extraction (UAE), an environmentally friendly technique designed to enhance the recovery of bioactive compounds. Two hundred grams of the

simplicia powder was sonicated in 2 L of deionized water at 40 °C for 30 min using a Branson 2510 ultrasonic bath (St. Louis, MO, USA) operating at 400 W. The resulting mixture was filtered and re-extracted to maximize compound yield. The filtrate was concentrated using a rotary evaporator at 100 °C to obtain a thick extract. This UAE-derived *E. acoroides* extract (E-UAE) was then stored in aluminum foil at 4 °C in a dry, dark environment until further phytochemical and pharmacological analyses were performed.

Zebrafish husbandry and experimental conditions

Adult zebrafish (*Danio rerio*), aged 2.5 to 3 months, were obtained from the Animal Laboratory of the Medical Research Center, Indonesia. Upon arrival, all fish were acclimatized and maintained under standardized laboratory conditions in a recirculating aquaculture system (RAS). The water used for zebrafish maintenance was dechlorinated tap water, filtered through activated carbon, and subjected to ultraviolet (UV) irradiation to eliminate microbial and chemical contaminants. Dissolved oxygen levels were maintained using a continuous aeration system provided by an air pump. Water parameters were strictly controlled: pH was maintained at 6.8–7.0, temperature at 28 ± 1 °C, and a photoperiod of 14 h light and 10 h dark was applied. The stocking density was maintained at 15 adult fish per 4 L tank to ensure optimal physiological conditions and reduce stress-related variability. All procedures adhered strictly to the Guidelines for Reporting In Vivo Experiments (ARRIVE) and have also been reviewed and accepted in the Preclinical Trials Study Register with number PCTE0000604 and received formal approval from the Institute for Research and Community Services, Sunan Kalijaga State Islamic University Yogyakarta, Yogyakarta, Indonesia with number 2380/Un.02/L3/TL/06/2025.

Induction of obesity and diabetes in zebrafish

To induce diet-induced obesity (DIO), zebrafish were randomly assigned to two groups: an overfeeding group and a control group, with ten fish housed in each 2-liter tank. The DIO group was subjected to an overfeeding protocol consisting of 120 mg of commercial zebrafish feed per fish per day (distributed in six feeding sessions). The control group received 20 mg of Otohime B2 feed per fish per day. Body weight and fasting blood glucose (FBG) levels were monitored weekly over 4 weeks to assess the development of obesity.

After the overfeeding phase, DIO zebrafish were immersed in a 110 mM D-glucose solution for 14 days to induce hyperglycemia. The glucose solution was freshly prepared and replaced daily to maintain sterility and glucose stability. Following previously established procedures, fasting blood glucose was measured at the end of

the induction period using a validated handheld glucometer. Diabetes was confirmed in zebrafish whose FBG levels were at least 1.5-fold higher than those of the control group.

Experimental design for SEAE anti-diabetic assessment

To evaluate the anti-diabetic potential of *Enhalus acoroides* seagrass extract (SEAE), zebrafish were randomly assigned to four treatment groups, each consisting of twelve fish (six males and six females), and maintained in 2-liter tanks for 20 days. All groups were fed a standard diet of 20 mg/fish/day throughout the experiment. The groups were as follows:

- Group A (NORM): Healthy control zebrafish with no intervention.
- Group B (DIABE): Diabetic zebrafish (DIO + glucose immersion).
- Group C (METFO): Diabetic zebrafish treated with Metformin at a concentration of 20 μ M (equivalent to 3.3 mg/L).
- Group D (SEAE): Diabetic zebrafish treated with SEAE at 5 mg/L. The SEAE dose selection was based on our previous *in silico* and *in vitro* studies demonstrating its effective bioactivity and pharmacological relevance [7]. Furthermore, this dosing level aligns with a recent study utilizing a comparable concentration of natural products in diabetic models [8], supporting its translational applicability and comparability to metformin in zebrafish.

Fasting blood glucose levels were measured every 5 days using a rapid blood glucose monitoring device to evaluate glycemic control. Additional outcome parameters included body weight, expression of key genes (GLP-1), and metabolic regulators (e.g., PPAR γ , SREBP-1c).

Quantitative real-time PCR (qRT-PCR) analysis

Following the treatment phase, whole-body samples of zebrafish were collected and immediately preserved at –80 °C for subsequent gene expression analysis. To investigate the impact of SEAE treatment on glucose and lipid metabolism, total RNA was isolated using TRIsure™ Reagent (Meridian Bioscience, USA) according to the manufacturer's protocol. The RNA pellet was air-dried and reconstituted in 500 μ L of RNase-free water. Quantification and purity assessment of RNA were performed by measuring the absorbance at 260/280 nm using a SmartSpec™ Plus Spectrophotometer (Bio-Rad).

An aliquot of 5 μ g total RNA was reverse transcribed into complementary DNA (cDNA) using the Tetro™ Reverse Transcriptase kit with Random Hexamer primers (Meridian Bioscience). The synthesized cDNA served

as the template for quantitative real-time PCR to determine the relative expression of target genes associated with metabolic regulation. Specifically, transcript levels of glucagon-like peptide-1 (GLP-1), peroxisome proliferator-activated receptor gamma (PPAR γ), and sterol regulatory element-binding protein-1c (SREBP-1c) were quantified. The β -actin gene was employed as an internal control to normalize gene expression levels.

Estimation of tissue lipid profile

The methods used for the estimation of lipid profile components, including Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL) levels, were adapted from a previously published protocol by Nagara A, et al. (2021) and Reshma A, et al. (2023) [9, 10].

The zebrafish gut microbiota analysis

Two hours post-feeding, zebrafish were euthanized through immersion in ice-cold water. Each specimen was externally sterilized using 70% ethanol before the extraction of intestinal content, which was obtained by gently squeezing the gut with sterilized forceps. The V3–V4 hypervariable regions of the 16 S rRNA gene were amplified and sequenced on the Illumina MiSeq platform (paired-end mode). Raw reads were quality-filtered and cleaned of adapter and primer sequences using Trimmomatic v0.39 and Cutadapt v2.10. High-quality reads were merged and denoised to generate Amplicon Sequence Variants (ASVs) using the q2-DADA2 plugin within the QIIME2 environment [11]. Taxonomic classification of ASVs was performed using the SILVA SSURef Nr99 database version 138, employing the q2-feature-classifier plugin with the classify-consensus-blast approach [12].

Sample size determination

The sample size was calculated using the Federer formula for biological replication:

$$(t - 1)(r - 1) \geq 15$$

Where t is the number of treatments (4) and r is the number of biological replicates. Solving the equation gives $r \geq 6$. To enhance the statistical power and robustness of the findings, we increased the number of zebrafish per group to 12. This was to account for expected sample loss due to mortality and technical exclusion during follow-up molecular and biochemical analysis (e.g., RNA degradation, abnormal gene expression profiles, or incomplete microbiome sequencing data). This increases the reliability of group comparisons and the overall validity of the experimental outcomes.

Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD). Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) post hoc test for pairwise comparisons. End-point comparisons of biomarkers were assessed by two-way ANOVA with Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$. Pearson's correlation analysis explored relationships among molecular biomarkers, lipid profiles, and gut microbiota composition in diabetic zebrafish treated with *Enhalus acoroides* seaweed extract (SEAE). All graphical outputs and statistical visualizations were generated using GraphPad Prism version 10 (GraphPad Software, San Diego, CA, USA).

Results

Anti-obesity property of *E. acoroides*

The anti-obesity effect of *Enhalus acoroides* extract (SEAE) in diabetic zebrafish is illustrated in Fig. 1. As shown in Fig. 1A, there were no significant differences in initial body weight among all groups, confirming similar baseline conditions. In Fig. 1B, diabetic fish exhibited a significantly higher final body weight than normal fish ($P \leq 0.0001$). However, treatment with either metformin or SEAE markedly reduced final body weight compared to untreated diabetic fish ($P \leq 0.001$ and $P \leq 0.0001$, respectively), with SEAE-treated fish displaying a lower mean body weight than those treated with metformin. Despite this, Fig. 1C shows no significant difference in body weight change (delta) between the metformin and SEAE groups, indicating similar therapeutic efficacy. These results demonstrate that SEAE effectively mitigates weight gain in diabetic zebrafish, comparable to the standard treatment, and holds promise as an anti-obesity agent.

The effects of SEAE treatment on lipid metabolism, specifically the Total Cholesterol (TC), Triglycerides (TG), High-Density Lipoprotein (HDL), and Low-Density Lipoprotein (LDL), are shown in Fig. 2. As shown in Fig. 2A and D, the Diabetic Fish group showed significantly higher levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL), alongside a significantly lower levels of high-density lipoprotein (HDL), compared to the normal control group ($P \leq 0.0001$), indicating a hyperlipidemic conditions. Treatment with either metformin or SEAE significantly improved the lipid profile, with reductions in TC, TG, and LDL levels and partial restoration of HDL levels ($P \leq 0.01$ to $P \leq 0.0001$). Notably, metformin showed slightly better efficacy in lowering LDL and TG, while SEAE exhibited a higher HDL-restorative potential. These findings suggest that SEAE, like metformin, exerts beneficial effects on

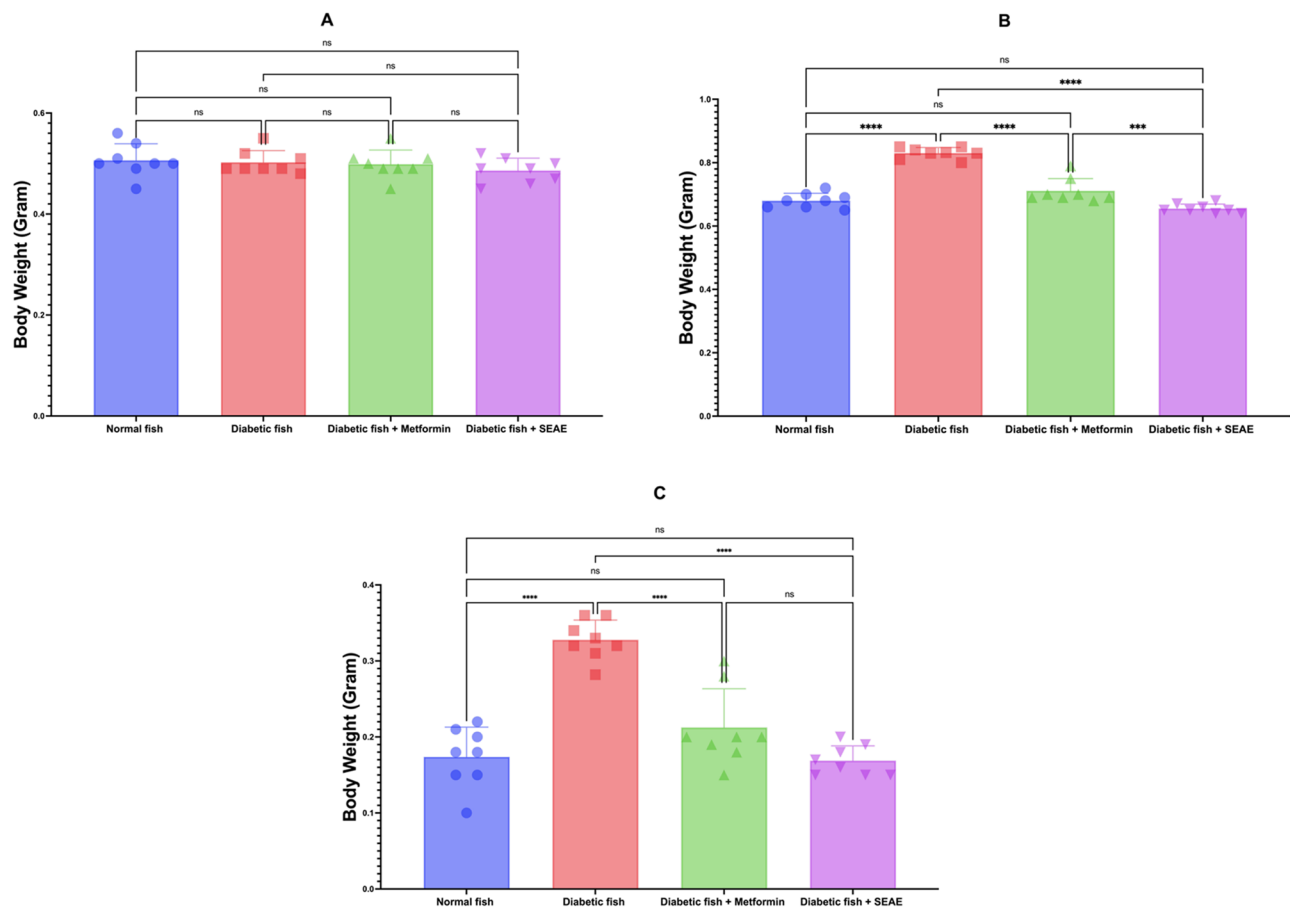


Fig. 1 Anti-obesity potential of *E. acoroides*. (A) Initial body weight amongst the four treatment groups. (B) Final body weight after 20 days. (C) Changes in body weight. ns. $P > 0.05$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$

lipid metabolism and may play a protective role against metabolic dysregulation in diabetic conditions.

Anti-hyperglycemic property of *E. acoroides*

Figure 3 illustrates the anti-diabetic effect of *Enhalus acoroides* extract (SEAE) in a diabetic zebrafish model over 20 days. Diabetic fish consistently exhibited significantly elevated blood glucose levels compared to normal controls at all time points ($P \leq 0.0001$). Treatment with either metformin or SEAE markedly reduced blood glucose levels relative to untreated diabetic fish at days 5, 10, 15, and 20 ($P \leq 0.0001$), demonstrating effective glycemic control. Notably, a significant difference was observed between the glucose-lowering effects of SEAE and metformin. SEAE showed a more substantial reduction in blood glucose levels compared to metformin-treated diabetic fish within 20 days, suggesting a potential higher efficacy. Throughout this study, SEAE maintained stable and significantly lower glucose levels in diabetic fish, supporting its potential as a promising anti-diabetic agent.

GLP-1/PPAR γ /SREBP-1c modulation

Figure 4 illustrates the modulatory effects of *Enhalus acoroides* extract (SEAE) on the expression of metabolic-related genes PPAR γ , GLP-1, and SREBP-1c in a diabetic zebrafish model. Diabetic fish exhibited a significant upregulation of PPAR γ and SREBP-1c and a marked downregulation of GLP-1 compared to normal fish ($P \leq 0.0001$), indicating disrupted metabolic regulation. Treatment with SEAE significantly reduced the expression of PPAR γ and SREBP-1c while significantly increasing GLP-1 expression, with effects comparable to metformin. Notably, SEAE-treated fish showed significantly higher GLP-1 and lower PPAR γ and SREBP-1c expression compared to metformin-treated fish ($P \leq 0.0001$), suggesting that SEAE may offer enhanced benefits in improving glucose metabolism and reducing lipogenesis.

Gut microbiome analysis

Figure 5A illustrates the Firmicutes/Bacteroidetes (F/B) ratio among the four groups. The diabetic fish group showed a significantly elevated F/B ratio compared to

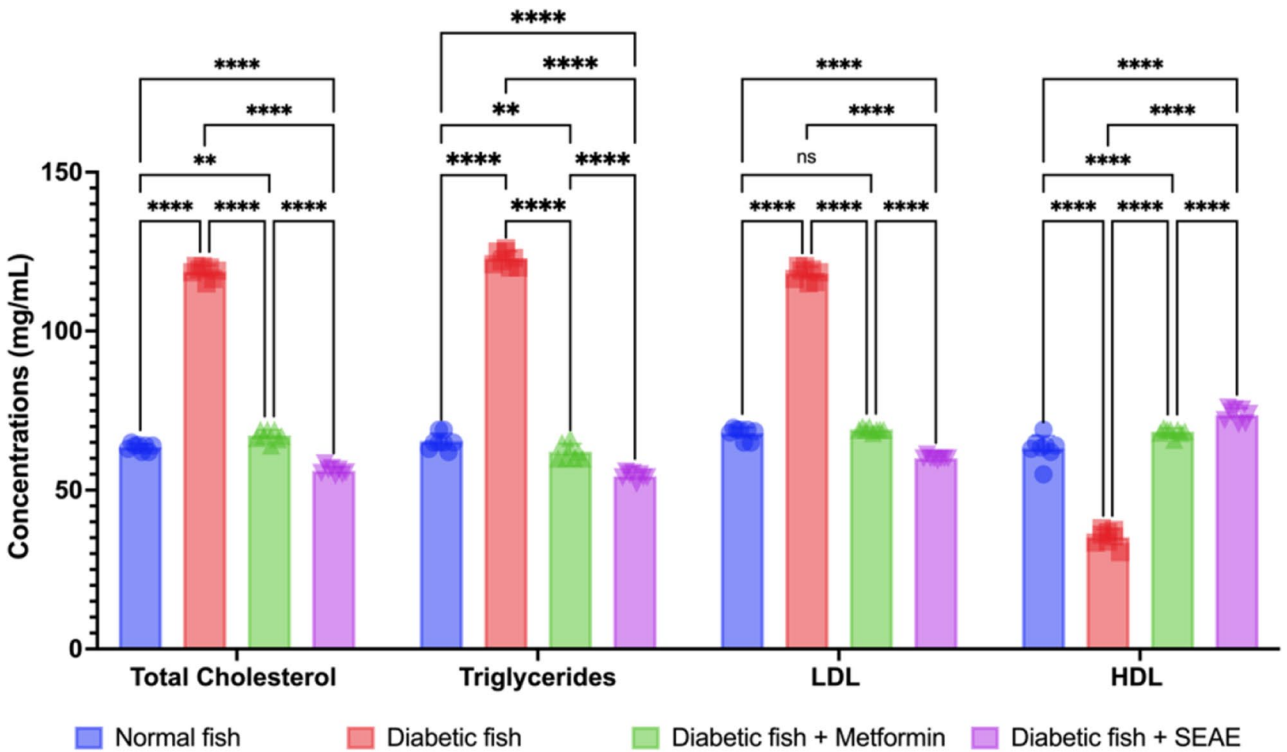


Fig. 2 Comparative analysis of lipid profile parameters across treatment groups. ns. $P > 0.05$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$

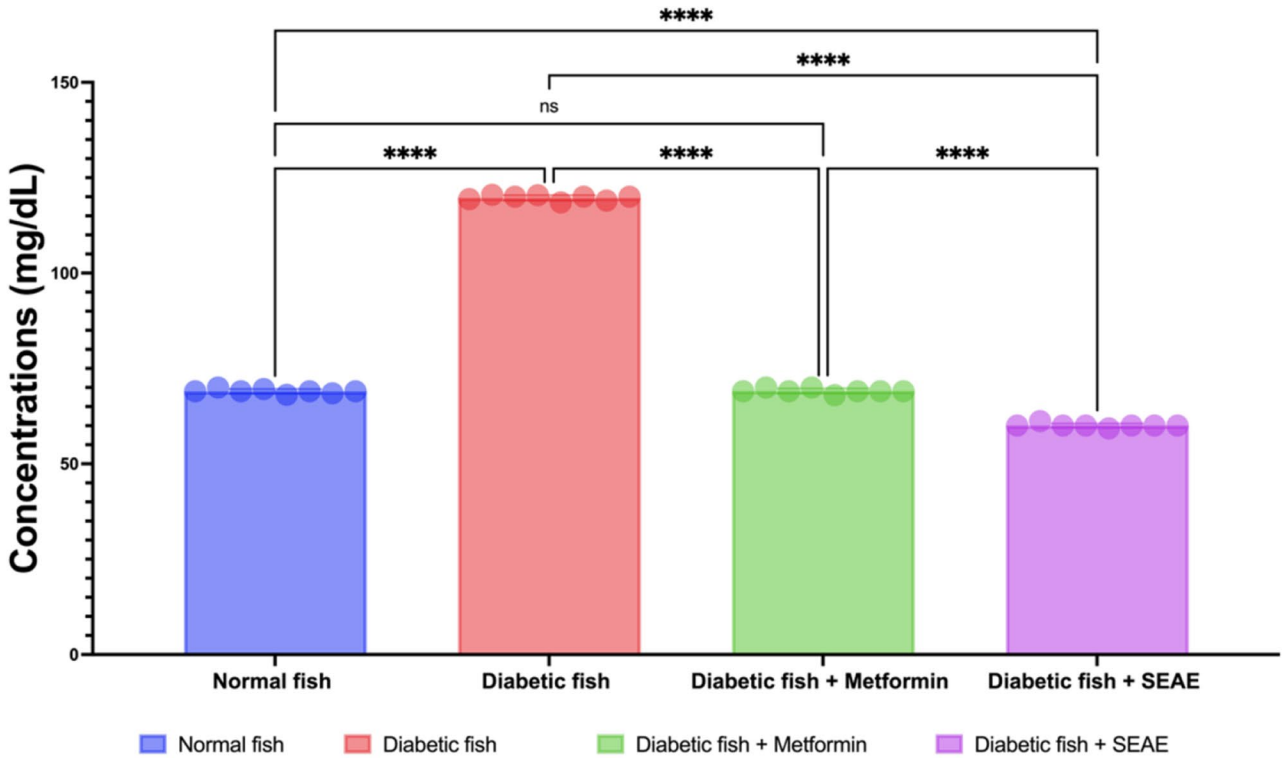


Fig. 3 Anti-hyperglycemic property of *E. acoroides* within the 20 days. ns. $P > 0.05$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$

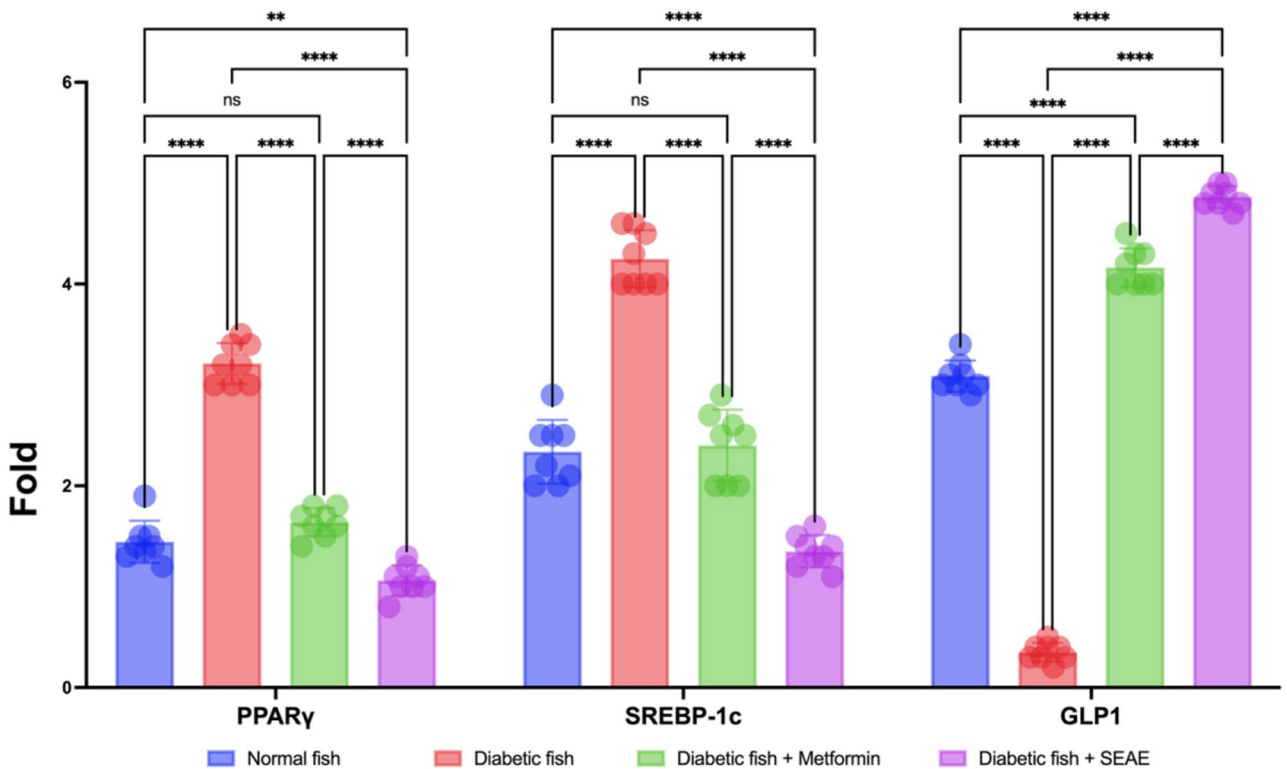


Fig. 4 Modulation of PPAR γ / GLP-1/SREBP-1c by *E. acoroides*. ns, $P > 0.05$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$

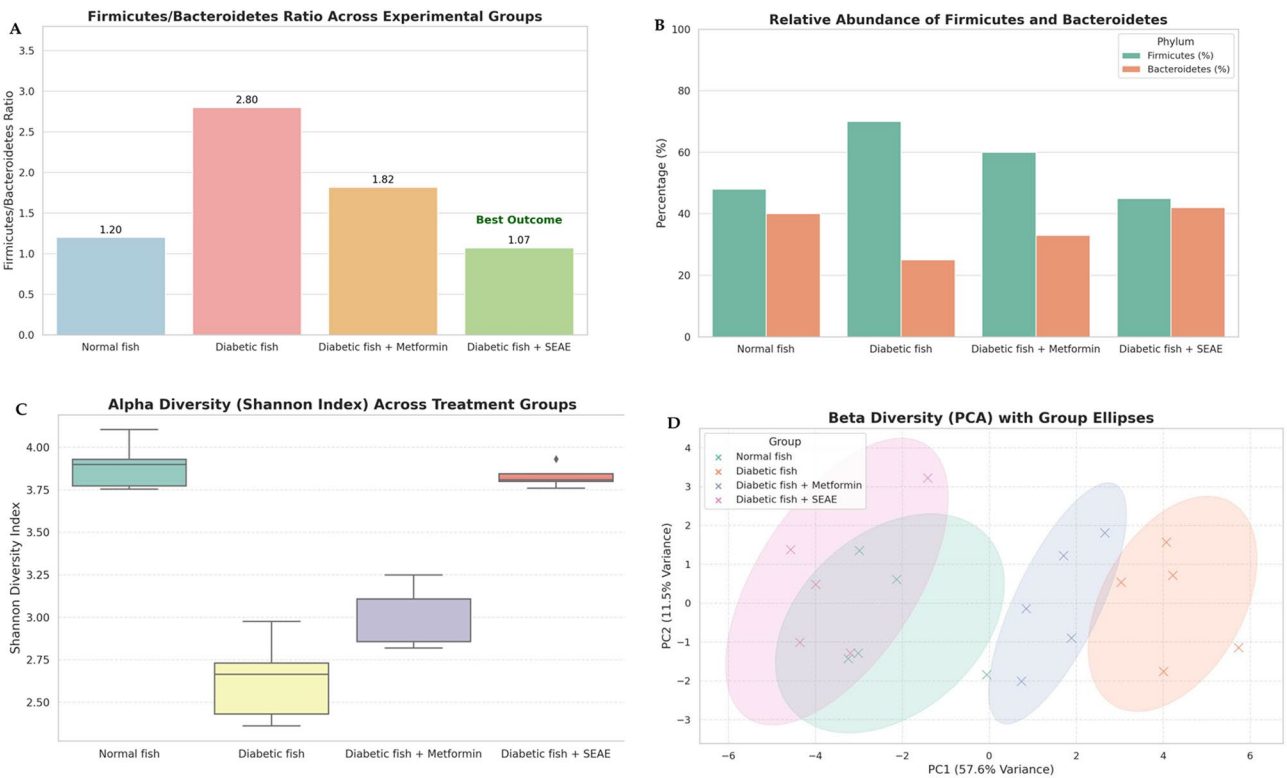


Fig. 5 Changes in the Gut Microbiome. (A) Firmicutes/Bacteroidetes (F/B) Ratio. (B) Proportion of Firmicutes and Bacteroidetes. (C) Alpha Diversity. (D) Beta Diversity

the normal fish group, thus suggesting gut dysbiosis is associated with diabetes. The diabetic fish treated with metformin showed a lower F/B ratio but did not reach normal levels. Meanwhile, the diabetic fish treated with SEAE showed the lowest F/B ratio, even compared to the healthy baseline, thus suggesting that SEAE extract could potentially restore the gut microbiota balance in diabetes.

Figure 5B illustrates the relative composition of the two dominant gut microbiota phyla: Firmicutes and Bacteroidetes. The normal fish group has a similar composition of the two dominant gut microbiota. Meanwhile, the diabetic fish group showed a high Firmicutes dominance and a marked reduction in Bacteroidetes, which reflects the total dysbiotic pattern seen in metabolic disorders. After being treated with metformin, the diabetic fish showed partial restoration, but the relative composition remained imbalanced. Interestingly, the diabetic fish treated with SEAE achieved a microbial composition closely resembling that of the healthy control, highlighting SEAE's role in restoring gut microbial homeostasis.

Figure 5C illustrates the Alpha Diversity (Shannon Index) across the treatment group. The Alpha diversity analysis revealed that the diabetic fish group had the lowest Shannon Index, indicating a significant loss in microbial richness and diversity due to diabetes. Although metformin treatment led to a modest increase, it failed to fully recover diversity levels. Remarkably, the SEAE-treated group displayed the highest Shannon Index, even exceeding that of the normal group, suggesting a protective and restorative effect of SEAE on gut microbial diversity.

Figure 5D illustrates the Beta Diversity (PCA + ellipses) across the treatment group. Beta diversity visualization using principal composition analysis (PCA) showed apparent group clustering and separation. The diabetic fish group was distinctly distant from the normal fish group, highlighting the major microbial composition shift. Metformin treatment caused a partial shift toward normal, but it remained in a separate cluster. In contrast, the SEAE group clustered closely with the healthy group, both visually and statistically, as indicated by overlapping ellipses, further supporting SEAE's ability to normalize the gut microbiota profile.

The associations between gut microbiota composition and key metabolic biomarkers in SEAE-treated diabetic zebrafish are shown in Fig. 6. As shown in the heatmap, the relative abundance of Firmicutes was positively correlated with the expression of PPAR γ and SREBP-1c, as well as with elevated levels of LDL and triglycerides, suggesting a strong association between increased Firmicutes and markers of dyslipidemia and adipogenic gene expression. In contrast, the abundance of bacteroidetes showed a significant positive correlation with the antidiabetic incretin GLP-1 and negative correlations

with SREBP-1c and lipid parameters, including LDL and TG. Furthermore, the Firmicutes/Bacteroidetes (F/B), a commonly used indicator of gut dysbiosis, exhibited positive correlations with PPAR γ and SREBP-1c expression and negatively correlated with GLP-1 levels. These findings suggest that the SEAE treatment modulated the gut microbiota composition by lowering the F/B ratio and enriching beneficial Bacteroidetes and induced favorable shifts in metabolic and inflammatory biomarkers, particularly by enhancing GLP1 expression and reducing markers associated with lipid accumulation. These results indicate a potential mechanistic link between gut microbial balance and the observed metabolic improvements in SEAE-treated diabetic zebrafish.

Discussion

Enhalus acoroides, a monotypic seagrass species from the Hydrocharitaceae family, is abundantly found in Indonesia's coastal ecosystems and is recognized for its ecological and medicinal value [13]. *E. acoroides* produces diverse metabolites such as carotenoids, polyphenols, and terpenoids with broad therapeutic properties—anti-diabetic, antimicrobial, antioxidant, anti-inflammatory, and cytotoxic—contributing to human disease prevention [6, 14]. This study extracted the bioactive compounds from *E. acoroides* using Ultrasound-Assisted Extraction (UAE). This green and efficient method utilizes ultrasonic waves to enhance the penetration of solvents into plant matrices, thereby improving yield and preserving bioactive components [7]. Through UAE, several potent carotenoids were identified in the extract from our previous study, including fucoxanthin (C1), lutein (C2), astaxanthin (C3), canthaxanthin (C4), zeaxanthin (C5), β -cryptoxanthin (C6), and β -carotene (C7), which are implicated in various metabolic and regulatory functions [7]. Other independent studies have also reported the presence of flavonoids such as apigenin, luteolin, three kaempferol derivatives, and azelaic acid, along with phenolic compounds like caffeic acid, sulfated apigenin, and sulfated luteolin [15–17].

This study revealed that *Enhalus acoroides* extract (SEAE) possesses notable anti-obesity effects, as evidenced by the significant reduction in body weight among SEAE-treated diabetic zebrafish compared to untreated diabetic controls. The changes in body weight were statistically similar to the metformin-treated group, supporting the extract's therapeutic potential. Although metformin is primarily used for glycemic control, several studies have reported weight-reducing effects in diabetic and non-diabetic individuals, attributed to mechanisms such as appetite regulation and modulation of gut microbiota [18, 19]. Meanwhile, the anti-obesity effect of SEAE is likely mediated through inhibiting pancreatic lipase, an enzyme responsible for breaking down

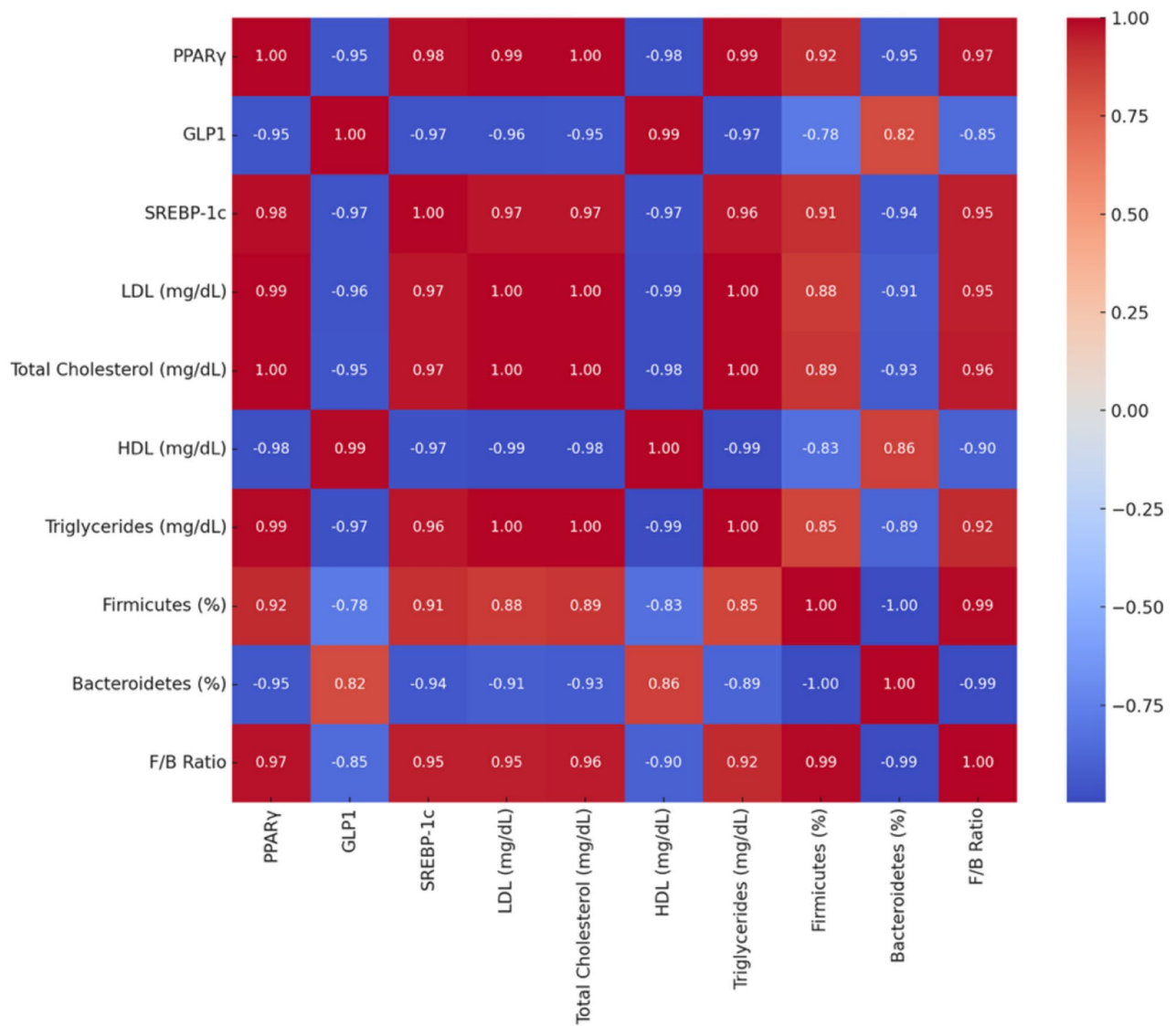


Fig. 6 Pearson Correlation Heatmap of Molecular Biomarkers and Lipid Profile in Relation to Gut Microbiota (*Firmicutes* and *Bacteroidetes*)

dietary triglycerides into absorbable free fatty acids [20]. By blocking this process, triglycerides are not converted into absorbable free fatty acids, leading to their excretion without being digested [20]. Our prior in vitro study revealed that SEAE, specifically its carotenoid component astaxanthin, displays pancreatic lipase inhibitory activity comparable to orlistat [7]. This suggests a possible mechanism whereby SEAE limits lipid digestion and absorption, contributing to decreased weight and triglyceride levels. These findings are consistent with other plant-based interventions, such as *Curcuma xanthorrhiza*, *Centella asiatica*, *Kaempferia angustifolia* Rosc., *Myristica fragrans*, and *Garcinia atroviridis*, which showed pancreatic lipase inhibition as well as a decrease in weight and triglyceride formation [21]. In addition to lipase inhibition, multiple in vitro studies have exhibited

its antioxidant effects [22, 23], further supporting the potential use of SEAE as a multifaceted natural therapeutic for metabolic disorders.

The extract’s antihyperglycemic effects are likely driven by its inhibiting key carbohydrate-digesting enzymes, namely α -glucosidase and α -amylase [7]. Inhibiting these enzymes slows the hydrolysis of complex carbohydrates into glucose, resulting in delayed glucose absorption and a reduced postprandial glycemic response [24]. Plant-derived compounds from *Garcinia mangostana*, *Rhizophora mucronata*, and marine fungi have shown similar α -glucosidase inhibition [25, 26]. At the same time, species such as *Costus igneus* and *Ocimum sanctum* have demonstrated α -amylase inhibition, reinforcing the therapeutic potential of natural enzyme inhibitors [27, 28]. The significantly lower blood glucose observed

in SEAE-treated diabetic zebrafish, even compared to metformin, underscores the extract's potent anti-diabetic activity.

This study provides deeper insight into the molecular mechanisms underlying the metabolic benefits of SEAE, mainly through its regulation of GLP-1, PPAR γ , and SREBP-1c. SEAE significantly increased GLP-1 expression, enhancing glycemic control and suppressing appetite [29]. Concurrently, its ability to downregulate PPAR γ suggests reduced adipocyte formation and improved insulin sensitivity. The decreased expression of SREBP-1c, a key regulator of lipid synthesis in the liver, implies reduced lipid accumulation and a protective effect against hepatic steatosis [3–5]. This regulatory effect may be partially mediated through the microRNA pathways; previous research has shown that UAE-derived extracts can downregulate microRNA-21, suppressing PPAR γ expression, offering a novel epigenetic mechanism for metabolic modulation [7]. The combined effect of enzyme inhibition and gene regulation suggests a multifaceted therapeutic potential of *E. acoroides* in managing obesity and diabetes. These findings highlight SEAE's dual action in promoting glucose metabolism and inhibiting lipogenesis, underpinning its anti-obesity and anti-hyperglycemic effects. SEAE showed comparable or even superior modulation of these metabolic markers compared to metformin. While metformin mainly improves insulin sensitivity and limits hepatic glucose output, it has a limited direct impact on lipid metabolism. In contrast, SEAE's broader mechanism of action suggests its potential as a natural therapeutic or adjunct treatment for metabolic syndrome, targeting both glucose and lipids.

The gut microbiome analysis revealed that SEAE treatment significantly reshaped the microbial landscape in diabetic zebrafish. Specifically, SEAE restored the Firmicutes/Bacteroidetes ratio to levels comparable to or better than healthy controls, suggesting a reversal of dysbiosis commonly observed in restoring metabolic health [30]. The more balanced Firmicutes/Bacteroidetes ratio in SEAE-treated fish, compared to the metformin group, may contribute to increased production of short-chain fatty acids (SCFAs), which are known to stimulate GLP-1 secretion and may partially explain the observed upregulation of GLP-1 expression [31]. Furthermore, SEAE enhanced alpha diversity, indicating an increase in microbial richness, and shifted beta diversity clustering closer to the normal group, which suggests a broader normalization of microbial communities. These changes were more pronounced than those observed with metformin, highlighting SEAE's superior modulatory effect. Considering the growing evidence that gut microbiota plays a crucial role in host metabolism, inflammation, and insulin resistance, the ability of SEAE to restore microbial

balance may represent a key mechanism underlying its anti-obesity and anti-diabetic efficacy [32].

This study's use of overfed and glucose-induced diabetic zebrafish provides a valuable model for examining metabolic diseases. To confirm the findings in this translational study, further study in mammalian systems and, eventually, in clinical settings is needed. Significantly, SEAE impacted several metabolic regulators, notably GLP-1, PPAR γ , and SREBP-1c, suggesting that it might aid in improving metabolic balance through various pathways. These results contribute to the growing body of research demonstrating the potential of plant-based medicines to address the intricate interactions between diabetes and obesity.

Strengths and Limitations

The major strength of this study lies in its integrative approach, combining physiological, molecular, and microbiota-level analyses in a well-established zebrafish model of metabolic dysfunction. The findings provide novel insights into the multi-targeted effects of *Enhalus acoroides* extract, including its capacity to modulate key genes (GLP-1, PPAR γ , SREBP-1c) and restore microbial balance. Furthermore, the extract demonstrated effects comparable to or exceeding those of metformin, highlighting its potential as a natural, marine-based therapeutic alternative.

Despite its valuable insights, this study has several limitations. While the zebrafish model provides translational insights, it may not fully capture the complexity of human metabolic responses. Incorporating additional analyses, such as oral glucose tolerance tests (OGTT) or insulin tolerance tests (ITT), could enable a more dynamic evaluation of glucose homeostasis. Future in vitro studies could also explore additional enzymes involved in carbohydrate metabolism, such as dipeptidyl peptidase-4 (DPP-4) or glucose-6-phosphatase, to further elucidate the antidiabetic mechanism of *E. acoroides* extract (SEAE). Furthermore, the current study's limited sequencing depth restricts microbiota characterization to the phylum level; future research could enhance this by conducting differential abundance analysis at deeper taxonomic levels. Additionally, while gene expression and microbiota analyses were performed, the absence of metabolomic and proteomic profiling represents a gap that warrants exploration in subsequent studies. Further validation in mammalian models and clinical settings is needed to confirm the therapeutic potential and safety of *E. acoroides* extract in humans.

Conclusions

This study demonstrates that *Enhalus acoroides* extract (SEAE) effectively ameliorates metabolic dysfunction in zebrafish models of diet-induced obesity and

glucose-induced diabetes. SEAE improved glycemic control, reduced body weight, and modulated key metabolic regulators—GLP-1, PPAR γ , and SREBP-1c—more effectively than metformin. Furthermore, SEAE significantly restored gut microbiota diversity and composition, aligning microbial profiles more closely with healthy control ones. These multifaceted actions suggest that SEAE is a promising natural therapeutic candidate for addressing the dual burden of obesity and diabetes. Further research in mammalian models and clinical trials is warranted to validate its translational potential as a novel functional food or nutraceutical intervention.

Abbreviations

E. acoroides	Enhalus acoroides
GLP-1	Glucagon-like peptide-1
PPAR γ	Peroxisome proliferator-activated receptor gamma
SREBP-1c	Sterol regulatory element-binding protein-1c
UAE	Ultrasound-assisted extraction
E-UAE	UAE-derived < Emphasis Type="Italic"> E. acoroides</ Emphasis> extract
SEAE	Enhalus acoroides seagrass extract
C1	Fucoxanthin
C2	Lutein
C3	Astaxanthin
C4	Canthaxanthin
C5	Zeaxanthin
C6	β -cryptoxanthin
C7	β -carotene
NCBI	National Center for Biotechnology Information
RAS	Recirculating aquaculture system (RAS)
UV	Ultraviolet
ARRIVE	Animal Research: Reporting of In Vivo Experiments
DIO	Diet-induced obesity
FBG	Fasting blood glucose
SD	Standard deviation
ANOVA	One-way analysis of variance
LSD	Least significant difference
F/B ratio	Firmicutes/Bacteroidetes ratio
PCA	Principal component analysis

Author contributions

Conceptualization, R.R.T., N.A.T. and F.N.; Methodology, F.N., N.A.T., R.R.T. and A.S.; Software, F.N., M.M.K. and R.A.S.; Validation, R.A.S., R.R., F.N., R.R.T. and N.A.T.; Formal Analysis, F.N., R.K., R.R., E.H., M.M.K. and R.A.S.; Investigation, F.N., N.A.T., M.M.K. and R.A.S.; Data Curation, F.N., R.K., M.M.K. and R.A.S.; Writing—Original Draft Preparation, F.N., M.M.K., N.A.T. and R.R.T.; Writing—Review and Editing, F.N., N.A.T., R.R., R.K., R.A.S., E.H. and A.S.; Visualization, A.S., F.N.; Supervision, F.N., R.R.T., R.R., A.S., R.A.S. and N.A.T. All authors have read and agreed to the published version of the manuscript.

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Data availability

The data presented in this study are available on request from the corresponding author. The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Declarations

Ethics and consent to participate

not applicable.

Consent to publish

Not applicable.

Informed consent

Not applicable.

AI statements

During the revision of this work the author(s) used ChatGPT in order to correct the language and paraphrase the necessary sections. After using this tool/service, the author(s) reviewed and edited the content as necessary and took full responsibility for the content of the published article. Authors are ultimately responsible and accountable for the contents of the work.

Institutional review board statement

All procedures adhered strictly to the Guidelines for Reporting In Vivo Experiments (ARRIVE) and have also been reviewed and accepted in the Preclinical Trials Study Register with number PCTE0000604.

Competing interests

The authors declare no competing interests.

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