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Carotenoid and peptide supplementation from *Caulerpa* sp. (sea grapes) extract mitigate metabolic syndrome in cholesterol-enriched diet rats via modulation of gut microbiota

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Abstract

Background Metabolic syndrome (MetS) is a multifactorial disorder characterized by obesity, dyslipidemia, insulin resistance, and hypertension, increasing the risk of cardiovascular diseases and type 2 diabetes mellitus (T2DM). Recent evidence suggests that gut microbiome dysbiosis plays a significant role in MetS pathogenesis. Functional foods enriched with bioactive compounds, particularly those derived from marine sources, have emerged as promising interventions.

Objective This study aimed to evaluate the effects of carotenoid and peptide supplementation from *Caulerpa* sp. (sea grapes) extract on metabolic syndrome and gut microbiome modulation in cholesterol-fat-enriched diet (CFED) rats.

Methods A randomized preclinical trial was conducted using CFED-fed rats, supplemented with carotenoid and peptide extracts from *Caulerpa* sp. Metabolic parameters, lipid profiles, enzymatic activities, and inflammatory biomarkers were assessed. Additionally, gut microbiota composition was analyzed using high-throughput sequencing techniques.

Results Supplementation with *Caulerpa* sp. extracts significantly improved metabolic markers, including reductions in body weight gain, triglycerides, total cholesterol, low-density lipoprotein (LDL), and blood glucose levels, while increasing high-density lipoprotein (HDL) ($p < 0.05$). Enzymatic analysis revealed suppression of key metabolic enzymes, such as α -glucosidase and lipase, suggesting potential regulatory effects on lipid and carbohydrate metabolism. Moreover, gut microbiome analysis indicated increased microbial diversity and favorable shifts in bacterial taxa. Specifically, *Faecalibacterium* and *Lactobacillus*, known for their anti-inflammatory and metabolic benefits, were enriched, while *Oscillospira*, which has been associated with metabolic disorders, showed a decline. These microbial changes suggest a potential role of *Caulerpa* sp. in modulating gut health and systemic metabolism.

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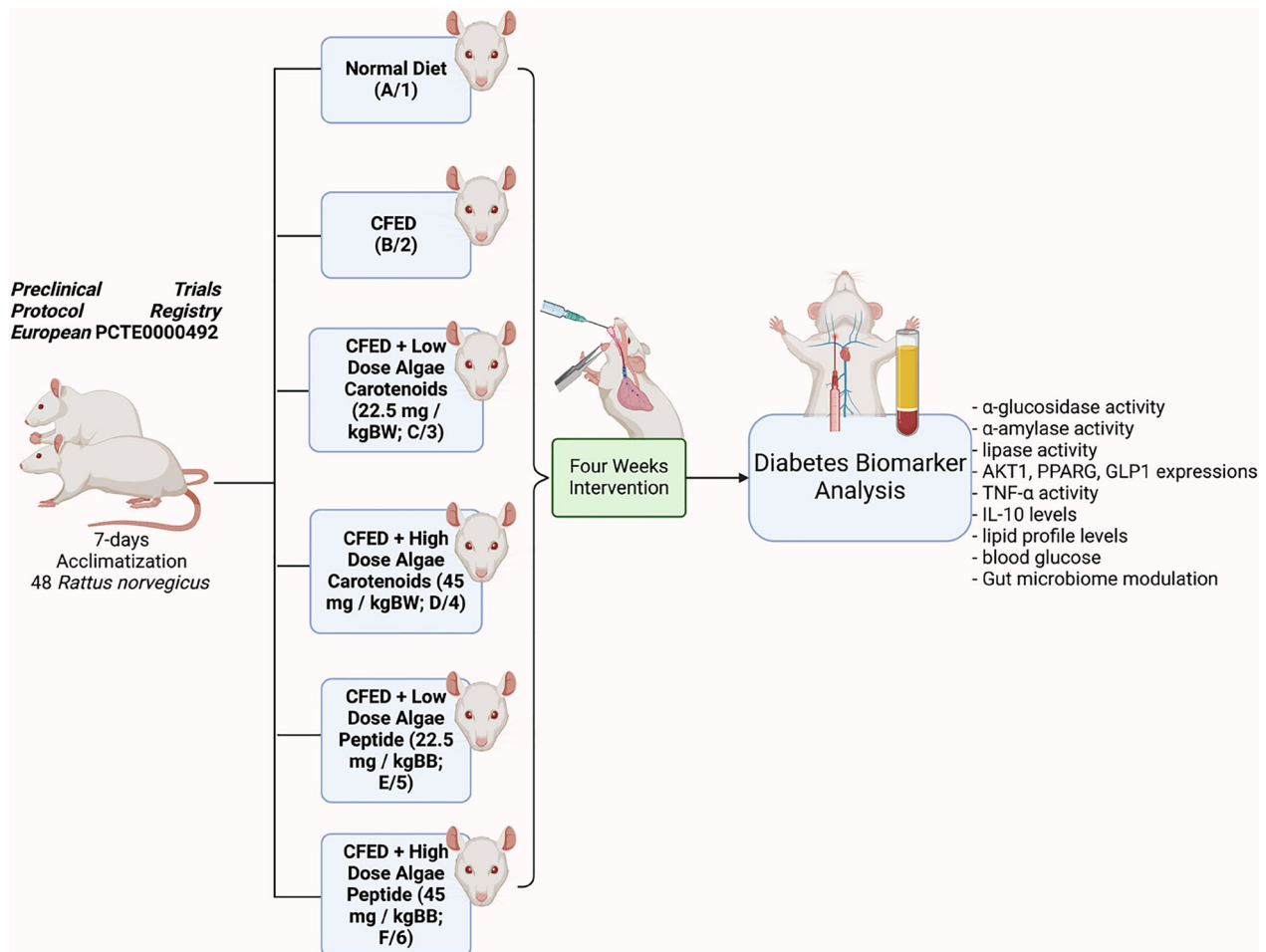


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Conclusion The findings demonstrate that carotenoid and peptide extracts from *Caulerpa sp.* effectively mitigate metabolic syndrome through metabolic regulation, gut microbiome modulation, and anti-inflammatory mechanisms. This study highlights *Caulerpa sp.* as a potential functional food for MetS management, paving the way for future clinical applications.

Clinical trial number Not applicable.

Graphical Abstract



Keywords Metabolic syndrome, Obesity, Diabetes, Functional food, Gut microbiota, Green algae

Introduction

Metabolic syndrome (MetS) is a complex disorder characterized by a cluster of metabolic abnormalities [1, 2], including central obesity, dyslipidemia, hypertension, and insulin resistance [3, 4], which significantly increase the risk of cardiovascular disease and type 2 diabetes mellitus (T2DM) [5, 6]. The prevalence of MetS has been rising globally, driven by sedentary lifestyles and unhealthy dietary patterns [7]. Current evidence demonstrates the role of gut microbiome dysbiosis in the pathogenesis of MetS [8, 9], highlighting the intricate relationship between dietary components and microbial diversity,

which, in turn, influence metabolic homeostasis [9–11]. Functional foods enriched with bioactive compounds have emerged as a promising strategy to mitigate MetS [12, 13]. These foods are characterized by their ability to provide health benefits beyond basic nutrition through the presence of bioactive compounds that can modulate physiological functions [14]. Among them, carotenoids and bioactive peptides derived from marine sources have gained attention due to their potent antioxidant [15, 16], anti-inflammatory, and metabolic regulatory properties [15].

Sea grapes (*Caulerpa* sp.), a marine algae rich in carotenoids and peptides [17], have been traditionally consumed in various coastal regions [18, 19], qualify as a functional food due to their rich content of carotenoids, peptides, polyphenols, and essential fatty acids that confer metabolic benefits beyond basic nutritional value [20]. *C. lentillifera* demonstrates superior bioactive compound concentrations compared to other *Caulerpa* species, with notably higher levels of fucoxanthin and bioactive peptides that contribute to its enhanced antioxidant capacity [21],[22]. Previous studies have shown that *C. lentillifera* consumption is associated with improved lipid profiles and reduced inflammatory markers in human subjects [23], [24], yet the specific mechanisms underlying these benefits remain poorly understood.

Limited research has explored the direct relationship between sea grape consumption and gut microbiome modulation. Preliminary studies suggest that marine algae consumption can influence gut microbial composition through prebiotic effects of alginate and other polysaccharides [25],[26]. The bioactive compounds in sea grapes, particularly carotenoids and peptides, may exert their metabolic effects through modulation of beneficial bacteria such as *Bifidobacterium* and *Lactobacillus*, while suppressing pathogenic strains associated with metabolic dysfunction [27], [28]. However, comprehensive mechanistic insights into how *C. lentillifera*-derived bioactives specifically influence gut-metabolic axis remain largely unexplored.

This study aims to investigate the effects of carotenoid and peptide supplementation extracted from sea grapes (*C. lentillifera*) on metabolic syndrome modulation through gut microbiome alterations in a cholesterol-enriched diet (CFED) rat model. While previous studies have evaluated the impact of dietary interventions on metabolic health [29, 30], this research provides comprehensive mechanistic insights into how specific marine bioactives influence host metabolism through gut microbiota modulation.

The novelty of this study lies in the comprehensive approach of individual carotenoid and peptide supplementation effects on both metabolic pathways and gut microbiome diversity. This approach allows for the identification of specific bioactive compounds responsible for metabolic improvements, providing targeted insights for functional food development. Furthermore, by employing high-throughput sequencing techniques and advanced statistical modeling, this study offers novel perspectives on gut microbial shifts associated with metabolic improvements, paving the way for targeted dietary strategies in managing MetS.

By elucidating the interplay between marine-derived bioactives, gut microbiota, and metabolic parameters, this study provides valuable insights for developing

functional foods and nutraceuticals aimed at addressing the growing burden of MetS. The findings could have far-reaching implications in precision nutrition and therapeutic strategies, particularly in regions where sea grapes are readily available as a sustainable dietary source.

Materials and methods

Preparation and procurement of peptide and carotenoids extracts of *Caulerpa*

Preparation of green algae (*Caulerpa racemosa*)

Caulerpa racemosa and *C. lentillifera* were selected based on their distinct bioactive profiles and traditional consumption patterns in Indonesian coastal communities. *C. racemosa* was specifically chosen for carotenoid extraction due to its documented high β -carotene and lutein content, while *C. lentillifera* was selected for peptide extraction based on its superior protein content and previously identified bioactive peptide sequences with potential metabolic benefits [21]. This species-specific selection strategy was adopted to maximize the extraction of target compounds from each algal source, as supported by preliminary phytochemical screening studies that demonstrated significantly higher carotenoid yields in *C. racemosa* and superior peptide diversity in *C. lentillifera*.

Samples of green algae (*Caulerpa racemosa* and *Caulerpa lentillifera*) were obtained from a cultivation pond in Jepara Regency, Central Java, Indonesia, with approval from both the pond owner and local authorities. The botanical identification and authentication were conducted at Hasanuddin University, Indonesia, using the National Center for Biotechnology Information (NCBI) Taxonomy ID (*C. racemosa* = 6968) and the Integrated Taxonomic Information System (ITIS) Report ID (*C. racemosa* = 6968). The collected green algae were thoroughly washed to eliminate any impurities, followed by sun-drying for two to three days. Subsequently, they were oven-dried at 40 °C to ensure complete dehydration. The dried algae were then finely chopped and ground using a blender to produce *Caulerpa* powder. The methodology followed in this study was adapted from previous research [31]. Maceration was employed to extract the bioactive components from the obtained powder for further investigation.

Extraction process for Carotenoid-Rich extract

To prepare the carotenoid-rich extract from *C. racemosa* [31], one kilogram of the powdered *Caulerpa* was mixed with 2 L of 96% ethanol in a 1:2 ratio and placed in a dark bottle. The mixture underwent three consecutive 24-hour soaking periods, during which the filtrate was stirred and filtered every 24 h using Whatman 41 filter paper. The remaining residue was re-extracted using fresh 96% ethanol, and the process was repeated. The extract was then

subjected to ultrasonication for 30 min at 40 °C using an ultrasound sonicator (400 W, Branson 2510 model, Danbury, CT, USA). After extraction, the solution was concentrated using a rotary evaporator under reduced pressure (100 millibars) for 90 min and further dried in an oven at 40 °C. The final extract, denoted as CrE: *Caulerpa racemosa*-ethanol (polar), was selected due to its previously reported high carotenoid content [31].

Preparation of P13 FDGIP peptide sample

Given that previous research has demonstrated that *Caulerpa lentillifera* possesses a rich profile of bioactive peptides with potential therapeutic applications, different *Caulerpa* species were selected based on their distinct bioactive compound profiles to maximize the diversity of compounds investigated in this study. The synthetic derivative of *C. lentillifera* FDGIP (Phe-Asp-Gly-Ile-Pro; pentapeptide), which demonstrated the most favorable docking score, was obtained from an Indonesian laboratory. Peptide synthesis was conducted using a Solid-Phase Peptide Synthesizer with Microwave-Assisted Technology and an internal temperature sensor. The synthesis began with the C-terminal amino acid as the first incorporated residue. Specifically, HD-Pro-2-CITrt-Resin (for FDGIP synthesis, FP-5) was swollen in 6 mL of DMF for 1 h before proceeding with the peptide assembly. The synthesis protocol followed the manufacturer's guidelines and was based on a well-established research methodology. The final peptide product achieved a purity level of 97%, as determined by the Average Local Confidence metric, consistent with previous studies [32].

In vivo preclinical trial study

Animal model and experimental setup

All experimental rats were provided unrestricted access to standard food and water *ad libitum*. A total of thirty-two male *Rattus norvegicus* rats (aged 3–5 weeks) were procured from the Animal Husbandry Farm in Indonesia. The animals were housed in groups within cages under controlled laboratory conditions, maintained at a temperature of 27 ± 2 °C, and subjected to a 12-hour light/dark cycle. To acclimatize to the experimental environment, the rats were kept under these conditions for seven days prior to the intervention. All procedures adhered strictly to the Guidelines for Reporting In Vivo Experiments (ARRIVE) and has also been reviewed-accepted in the Preclinical Trials Study Register with number PCTE0000492 and has obtained ethical approval from UIN Sunan Kalijaga Yogyakarta (Approval No. 2390.3/Un.02/L3/TL/06/2025). Daily observations were conducted by licensed veterinarians to monitor the animals' welfare, assessing for signs such as reduced appetite, ruffled fur, lethargy, withdrawal behavior, hiding, or curling up. Weekly evaluations of body weight and general

health markers were also performed. The sample size for the study was determined using the Federer formula. According to the Federer formula, $(t-1)(r-1) \geq 15$ [29, 30], where t represents the number of treatments and r is the minimum number of rats per treatment. Given that there are six treatments ($t=6$), the equation simplifies to $5(r-1) \geq 15$, which leads to $r \geq 4$. To ensure the reliability of the study and account for potential animal losses or mortality, eight rats were selected for each treatment group.

Experimental groups and treatments

The study employed a randomization process using a random number table for simple randomization to allocate subjects into groups [33]. Blinding was implemented at multiple levels to minimize bias. Intervention providers and diet providers were not informed of the specific type of diet or feed administered to the animals. Similarly, data processors responsible for analyzing the results were blinded to the dietary or feed interventions to ensure unbiased data assessment. The rats were randomly divided into four groups, each receiving a specific dietary and treatment protocol:

- **Control-Normal (CON-NORM, Group A):** Standard pellet diet and water *ad libitum* (no CFED or Extracts).
- **Control-Negative (CON-NEG, Group B):** Cholesterol- and Fat-Enriched Diet (CFED) with water *ad libitum*.
- **Low-Dose Carotenoid Extract (Carotenoid-Low, Group C):** CFED + Low Dose Carotenoids (22.5 mg / kgBW; C/3) and water *ad libitum*.
- **High-Dose Carotenoid Extract (Carotenoid-High, Group D):** CFED + High Dose Carotenoids (45 mg / kgBW; D/4) and water *ad libitum*.
- **Low-Dose Peptide Extract (Peptide-Low, Group E):** CFED + Low Dose Peptide (22.5 mg / kgBB; E/5) and water *ad libitum*.
- **High-Dose Peptide Extract (Peptide-High, Group F):** CFED + High Dose Peptide (45 mg / kgBB; F/6) and water *ad libitum*.

The dosage selection was based on the conversion factor proposed by Laurence and Bacharach (2013) [24], using a human-to-rat conversion factor of 0.018, and supported by previous safety studies demonstrating no adverse effects at doses up to 90 mg/kg BW in rodent models [27, 28]. The selected dosages (22.5 and 45 mg/kg BW) correspond to approximately 250–500 mg daily human equivalent doses, which align with traditional consumption patterns and previous efficacy studies in metabolic syndrome interventions. Control groups received equivalent volumes of vehicle (distilled water) to match the administration volume of treatment groups, ensuring consistent

handling and gavage procedures across all experimental groups. Metabolic syndrome (MetS) was verified after two weeks of CFED administration by measuring fasting blood glucose (≥ 126 mg/dL), triglycerides (≥ 150 mg/dL), and total cholesterol (≥ 200 mg/dL) levels, with animals meeting at least two of these criteria being included in the study groups.

Extract administration was conducted orally by certified personnel. Throughout the study, the daily intake of food and water was monitored to ensure consistency between the control and experimental groups. The dosage selection was based on the conversion factor proposed by Laurence and Bacharach (2013) [34], using a human-to-rat conversion factor of 0.018.

Blood and tissue sample collection

After four weeks of dietary intervention, blood samples were collected following an overnight fasting period. Anesthesia was administered using ketamine hydrochloride (100 mg/kg BW) delivered via intraperitoneal injection, with anesthetic depth confirmed by absence of pedal withdrawal reflex before proceeding with blood collection. Samples were transferred into sterile, anticoagulant-free tubes and allowed to coagulate at room temperature before being centrifuged at 1000 g for 20 min to separate the serum.

Blood glucose and cholesterol levels were analyzed using a COBAS Integra® 400 plus analyzer (Roche). Liver tissue samples were washed with 1% Phosphate Buffered Saline (PBS, pH 7.4) until clear. These samples were centrifuged (3,000 rpm, 20 min) to separate the pellet and supernatant, the latter being used for the analysis of PGC-1 α levels. PGC-1 α concentrations were quantified using the Mouse PGC-1 α ELISA Kit (Sunlong Biotech Co., Ltd.).

Biomarker assessment

The study evaluated several biomarkers, including:

- **Metabolic Profile:** High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), Triglycerides (TG), Total Cholesterol (TC), and Blood Glucose (BG).
- **Enzymatic Activity:** α -Amylase, α -Glucosidase, Lipase, and AST.
- **Other Biomarkers:** AKT1, PPARG, IL-10, TNF- α , and PGC-1 α .

Lipid profile markers (HDL, LDL, TG, TC) and blood glucose were measured using automated enzymatic assays on the COBAS Integra® 400 plus analyzer. Enzymatic activities (α -Amylase, α -Glucosidase, Lipase, AST) were determined using spectrophotometric assays with specific substrate reactions. Molecular biomarkers (AKT1, PPARG, IL-10, TNF- α , PGC-1 α) were quantified

using enzyme-linked immunosorbent assays (ELISA) with commercially available kits, following manufacturer protocols. AKT1, PPARG, and PGC-1 α were selected as molecular markers due to their central roles in metabolic syndrome pathophysiology. AKT1 serves as a key regulator of glucose uptake and lipid metabolism, PPARG functions as a master regulator of adipogenesis and insulin sensitivity, while PGC-1 α controls mitochondrial biogenesis and energy metabolism, making these biomarkers mechanistically relevant to MetS assessment and treatment response evaluation. The experimental protocols for biomarker assessment followed previously established methods [29, 30].

Dietary Preparation

The standard control diet consisted of commercial rat chow containing 12% moisture, 20% protein, 4% fat, 14% calcium, 1% fiber, 0.7% phosphorus, 11.5% total ash, 0.3% vitamin C, and 0.1% vitamin E. The Cholesterol- and Fat-Enriched Diet (CFED) consisted of 1% cholic acid, 2% pure cholesterol powder, 20% animal fat (pork oil), and 2% corn oil. These components were homogeneously mixed with standard rat chow, formed into a dough using 1,000 mL of distilled water, shaped into small pellets, and air-dried under sterile conditions. CFED batches were prepared weekly and stored at 4 °C to minimize oxidation.

Analysis of rat feces for gut microbiota

Fecal samples were collected from live animals using sterile collection tubes during the final week of the intervention period, immediately placed on ice, and were stored at -80 °C prior to conducting gut microbiome analysis. Intestinal bacterial genomes were extracted from these samples using the OMG Soil Extraction Kits provided by Shanghai Meiji Biopharmaceutical Technology Co., Ltd. (Shanghai, China). The V3-V4 variable regions of the 16 S rRNA gene were amplified via polymerase chain reaction (PCR) using the primers 338 F (5'-ACTCCTA CGGGAGGCAGCAG-3') and 806R (5'-GGACTACH-VGGGTWTCTAAT-3'). Sequencing was carried out on the Illumina Miseq PE300 platform. Raw sequencing data were processed using FAST software (version 3.5.2), and sequences were trimmed with FLASH software. High-quality reads were clustered into Operational Taxonomic Units (OTUs) at 97% similarity using UPARSE software (version 7.1). Potential chimera sequences were removed through specialized search algorithms. This was achieved by comparing sequences against the Silva 16 S rRNA database (Version 138, maintained by the Max Planck Institute for Marine Microbiology and Jacobs University, Bremen, Germany) with a 70% similarity threshold.

Data analysis and management

Prior to statistical analysis, normality of data distribution was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated using Levene's test. Data meeting these assumptions were analyzed using parametric tests, while non-parametric alternatives were employed when assumptions were violated. Multivariate ANOVA was applied to analyze several parameters, including lipid profile markers such as LDL, HDL, TG, TC, and BG; inflammatory biomarkers IL-10, TNF- α , and PGC-1 α ; enzymatic indicators α -Amylase, α -Glucosidase, Lipase, AST; and molecular biomarkers AKT1, PPARG, IL-10, TNF- α , and PGC-1 α in an in vivo experimental setting. One-way ANOVA was used to examine variations in secondary parameters, such as water intake (mL), food intake (g), food efficiency ratio (FER), and changes in body weight (initial, final, and weight gain in g) among the groups. Data were reported as mean $\pm$ standard error of the mean (SEM) with a 95% confidence interval. Statistical analyses for both in vitro and in vivo data were performed using GraphPad Prism software version 10.4.1 (Boston, MA, USA) on a MacBook/Mac Os.

Results

The study investigated the effects of *Caulerpa* (Sea Grapes) extract on various physiological and biochemical parameters in rats fed a high-fat, high-carbohydrate diet (CFED). The results were categorized into body weight changes, lipid profiles, enzymatic activities, and molecular biomarker expressions.

Body weight characteristics and nutritional intake measurements are presented in Fig. 1. Initial body weights showed no significant differences between groups ($p > 0.05$). However, final body weights demonstrated significant variations, with the CFED control group showing the highest final weight compared to treatment groups. The change in body weight (Δ) revealed that all CFED treatment groups experienced significant weight reduction compared to the normal diet (ND) group, with the CFED + Carotenoid-High group showing the most pronounced reduction. This weight reduction in treatment groups may be attributed to the metabolic effects of *Caulerpa* extract components, which potentially enhanced lipid metabolism and reduced adipose tissue accumulation despite the high-fat, high-carbohydrate diet. Food intake and water intake measurements showed no significant differences between groups ($p > 0.05$), indicating that

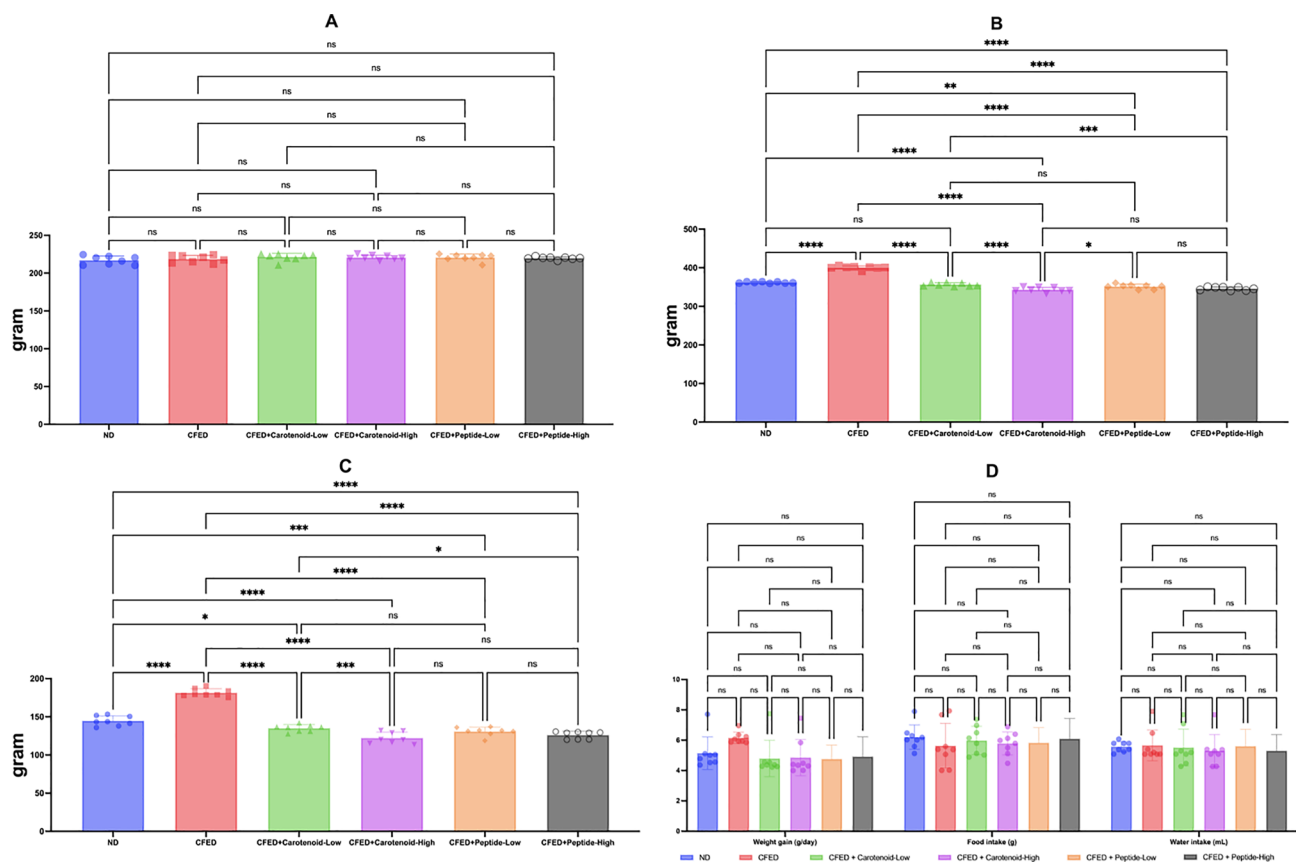


Fig. 1 Characteristic of Rats Markers. **A.** Initial body weight in all groups. **B.** Final body weight in all groups. **C.** Change in body weight (Δ). **D.** Weight gain, Food Intake and Water Intake Data. ****, ***, **, * $p < 0.05$, not significant (NS) $p > 0.05$

the observed weight changes were not due to altered consumption patterns but rather to metabolic modifications induced by the extract.

Figure 2 presents the effects of *Caulerpa* extract on the lipid profile and blood glucose levels in CFED-fed rats. The intervention significantly influenced lipid metabolism, as evidenced by alterations in high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), and total cholesterol (TC) levels. In addition, blood glucose (BG) levels were assessed to determine the extract's potential impact on glucose homeostasis. The results indicate that *Caulerpa* extract may play a role in modulating dyslipidemia and hyperglycemia, two major metabolic disorders associated with high-fat and high-carbohydrate diets. The statistical analysis confirmed significant differences ($p < 0.05$) between groups, reinforcing the potential metabolic benefits of the extract.

The effects of *Caulerpa* extract on lipid metabolism were highly significant, as reflected by changes in high-density lipoprotein (HDL), low-density lipoprotein (LDL), triglycerides (TG), and total cholesterol (TC)

(Fig. 2). In the CFED group, LDL, TG, TC, and blood glucose all increased, accompanied by a decline in HDL. Both high- and low-dose carotenoid supplementation raised HDL levels, with the greatest improvement seen in the high-dose peptide group (MD = 24.79, $p < 0.0001$). LDL levels fell dramatically and significantly in the high-dose peptide group (MD = -16.22, $p < 0.0001$) and the high-dose carotenoid group (MD = -16.35, $p < 0.0001$), whereas low-dose treatments produced only modest reductions. Triglycerides decreased in all intervention groups, most notably in the high-dose carotenoid group (MD = -14.31, $p < 0.0001$). The elevated total cholesterol induced by the high-fat, high-cholesterol diet was significantly lowered by high-dose peptide supplementation ($p < 0.0001$). Blood glucose levels also dropped across all groups, with the largest decrease in the high-dose peptide group (MD = -21.85, $p < 0.0001$). Collectively, high-dose peptide supplementation yielded the most consistent and effective improvements in both lipid and glucose profiles.

The enzymatic profile, illustrated in Fig. 3, highlights the effects of *Caulerpa* extract on key metabolic enzymes

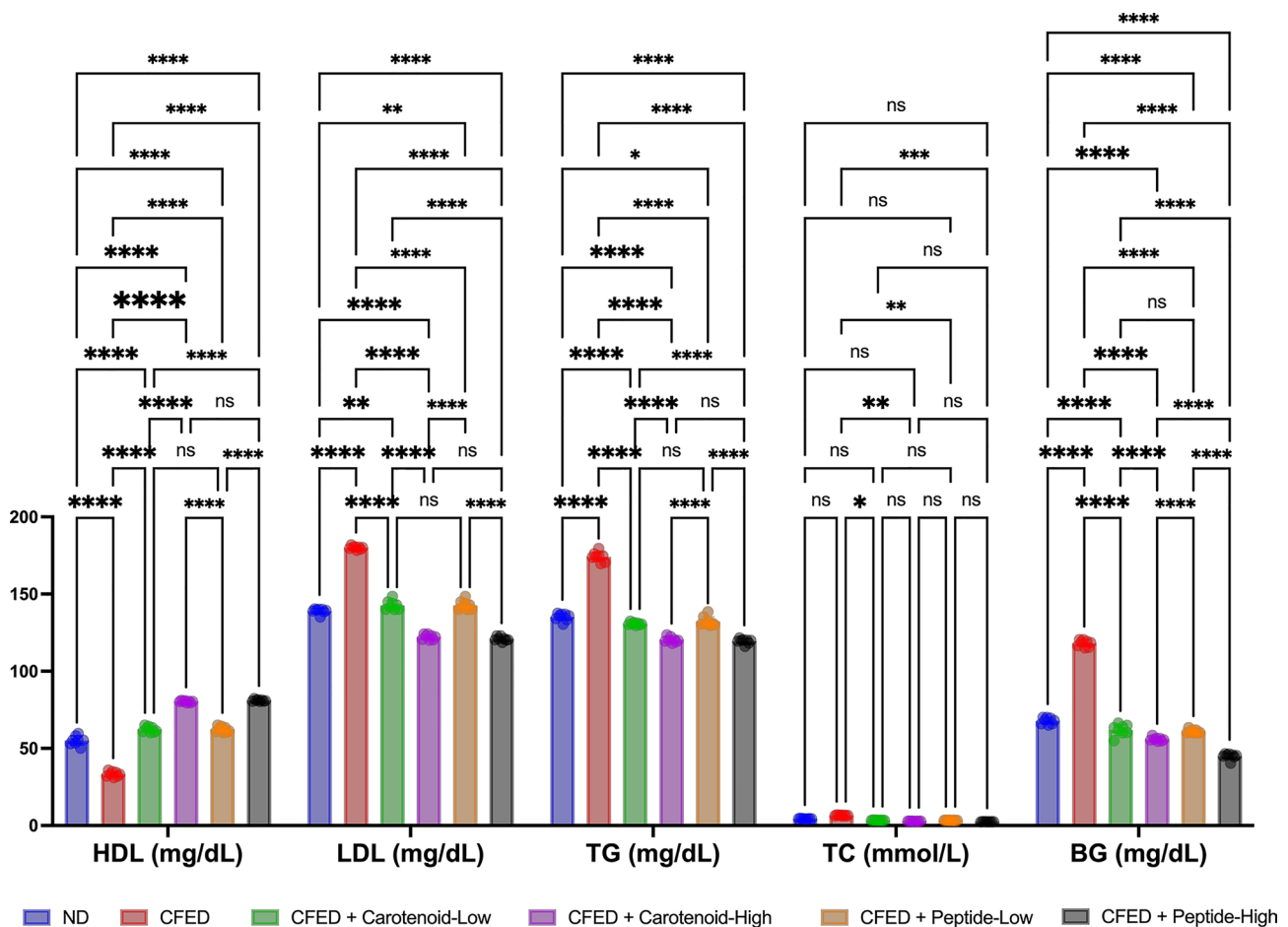


Fig. 2 Effect of Sea Grapes Extracts on lipid profile and blood glucose in rats fed by CFED. High-density lipoprotein (HDL); low-density lipoprotein (LDL); triglycerides (TG); total cholesterol (TC); blood glucose (BG). ****, ***, **, * $p < 0.05$, not significant (NS) $p > 0.05$

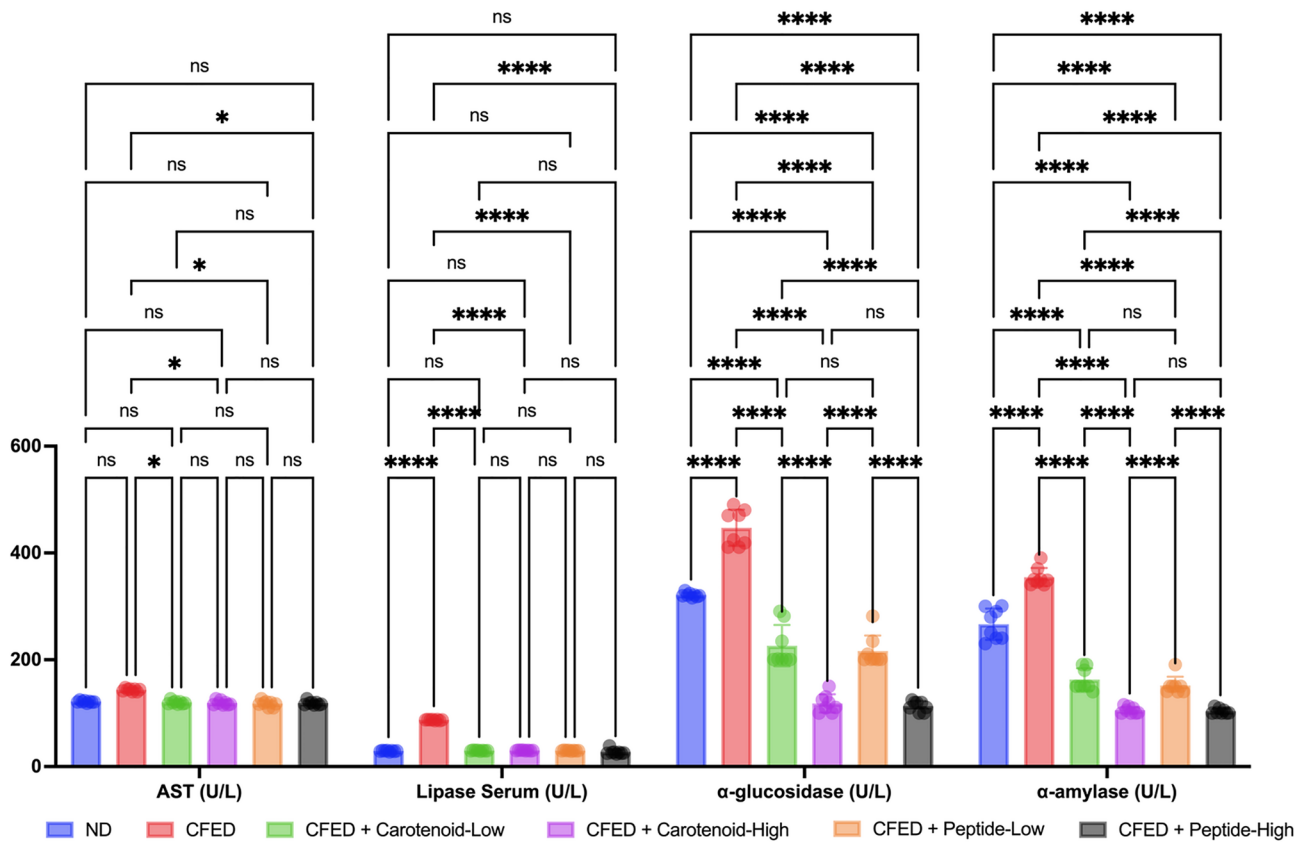


Fig. 3 Effect of Sea Grapes Extracts on serum enzyme levels in rats fed by CFED. aspartate transaminase (AST); Lipase; α-glucosidase; α-amylase. ****, $p < 0.0001$; not significant (NS) $p > 0.05$

in serum. Specifically, the study measured aspartate transaminase (AST), lipase, α-glucosidase, and α-amylase levels. The results demonstrate significant variations ($p < 0.0001$) in enzyme activities, suggesting that *Caulerpa* extract may influence both lipid and carbohydrate metabolism, potentially reducing metabolic stress caused by CFED feeding.

Lastly, Fig. 4 depicts the molecular biomarker expression in response to *Caulerpa* extract administration. Several critical biomarkers related to inflammation, metabolism, and insulin signaling were evaluated, including tumor necrosis factor-α (TNF-α), peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PGC-1α), interleukin-10 (IL-10), glucagon-like peptide-1 (GLP-1), AKT1, and PPARG. The expression levels of AKT1 and PPARG, which are critical regulators of glucose homeostasis and lipid metabolism, were also analyzed. The statistical significance ($p < 0.05$) highlights the potential molecular mechanisms through which *Caulerpa* extract exerts its beneficial effects.

The taxonomic composition at the genus level is depicted in Fig. 5, illustrating the relative abundance of different microbial taxa in response to the experimental conditions. Each colored bar represents the proportion of various microbial genera within the total microbiota,

providing insights into the shifts in microbial community structure. The distribution of these genera reflects the impact of dietary interventions, environmental factors, or treatments on the gut microbiome composition. The analysis indicates notable variations in microbial taxa across different groups, suggesting a potential influence of *Caulerpa* extract on the microbial ecosystem. Certain genera may exhibit increased abundance, indicating their potential role in modulating gut health, while others may show a decline, possibly due to competitive exclusion or shifts in nutrient availability. These changes in taxonomic composition provide a foundational understanding of how dietary components influence microbial diversity and function. By examining the relative proportions of key bacterial genera, this data offers crucial insights into the potential prebiotic or antimicrobial effects of the intervention. Further statistical analysis and functional profiling may be necessary to elucidate the precise biological implications of these taxonomic shifts.

The analysis of gut microbiome diversity across different treatment groups revealed interesting patterns in both Shannon and Simpson diversity indices (Fig. 6). The Shannon diversity index showed that the CFED + Carotenoid-High group exhibited the highest mean diversity (1.89 ± 0.14), indicating a greater species richness and

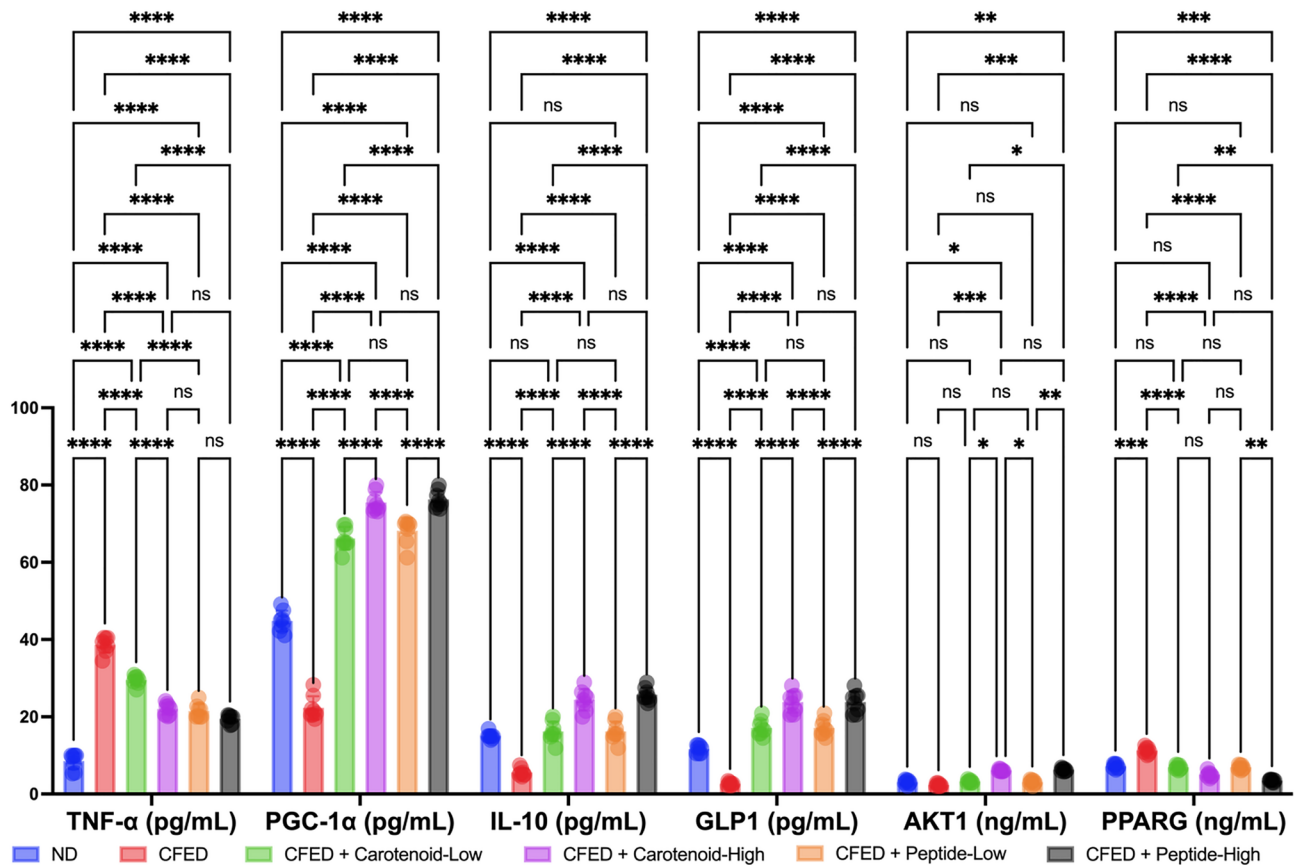


Fig. 4 Effect of Sea Grapes Extracts on molecular biomarkers in rats fed by CFED. Tumor necrosis factor alpha (TNF-α); Pparg coactivator-1 alpha (PGC-1α); interleukin-10 (IL-10); glucagon-like peptide-1 (GLP1); AKT1, PPARG. ****, ***, **, * $p < 0.05$, not significant (NS) $p > 0.05$

evenness compared to other groups. In contrast, both the CFED control group and CFED+Carotenoid-Low group displayed lower diversity values (approximately 1.58 ± 0.13 and 1.59 ± 0.19 , respectively). The CFED + Peptide-High treatment showed intermediate diversity levels (1.72 ± 0.17), suggesting a moderate effect on microbial community structure. The Normal Diet (ND) group, after data correction, demonstrated comparable diversity to the CFED+Peptide-High group (1.72 ± 0.31), though with notably higher variability as indicated by the larger standard deviation. Regarding the Simpson diversity index, which emphasizes species dominance, the differences between groups were less pronounced. The CFED + Carotenoid-High group maintained the highest values (around 0.76), suggesting a more balanced distribution of dominant species. However, the statistical significance of these differences was less evident compared to the Shannon index results. The visualization through box plots combined with jitter points effectively illustrated the distribution of individual samples within each group, highlighting both the central tendencies and the variation in microbial diversity. These findings suggest that high-dose carotenoid supplementation in the CFED diet may promote a more diverse gut microbiome

community, particularly as shown by the Shannon index. The effect appears to be dose-dependent, as the low-dose carotenoid group showed diversity levels similar to the CFED control. The peptide treatments showed intermediate effects, while the normal diet maintained relatively high diversity levels despite greater variability among samples. This pattern indicates that dietary interventions, particularly high-dose carotenoid supplementation, may have significant impacts on gut microbiome diversity, potentially influencing host health through altered microbial community structures.

Analysis using NMDS generated a two-dimensional ordination of the microbial communities with an extremely low stress value (approximately 9.33×10^{-5} – 59.33×10^{-5}), indicating an excellent representation of the original Bray-Curtis dissimilarities in the reduced dimensions (Fig. 7). The ANOSIM test produced an R statistic of about 0.78 with a p-value of 0.001, strongly suggesting that the differences in microbial community composition among the treatment groups are statistically significant. In the NMDS plot, samples belonging to different treatment groups are distinctly clustered. While the CFED group tends to occupy a separate space compared to the Carotenoid and Peptide treatment groups,

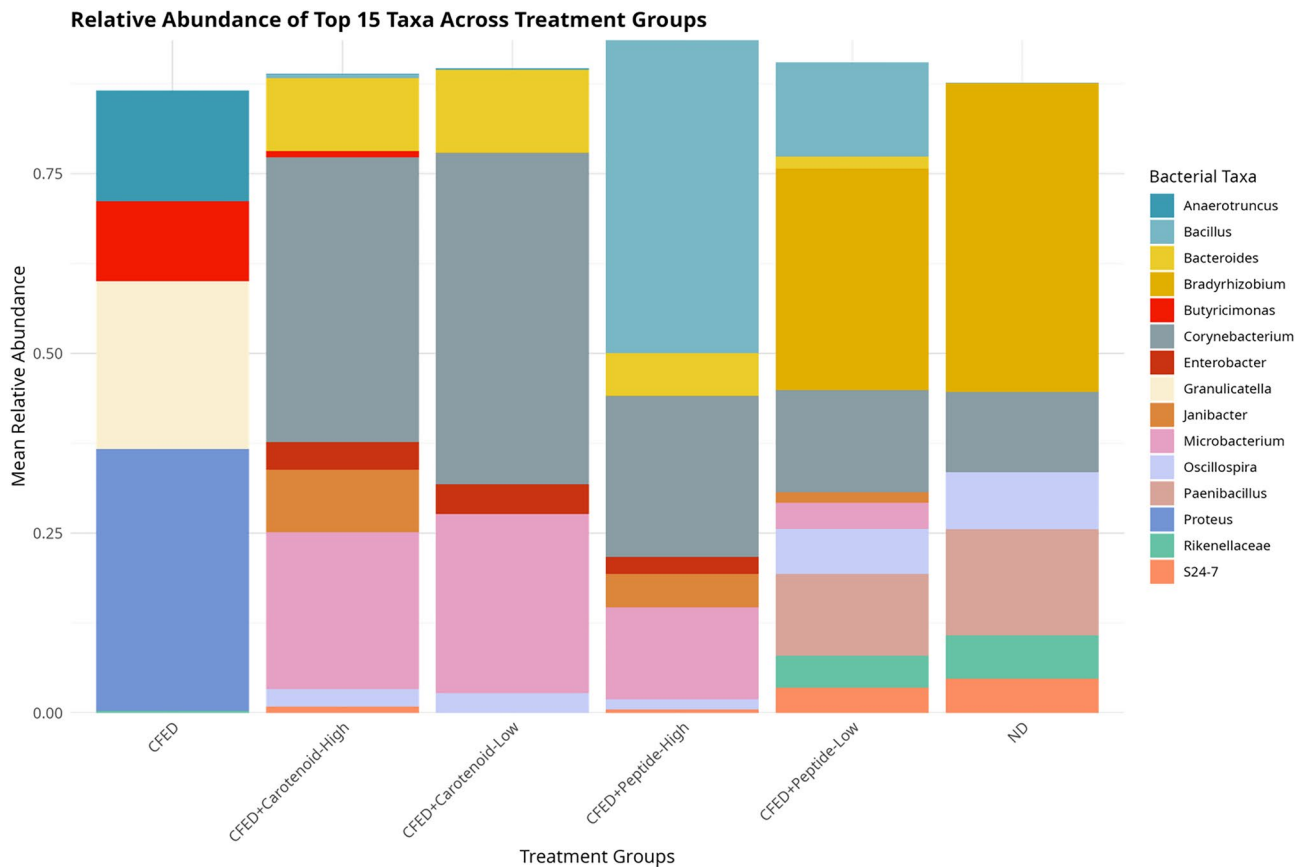


Fig. 5 Taxonomic composition at the genus level. Each colored bar represents the percentage of each phylum or genus relative to the total microorganisms

the latter tend to cluster closer together in the ordination space. The confidence ellipses—which were computed using a t-distribution—provide a visual estimate of the variability within each group, supporting the overall finding of significant group separation. In summary, the combined NMDS and ANOSIM analyses confirm that the dietary treatments have a substantial effect on the microbiome composition, with clear and statistically significant differences between groups.

The Linear Discriminant Analysis (LDA) Effect Size (LEfSe) analysis revealed significant differences in gut microbiota composition across different dietary treatments (Fig. 8). The analysis identified several discriminative bacterial genera, with particularly strong signals from *Faecalibacterium*, *Enterococcus*, and *Prevotella*. In the CFED group, *Faecalibacterium* emerged as the most discriminative genus with the highest LDA score (3.88), suggesting its significant association with this dietary condition. The CFED+Carotenoid-High group showed *Enterococcus* as its primary discriminative feature, achieving the highest overall LDA score (3.91) among all groups, indicating a strong relationship between carotenoid supplementation and *Enterococcus* abundance. The CFED+Carotenoid-Low treatment demonstrated the most diverse response, with five significant features,

including *Prevotella* as its strongest discriminative genus (LDA score 3.77). This suggests that lower carotenoid concentrations might influence a broader range of bacterial taxa. In contrast, the CFED+Peptide-High group showed more focused effects, with *Clostridium* as its sole significant feature, albeit with a lower LDA score (2.09). The CFED+Peptide-Low group was characterized by significant changes in *Lactobacillus* populations (LDA score 3.58), while the ND (Normal Diet) group showed distinctive patterns in *Eubacterium* abundance (LDA score 3.15).

The heatmap visualization revealed distinct clustering patterns of these genera across dietary groups, suggesting diet-specific microbial community structures (Fig. 9A). The bubble plot further illustrated the relative abundance distributions, highlighting the differential responses of specific genera to various dietary interventions (Fig. 9B). These patterns were particularly evident in the varying sizes of bubbles representing bacterial abundance across different diet groups. Collectively, these results demonstrate that dietary interventions, particularly those involving carotenoids and peptides, can significantly modulate the gut microbiota composition at the genus level, with specific bacterial groups showing strong associations with particular dietary treatments. These

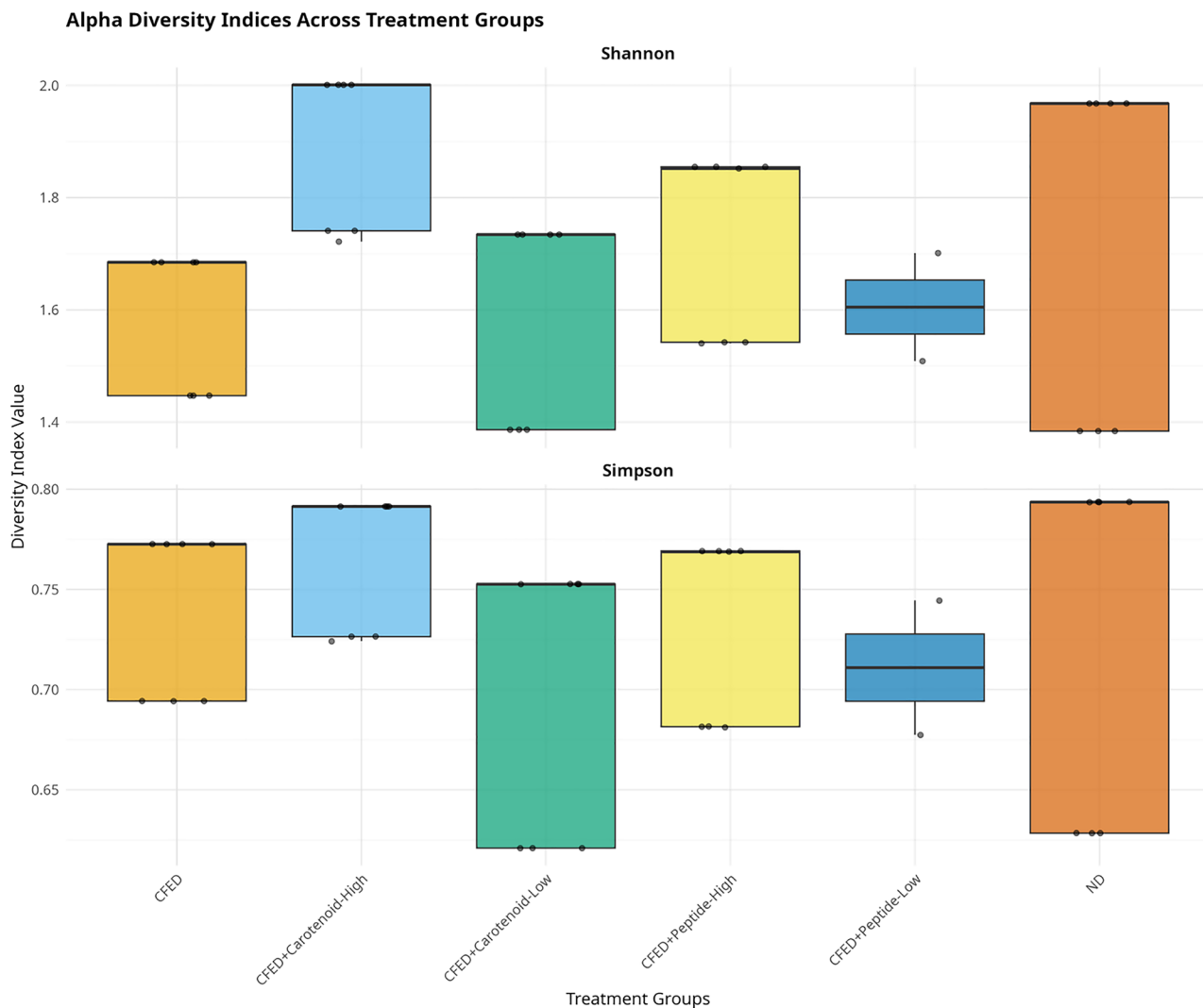


Fig. 6 Shannon and Simpson alpha diversity index

findings provide valuable insights into the diet-dependent modulation of gut microbiota and may help inform future dietary interventions targeting specific microbial populations. The statistical summary reinforces these observations, showing varying numbers of significant features across diet groups, with mean LDA scores ranging from 2.09 to 3.52, indicating different degrees of discriminative power among the identified bacterial genera. This comprehensive analysis not only identifies key bacterial players in response to different dietary interventions but also provides quantitative measures of their discriminative importance, offering valuable insights for future research in dietary modulation of gut microbiota.

The correlation analysis between gut microbiome composition and biomarkers revealed several significant relationships (Fig. 10). The most notable correlation was observed between Blood Glucose (BG) levels and several bacterial genera, showing a strong positive correlation

($r=0.92$) with *Oscillospira* and similarly strong negative correlations ($r = -0.92$) with *Corynebacterium* and *Bradyrhizobium*. Another substantial relationship was identified with α -amylase levels, which demonstrated strong negative correlations ($r = -0.87$) with multiple bacterial genera. Triglycerides (TG) showed moderate negative correlations ($r = -0.43$) with several bacterial populations. The analysis also revealed that α -glucosidase exhibited moderate positive correlations ($r=0.40$) with certain bacterial genera. Interestingly, some biomarkers such as TNF- α and AST showed relatively weak correlations ($r = -0.01$ and $r = -0.06$ respectively) with the gut microbiota. PPARG and Lipase Serum both demonstrated moderate negative correlations ($r = -0.31$) with various bacterial populations. The final body weight showed a weak to moderate positive correlation ($r=0.25$) with several bacterial genera. These findings suggest that certain gut bacteria may play important roles in metabolic processes,

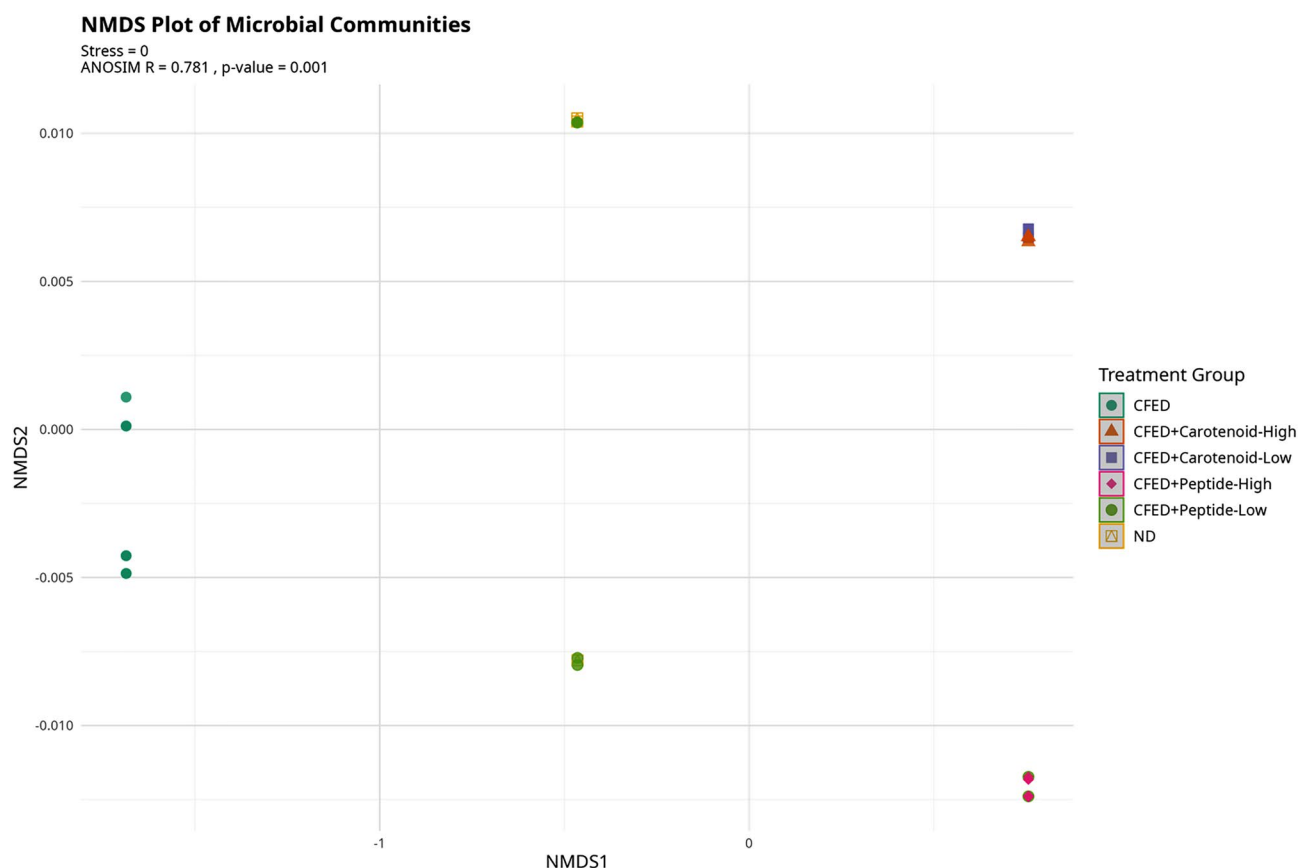


Fig. 7 Non-metric multidimensional scaling (NMDS) plot of all samples using the Bray-Curtis resemblance matrix by ANOSIM analysis

particularly in glucose metabolism and enzymatic activities.

Discussion

*The findings of this study demonstrate that supplementation with carotenoid and peptide extracts from *Caulerpa* sp. significantly mitigates metabolic syndrome (MetS) through both metabolic regulation and gut microbiota modulation in CFED rats (Fig. 11). These results align with prior research on *Caulerpa racemosa* extracts, particularly those evaluating its sulfated polysaccharides (SPCr) and aqueous extracts (AEC) in ameliorating cardiometabolic syndrome through oxidative stress reduction, lipid metabolism regulation, and inflammation attenuation [29, 30].*

Metabolic regulation and cardiometabolic biomarkers

Our study indicates that the administration of *Caulerpa* sp. extracts (peptide and carotenoid extracts) resulted in significant improvements in lipid profiles and glucose homeostasis, as evidenced by reduced low-density lipoprotein (LDL), triglycerides (TG), total cholesterol (TC), and blood glucose (BG), alongside increased high-density

lipoprotein (HDL) (Fig. 11). These effects were more pronounced in the high-dose supplementation group, consistent with previous reports demonstrating the efficacy of SPCr in modulating metabolic markers via the PRMT-1/DDAH/ADMA pathway and the mTOR-SIRT1-AMPK axis [29]. Similarly, the aqueous extract of *Caulerpa racemosa* has been found to exert significant antioxidant and anti-inflammatory effects, contributing to improved endothelial function and reduced metabolic dysfunction [30]. Our findings further support the role of *Caulerpa* sp. as a nutraceutical with therapeutic potential in addressing metabolic disorders.

Enzymatic activities and lipid/carbohydrate metabolism

The inhibition of key metabolic enzymes, including pancreatic lipase, α -amylase, and α -glucosidase, observed in this study aligns with findings from previous research on *Caulerpa* sp. Extracts [35, 36]. The ability of these extracts to inhibit digestive enzymes involved in lipid and carbohydrate metabolism suggests a potential mechanism for reducing postprandial glucose spikes and lipid accumulation [37], thereby preventing insulin resistance and obesity-related metabolic complications [30]. Notably, our results demonstrate a dose-dependent effect,

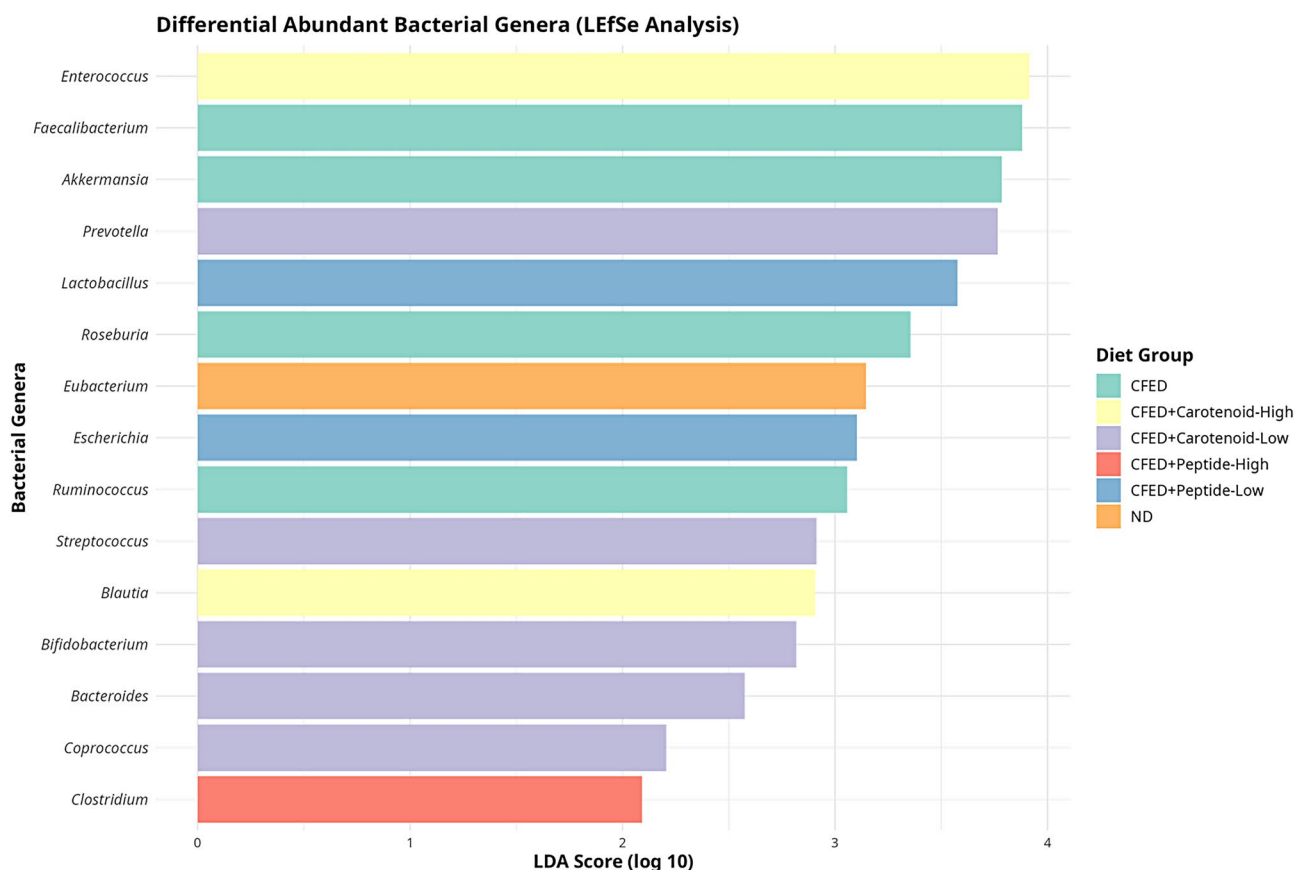


Fig. 8 Linear discriminant analysis (LDA) effect size (LEfSe) analyses of gut microbiota according to diet at the genus level

with higher doses of *Caulerpa sp.* extract exerting more significant metabolic benefits.

Gut microbiota modulation

The role of gut microbiota in metabolic health has been widely acknowledged, with dysbiosis often linked to MetS and associated metabolic disorders [9, 38]. Our findings reveal that supplementation of peptide and carotenoid extracts from *Caulerpa* resulted in distinct shifts in gut microbial composition, with increased microbial diversity and favorable changes in bacterial taxa associated with metabolic health. Specifically, we observed a reversal of the Firmicutes/Bacteroidetes imbalance induced by CFED, a trend consistent with findings from previous studies on *Caulerpa racemosa* polysaccharides [29]. The ability of *Caulerpa sp.* to restore microbial homeostasis suggests that its bioactive compounds exert prebiotic effects, potentially enhancing gut barrier integrity and metabolic function.

Anti-Inflammatory and antioxidant mechanisms

Chronic inflammation and oxidative stress are central contributors to metabolic dysfunction [39–41], and our findings indicate that peptide and carotenoid extracts from *Caulerpa sp.* extracts significantly modulate

inflammatory biomarkers such as TNF- α , IL-10, and PGC-1 α . AST serves as a biomarker for liver function, with elevated activity indicating hepatocellular damage or metabolic stress. Lipase represents a critical enzyme involved in lipid digestion and metabolism. α -glucosidase and α -amylase are essential enzymes for carbohydrate metabolism, with their activities reflecting the efficiency of carbohydrate processing. TNF- α functions as a pro-inflammatory cytokine associated with metabolic syndrome and chronic inflammation. IL-10 acts as an anti-inflammatory marker that counteracts pro-inflammatory responses. PGC-1 α serves as a key regulator of mitochondrial biogenesis and energy metabolism, playing crucial roles in cellular energy production. GLP-1 is involved in glucose regulation and insulin secretion, contributing to glucose homeostasis. AKT1 and PPARG function as critical regulators of glucose homeostasis and lipid metabolism, with their expression levels indicating metabolic efficiency and insulin sensitivity. These results corroborate previous studies highlighting the antioxidant and anti-inflammatory properties of SPCr and AEC, which act through multiple pathways, including the PRMT-1/DDAH/ADMA and mTOR-SIRT1-AMPK axes [29, 30]. The observed reduction in oxidative stress markers and enhancement of anti-inflammatory cytokines

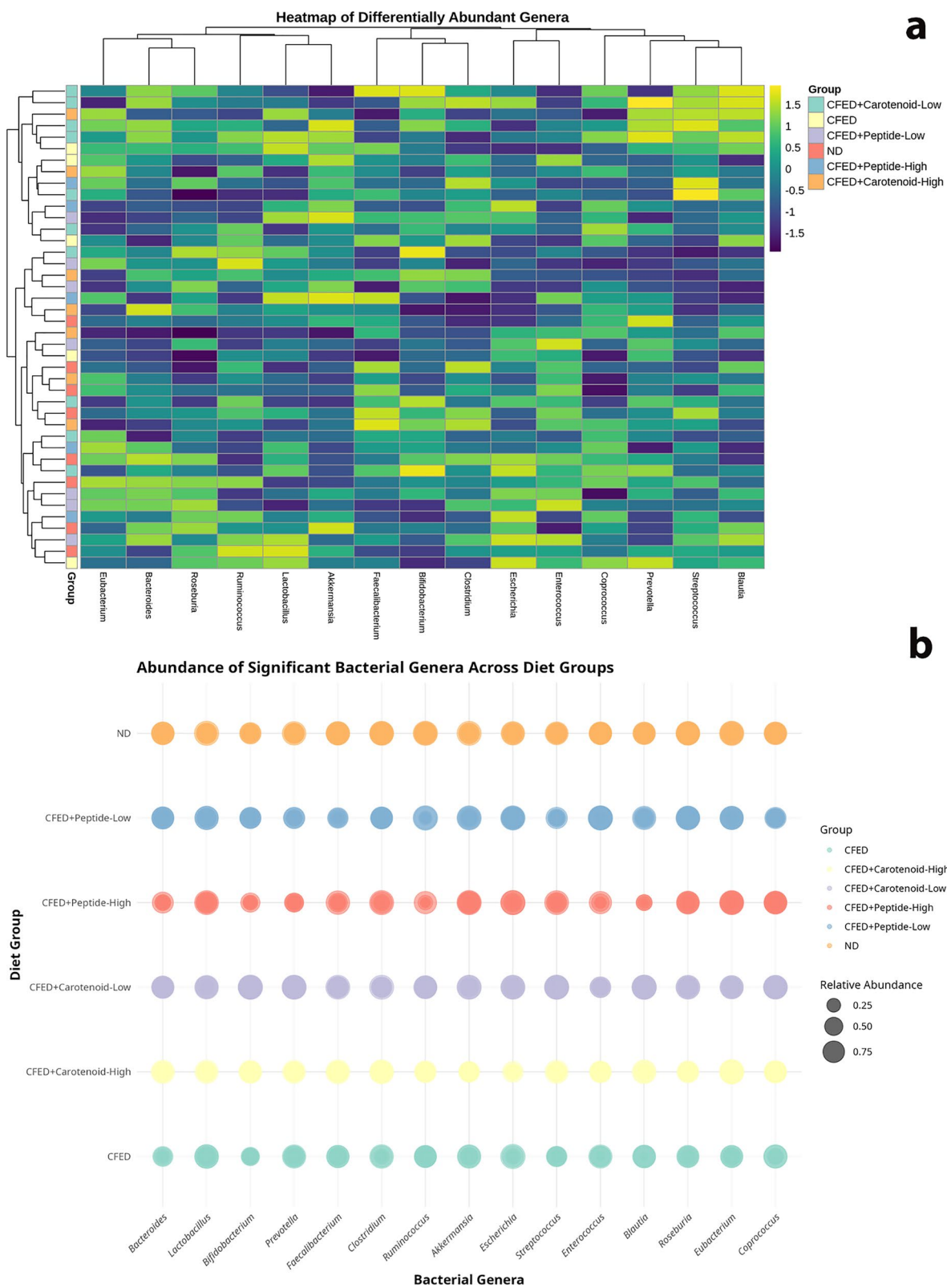


Fig. 9 **A** Heatmap visualization of bacterial genera abundance across different dietary treatments. Each row represents a bacterial genus, while each column represents a dietary group. The intensity of the color indicates the relative abundance of each genus, with hierarchical clustering demonstrating the grouping of bacterial taxa based on similarity in abundance patterns. **B**. Bubble plot illustrating the relative abundance of key bacterial genera in different dietary intervention groups. The size of each bubble corresponds to the relative abundance of the bacterial genus, providing a comparative visualization of microbial shifts in response to dietary interventions

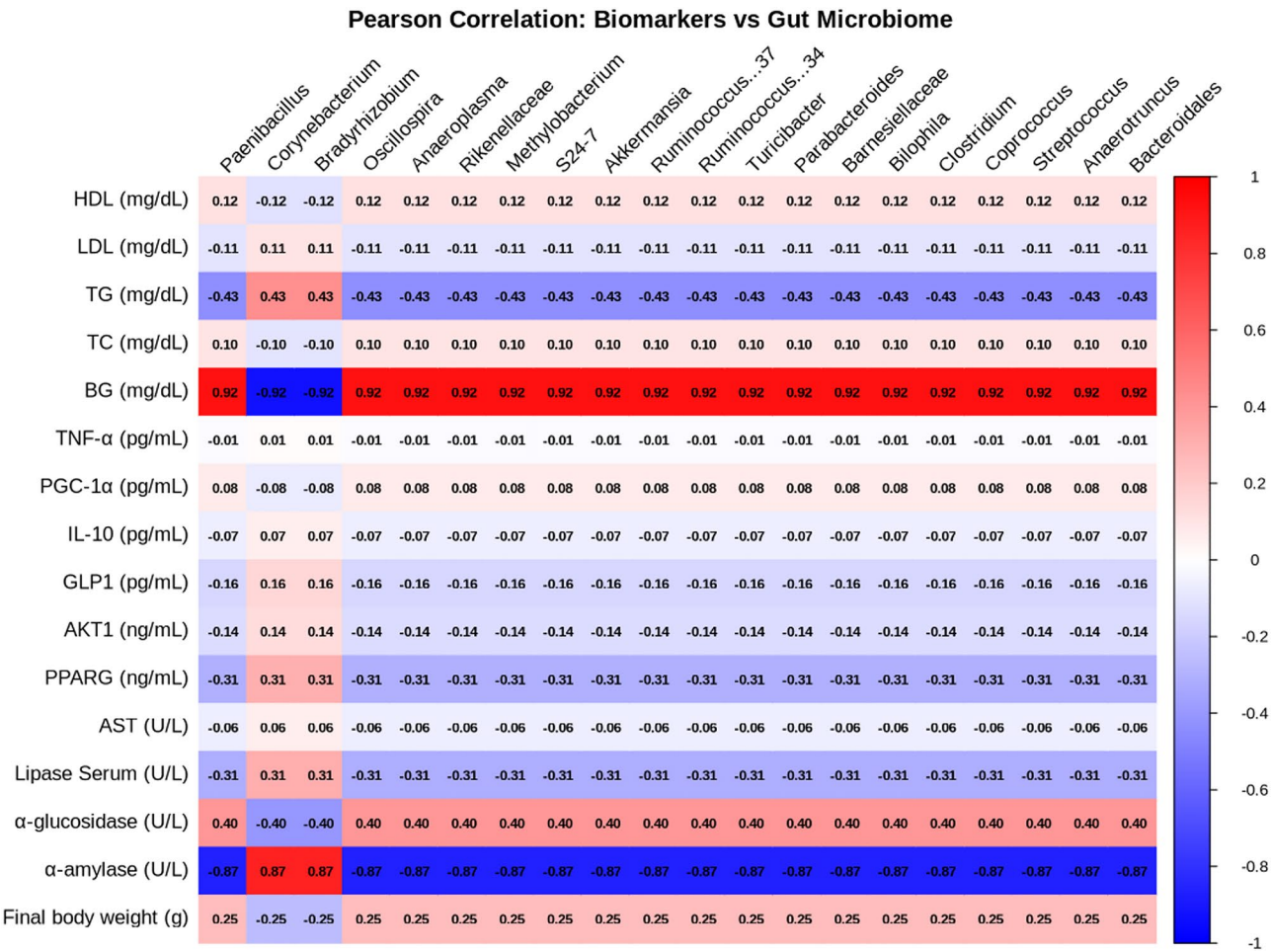


Fig. 10 Heatmap illustrating the Pearson correlation coefficients between gut microbiome composition and blood metabolic profiles in rats, providing insights into the relationships between specific microbial taxa and key metabolic biomarkers

further underscore the therapeutic potential of *Caulerpa racemosa* in metabolic disease management.

Clinical and translational implications

The translational potential of *Caulerpa* supplementation in human populations remains an area for future investigation. While our preclinical findings provide compelling evidence of its metabolic benefits, further clinical trials are required to establish optimal dosing, safety profiles, and efficacy in human subjects. Moreover, the integration of *Caulerpa*-derived bioactives into functional food formulations presents an exciting avenue for dietary interventions targeting metabolic syndrome. Future research should explore the synergistic effects of carotenoids and peptides with other bioactive compounds in *Caulerpa*, as well as their long-term impacts on metabolic health and gut microbiome stability.

Study limitations

Several limitations should be acknowledged in the interpretation of our findings. First, the absence of functional

metagenomic analysis limits our understanding of the microbial metabolic capacity and functional changes associated with *Caulerpa* supplementation. Second, our taxonomic analysis lacked strain-level resolution, which could provide more detailed insights into specific microbial species contributing to the observed metabolic effects. Third, we did not measure short-chain fatty acids (SCFAs), which represent crucial metabolites mediating host-microbiome metabolic crosstalk and could provide mechanistic insights into the observed benefits. Fourth, while our discussion references several molecular pathways (e.g., mTOR-SIRT1-AMPK, PRMT-1/DDAH/ADMA), we did not conduct gene or protein expression analyses (e.g., RT-qPCR, Western blot) to directly validate these mechanistic pathways. This mechanistic gap should be considered when interpreting the proposed mechanisms of action. Future studies should incorporate these analytical approaches to provide more comprehensive mechanistic insights and validate the proposed bioactive pathways.

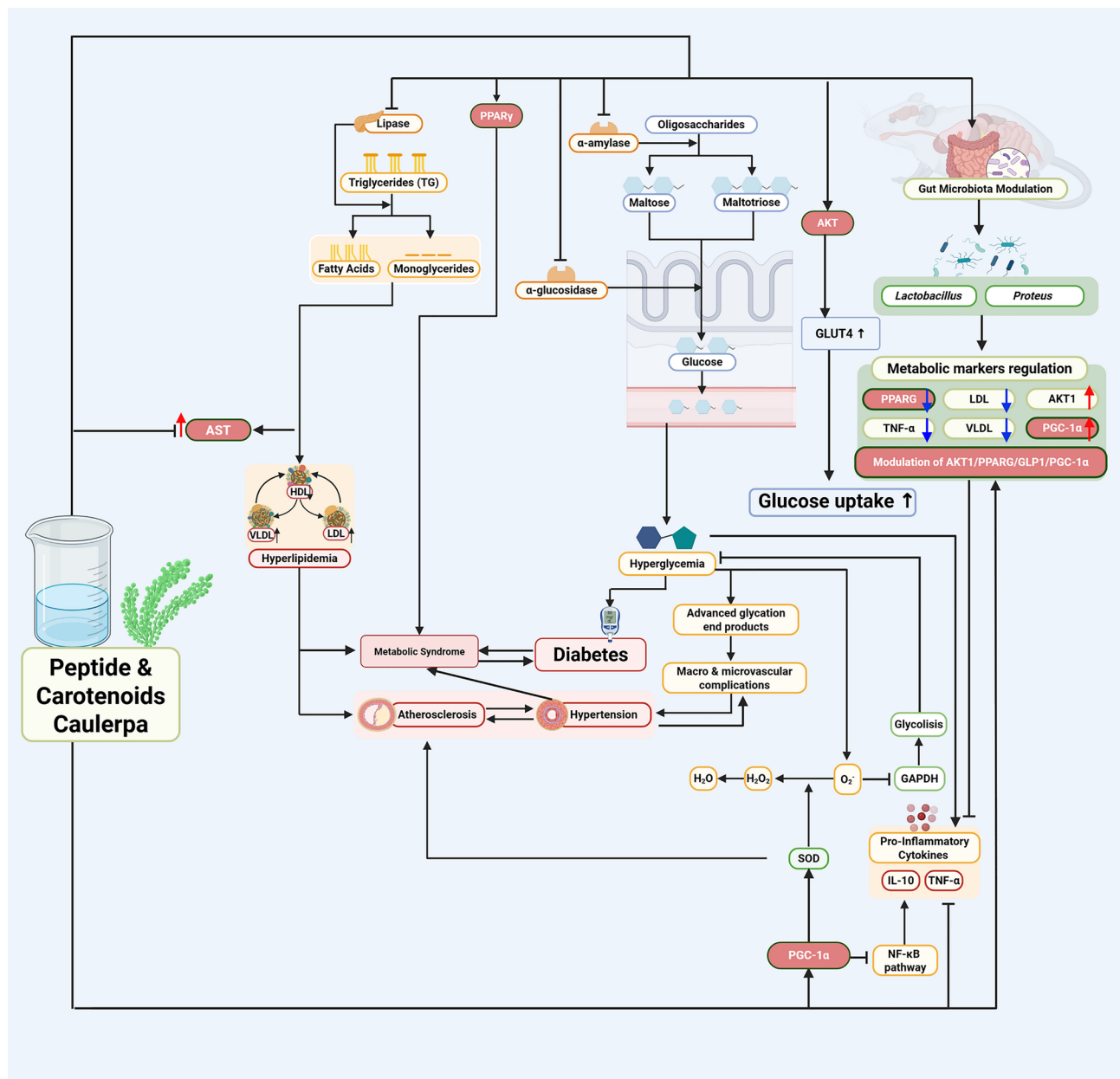


Fig. 11 Biomechanism of the Mitigation of Metabolic Syndrome and Gut Microbiome Modulation by the Carotenoid and Peptide Extracts from Sea Grapes in Cholesterol-Enriched Diet Rats

Conclusion

In summary, this study reinforces the therapeutic potential of *Caulerpa* sp. (peptide and carotenoid extracts) in mitigating metabolic syndrome through a multifaceted mechanism involving metabolic regulation, enzymatic inhibition, gut microbiota modulation, and anti-inflammatory effects. Our findings, supported by prior research, highlight peptide and carotenoid extracts from *Caulerpa* sp. as a promising functional food for metabolic health management. Future investigations should focus on clinical validation and mechanistic insights to

facilitate its translational application in human nutrition and disease prevention.

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Author contributions

Conceptualization, RK., A.M.A., N.A.T. and F.N.; Methodology, F.N., A.B., N.A.T., A.Y.S. and R.K.; Software, F.N. and R.K.; Validation, A.Y.S., F.N., A.M.A., A.B., B.B. and N.A.T.; Formal Analysis, F.N. and R.K.; Investigation, F.N., N.A.T. and R.K.; Data Curation, F.N. and R.K.; Writing—Original Draft Preparation, F.N., R.K., N.A.T. and A.M.A.; Writing—Review and Editing, F.N., N.A.T., A.Y.S., B.B. and A.B. Visualization, F.N.; Supervision, F.N., A.Y.S., B.B., A.M.A., A.B. 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 this article or/and can be request from the corresponding author.

Declarations

Ethics, consent to participate, and consent to publish declarations

Not applicable.

Human ethics and consent to participate declarations

Not applicable.

Informed consent

Not applicable.

Institutional review board statement

All procedures adhered strictly to the Guidelines for Reporting In Vivo Experiments (ARRIVE) and has also been reviewed-accepted in the Preclinical Trials Study Register with number PCTE0000492, and has obtained ethical approval from UIN Sunan Kalijaga Yogyakarta (Approval No. 2390.3/Un.02/L3/TL/06/2025).

Competing interests

The authors declare no competing interests.

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