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Spirulina platensis polysaccharide and its selenide ameliorate lipid metabolism disorders and gut microbiota dysbiosis in type 2 diabetic mice

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ABSTRACT: Type 2 diabetes mellitus (T2DM) is a chronic endocrine disorder characterized by metabolic disturbances. Currently, the treatment strategies for T2DM mainly focus on improving insulin sensitivity and regulating blood glucose homeostasis. However, due to the incomplete understanding of the interaction network mediating gene-metabolism-microbiota regulation, breakthroughs in targeted therapy have been limited. In this study, the hypoglycemic effects of *Spirulina platensis* polysaccharide (SPP) and its selenium-modified polysaccharide (SeSPP) on a high-fat diet/streptozotocin-induced T2DM mice model were thoroughly investigated, along with their underlying mechanisms. Transcriptomics and metabolomics analysis revealed that SPP and SeSPP exerted their hypoglycemic effects primarily by regulating lipid metabolism, carbohydrate metabolism, and cellular signaling pathways. They resulted in a reduction of Propionyl-CoA and Lyso-PI (0:0/18:1(9Z)) levels, while simultaneously increasing the level of 12,13-diHOME. Furthermore, they upregulated the expression of the *Acot3* gene and downregulated the expression of the *Acsn2* gene, thereby activating the fatty acid metabolic pathway. SPP and SeSPP significantly ameliorated the gut microbiota imbalance induced by T2DM by enhancing the abundance of *Lactobacillus* and *Faecalibaculum*, while simultaneously reducing the levels of *Alloprevotella* and *Bacteroides*. In conclusion, SPP and SeSPP have the potential to reshape the gut microbiota, thereby restoring microecological balance, including disorders in glucose and lipid metabolism, chronic low-grade inflammation, and oxidative stress.

Keyword: *Spirulina platensis*; Selenium; Polysaccharides; Type 2 diabetes; Gut microbiota

1. Introduction

Worldwide modernization and lifestyle changes contribute to increased high-fat food consumption, which have become an integral part of the global dietary structure [1]. However, long-term intake of high-fat diets (HFD) is closely associated with the burden of various metabolic diseases, including hyperlipidemia, non-alcoholic fatty liver disease, obesity, and type 2 diabetes mellitus (T2DM) [2]. T2DM, as a complex chronic metabolic disease, poses a major challenge to global health [3]. It is characterized by persistent hyperglycemia, insulin resistance, and impairment of pancreatic β -cells. These abnormal metabolic states can lead to serious complications, including cardiovascular diseases, nephropathies, and retinopathies, which significantly affect patients' quality of life and increase the social and economic burden on society [4, 5]. Despite significant advancements in drug development, including the introduction of new hypoglycemic

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agents including GLP-1 receptor agonists and SGLT-2 inhibitors, these medications may still have potential adverse effects, such as gastrointestinal reactions and urinary tract infections [6, 7]. Their long-term safety and cost-effectiveness need further validation, driving global research into safe, effective natural compounds for glucose regulation. These compounds exhibit considerable promise in the treatment and management of T2DM due to their diverse sources, multi-target effects, and favorable safety profile.

Numerous studies have indicated that natural polysaccharides can lower glucose levels through various targets and pathways, including enhancing insulin sensitivity, promoting insulin secretion, protecting pancreatic β -cell function, regulating gut microbiota homeostasis, and inhibiting inflammatory responses [8–12]. Among these, algae polysaccharides have been demonstrated to possess a range of significant bioactivities, including antioxidant, anti-inflammatory, immunomodulatory, and metabolic regulatory effects [13–15]. In recent years, some studies have elucidated the unique mechanism to lower blood glucose levels. Notably, compared to natural *Sargassum pallidum* polysaccharides, sulfation-modified *Sargassum pallidum* polysaccharides exhibited stronger α -glucosidase inhibitory activity and promoted glucose consumption in an insulin-resistant cell model [16]. Additionally, *Laminaria japonica* polysaccharides significantly ameliorated the abnormal inflammatory factors and gut microbiota dysbiosis induced by HFD [17]. Furthermore, Fucoidan was demonstrated to markedly reduce blood sugar levels of diabetic nephropathic mice by modulating the gut microbiota, specifically short-chain fatty acids (SCFAs)-producing bacteria, thereby alleviating mitochondrial oxidative stress and inhibiting the over-activation of the MAPK signaling pathway [18]. *Spirulina platensis* polysaccharide (SPP) was shown to increase lipolysis and decrease lipogenesis in the liver. It also played a role in correcting gut dysbiosis, by increasing beneficial microbiota such as *Clostridia* and *Bacterioidia* and reducing unfavorable microbiota such as *Actinobacteria* and *Verrucomicrobia* [13, 19]. These findings underscore the significant developmental potential of SPP for treating diseases related to glucolipid metabolism disorders. To further enhance the bioavailability and hypoglycemic efficacy of SPP, we explored structural modifications. Among these, selenium modification has emerged as an effective strategy, significantly enhancing the bioactivity of polysaccharides [20, 21]. Studies indicated that selenium-modified polysaccharides outperform their natural counterparts in improving insulin resistance, protecting β -cell function, and regulating glucose and lipid metabolism, thereby providing a vital theoretical foundation for new functional polysaccharides [22–24].

In previous work, we investigated that SPP and selenium-modified SPP (SeSPP) could improve the overall glucose metabolism and oxidative stress in mice with T2DM signs by regulating insulin resistance and glucose secretion. Selenium-modification could effectively enhance the improvement effect of SPP on T2DM mice. However, there are relatively few reports on the treatment of metabolic disorders in T2DM mice with SPP and SeSPP, and their therapeutic mechanisms remain unclear. In this study, through comprehensive analysis of transcriptome and metabolome, we evaluated the intervention effects of SPP and SeSPP on differentially expressed genes and metabolites in the liver of T2DM mice, explored key regulatory nodes, and systematically revealed the multi-dimensional regulatory network of “components-targets-pathways” of SPP

and SeSPP. In addition, the effects of SPP and SeSPP on the diversity of gut microbiota were evaluated by 16S rRNA, and key functional genera were screened. This study provided a theoretical basis for the development of polysaccharide-based T2DM therapeutic agents targeting the “gut-liver” axis.

2. Materials and methods

2.1 Preparation of SPP and SeSPP

Food-grade *Spirulina platensis* powder was procured from Fujian Xin Da Ze Co., Ltd. SPP and SeSPP were prepared using previously established methods [25]. Briefly, the *Spirulina platensis* powder was initially degreased and decolorized, followed by air-drying in a ventilated area. Subsequently, it was mixed with deionized water and stirred thoroughly to extract the crude polysaccharides using ultrasound at 200 W. Proteins and pigments were removed by the Sevag method and acetone. The resulting solution was then re-solubilized and dialyzed with dialysis membrane of 20 kDa, followed by lyophilization to obtain SPP. SPP was dissolved in 0.5% HNO₃ solution, and BaCl₂ and Na₂Se₂O₃ were added, reacting at 70 °C for 8 h. At the end of the reaction, Na₂SO₄ was introduced until no precipitate remained. Subsequently, the solution was dialyzed with dialysis membrane of 20 kDa. After concentration and lyophilization, SeSPP was obtained. The supplemental materials provide the main components of SPP and SeSPP [25]. The SPP and SeSPP extracted in the study were crude extracts.

2.2 Animal experiment

Dose experiments of 200 mg/kg bw and 400 mg/kg bw were carried out in the early stage, and it was concluded that 400 mg/kg bw had a better effect on regulating glucose homeostasis and insulin resistance, and no death occurred. Therefore, the dose of 400 mg/kg bw was selected for multiomic analysis. Five-week-old C57BL/6J male mice (20 ± 2 g, SPF-grade) were obtained from Beijing Viton Lihua Biotechnology Co. Ltd (20240605Abzz0619000134). The animals were kept under controlled conditions: temperature (25 ± 2 °C), humidity (50 ± 10%), and a 12 h light/dark cycle. Following a one-week acclimatization period, eight mice were randomly assigned to the normal control group (NC group) and fed 10% low-fat diet, while the remaining mice received a 60% HFD. After five weeks, all HFD mice underwent a 12 h fast and were then intraperitoneally administered streptozotocin (STZ) at 100 mg/kg bw, dissolved in 0.1 M citrate buffer (pH 4.5). The NC group received an equivalent volume of citrate buffer. The fasting blood glucose (FBG) was measured 72 h post-injection, and mice with FBG levels exceeding 11.1 mmol/L were classified as T2DM models. The T2DM mice were then randomly allocated into three groups: the model control group (MC group, treated with 0.9% saline), the high-dose SPP group (SPPH group, 400 mg/kg bw SPP), and the high-dose SeSPP group (SeSPPH group, 400 mg/kg bw SeSPP). The study protocol was approved by the Henan Provincial Laboratory Animal Center Committee (ZZU-LAC20240611[01]). The design of the animal experiment is shown in Fig. 1.

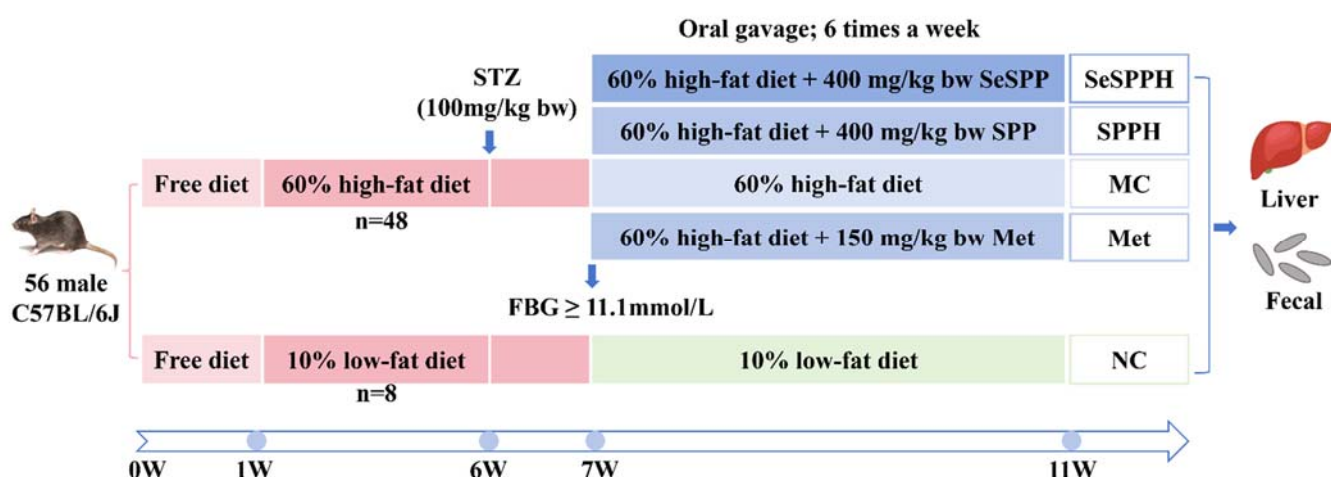


Figure 1 Animal experimental design.

2.3 Transcriptomic analysis of liver

Transcriptome sequencing was conducted at Genepioneer Biotechnologies Co. Ltd. Total RNA was extracted from 50 mg liver samples using the HiPure RNA kit. The concentration and purity of the extracted RNA were assessed using a Nanodrop 2000, and the integrity of the RNA was evaluated through agarose gel electrophoresis. All samples met the quality control standards prior to library construction. Following the extraction of total RNA, mRNA was enriched and fragmented using Oligo (dT) beads. Subsequently, complementary DNA (cDNA) was synthesized, purified, end-repaired, ligated into the splice header, and amplified via PCR to construct the library. Sequencing was performed on an Illumina NovaSeq 6000 platform. Differential expression analysis was conducted using the DESeq2 R package (1.20.0). Genes that met the criteria of fold change (FC) > 1.5 and $p\text{-adjust} < 0.05$ were classified as differentially expressed genes (DEGs).

2.4 Metabolomics analysis of the liver

The liver tissue was thawed slowly at 4 °C, and 100 mg of samples was accurately weighed into a centrifuge tube. Subsequently, 200 μ L of pre-cooled water was added, followed by vortexing for 60 s. An equal volume of a pre-cooled methanol-acetonitrile mixture (1:1, v/v) was then added, and the mixture was vortexed again for 60 s before being subjected to low-temperature ultrasound extraction for 30 min. To precipitate the proteins, the samples were placed in a centrifuge tube at -20 °C for 1 h. The supernatant was collected and subjected to vacuum drying. Then, 200 μ L 30% acetonitrile solution was added for re-solubilization. Following centrifugation at 12,000 rpm (Eppendorf 5425R, Germany) for 10 min at 4 °C, the supernatant was again vacuum dried. Another 200 μ L 30% acetonitrile solution was added for re-solubilization, followed by vortexing and centrifugation at 14,000 rpm (Eppendorf 5425R, Germany) for 15 min at 4 °C. The resultant supernatant was then used for on-line detection. The liver metabolites were analyzed using ultra-performance liquid chromatography-tandem electrostatic field orbit trap mass spectrometry (UHPLC-Q Exactive HFX, Thermo, USA).

Raw mass spectral data were acquired using Xcalibur 4.1 (Thermo Scientific) and subsequently processed using Origin QI (Waters Corporation, Milford, USA). Data analysis was conducted using the R

software package. Metabolites with variable importance values (VIP)>1 in the projection will undergo a one-way t-test to assess the metabolite's significance, with p-value<0.05 considered significant. Differential metabolites (DMs) were analyzed in MetaboAnalyst, incorporating both pathway enrichment and topological analysis.

2.5 16s rRNA sequencing of fecal

DNA was extracted from fecal samples using the HiPure DNA Kit, and the DNA concentration was measured using the Qubit® dsDNA HS assay kit. Two highly variable regions, V3 and V4, on prokaryotic 16S rDNA were amplified using PCR primers 338F and 806R, with 20–50 ng of DNA as the template. Additionally, an index was added to the end of the PCR product of 16S rDNA through PCR for next-generation sequencing. The final PCR product libraries were assessed using 1.5% agarose gel electrophoresis to confirm the target fragment at 600 bp. The library concentration was measured using enzyme labeling (Tecan, Infinite 200 Pro), and PE250/Novaseq (Illumina, San Diego, CA, USA) instruments were employed for PE250/FE300 double-end sequencing. After quality filtering, the final sequences obtained were utilized for operational taxonomic unit (OTU) clustering using VSEARCH (1.9.6), with sequence similarity set to 97%. The Silva 138 database served as the reference for the 16S rRNA comparison. The OTU representative sequences were examined for species taxonomy through the Bayesian algorithm utilized by the Ribosomal Database Program classifier, and the composition of the community in each sample was quantified across different species classification levels.

3. Results

3.1 Transcriptomic analysis of liver tissue

To elucidate the mechanisms of SPP and SeSPP in the treatment of HFD/STZ-induced T2DM, we conducted a comprehensive transcriptome analysis of liver tissues. This systematic investigation aimed to identify the genes affected by SPP and SeSPP treatments, both before and after intervention. We assessed the differences in transcriptional profiles among the SPPH, SeSPPH, and MC groups by OPLS-DA (Fig. 2A-B). The analysis revealed that both the SPPH and SeSPPH groups formed distinct clusters separate from the MC group, indicating that the SPP and SeSPP interventions significantly altered gene expression patterns in T2DM mice.

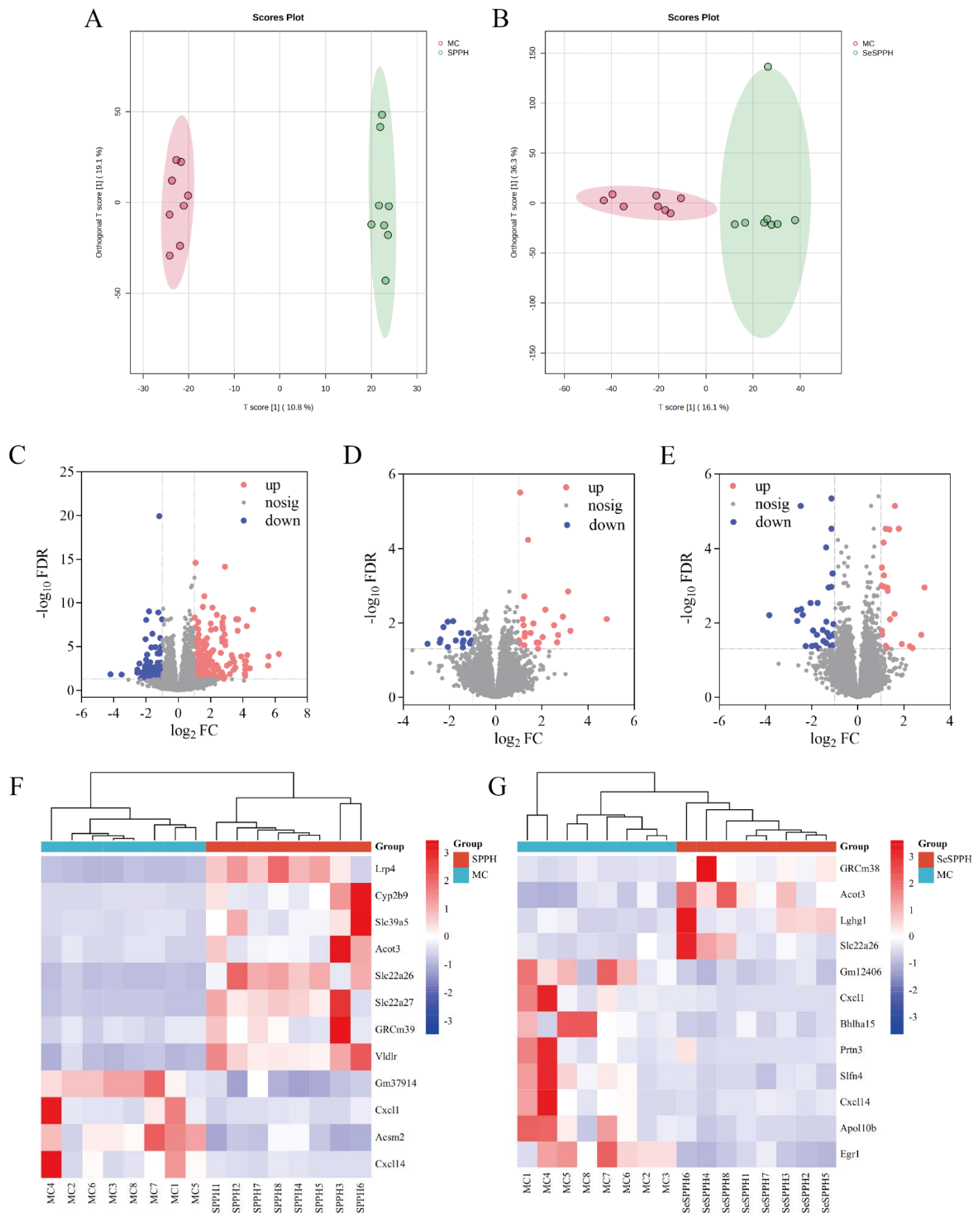


Figure 2 Effects of SPP and SeSPP on the DEGs in the liver of T2DM mice. (A) OPLS-DA analysis of hepatic genes identified between SPPH vs MC groups; (B) OPLS-DA analysis of hepatic genes identified between SeSPPH vs MC groups; (C) Volcanic map of DEGs between MC vs NC groups; (D) Volcanic map of DEGs between SPPH vs MC groups; (E) Volcanic map of DEGs between SeSPPH vs MC groups; mice. (F) Heatmap of DEGs between SPPH vs MC groups; (G) Heatmap of DEGs between SeSPPH vs MC groups.

We observed a significant number of DEGs in the MC group compared to the NC group (Fig. 2C). Notably, several genes were found to be either up-regulated or down-regulated in both the SPPH and SeSPPH groups when compared to the MC group. This finding suggested that SPP and SeSPP interventions may mitigate the aberrant expression of hepatic genes induced by T2DM (Fig. 2D-E). According to our screening criteria of $p\text{-adjust} < 0.05$ and $FC > 1$, the MC group exhibited abnormal hepatic gene expression, with a total of 295 DEGs identified, comprising 210 up-regulated and 85 down-regulated genes, which indicated that hepatic genes were significantly altered in T2DM mice. Following a period of SPP intervention, we identified 41 DEGs in the MC group compared to itself, with 24 genes up-regulated and 17 down-regulated. The SeSPPH group displayed a similar trend, revealing 55 DEGs, including 22 up-regulated and 33 down-regulated genes. Additionally, heatmaps were constructed to visually represent the changes in the core DEGs mentioned above (Fig. 2F-G). The gene expressions of SPPH group and SeSPPH group were similar and were grouped separately from the MC group. Collectively, these results implied that SPP and SeSPP may effectively ameliorate the aberrant gene expression patterns in T2DM by directly or indirectly regulating these key DEGs.

To gain a deeper understanding of the biological functions of the DEGs, we conducted Gene Ontology (GO) functional enrichment analysis by the clusterProfiler software package. The GO analysis systematically annotated the functional characteristics of the DEGs across three categories: Biological Process (BP), Molecular Function (MF), and Cellular Component (CC). In the SPP-MC comparison group, DEGs were enriched in 1,282 BP subclasses, with 112 showing significant enriched ($p\text{-adjust} < 0.05$), 140 CC subclasses, with 5 significantly enriched ($p\text{-adjust} < 0.05$), and 194 MF subclasses, with 47 significantly enriched ($p\text{-adjust} < 0.05$). Similarly, in the SeSPPH_MC group, DEGs were enriched in 1,729 BP subclasses, with 30 showing significant enriched ($p\text{-adjust} < 0.05$), 165 CC subclasses, and 251 MF subclasses with 19 showing significant enriched ($p\text{-adjust} < 0.05$). To present the analysis results more intuitively, we filtered the top 10 GO functional terms from the overall enrichment results and visualized those by bar charts (Fig. S1). Notably, the DEGs in both SPPH and SeSPPH groups were significantly enriched in key metabolic processes, including Fatty acid metabolism, Bile acid metabolism and biosynthesis, and Steroid metabolism. These results implied that SPP and SeSPP may ameliorate the metabolic dysregulation inherent to T2DM by modulating key metabolic pathways, particularly Fatty acid metabolism, and Bile acid metabolism, which are closely linked to lipid metabolism disorders commonly observed in T2DM.

KEGG enrichment analysis was further employed to investigate the enriched pathways and genes regulated by SPP and SeSPP in T2DM mice. In the SPPH_MC comparison group, the DEGs were significantly enriched in 89 KEGG pathways, whereas in the SeSPPH_MC comparison group, they were enriched in 123 KEGG pathways. Figures 3A-B illustrated the top 15 KEGG pathways with significant enrichment. Notably, the DEGs in both the SPPH and SeSPPH groups were significantly enriched in several key pathways closely associated with glycolipid metabolism, including Bile acid secretion and biosynthesis, Taurine metabolism, Fatty acid metabolism, and Steroidogenesis. This finding was highly consistent with the

results of the GO enrichment analysis, further confirming that SPP and SeSPP may ameliorate metabolic disorders in HFD/STZ-induced T2DM mice by regulating these core metabolic pathways.

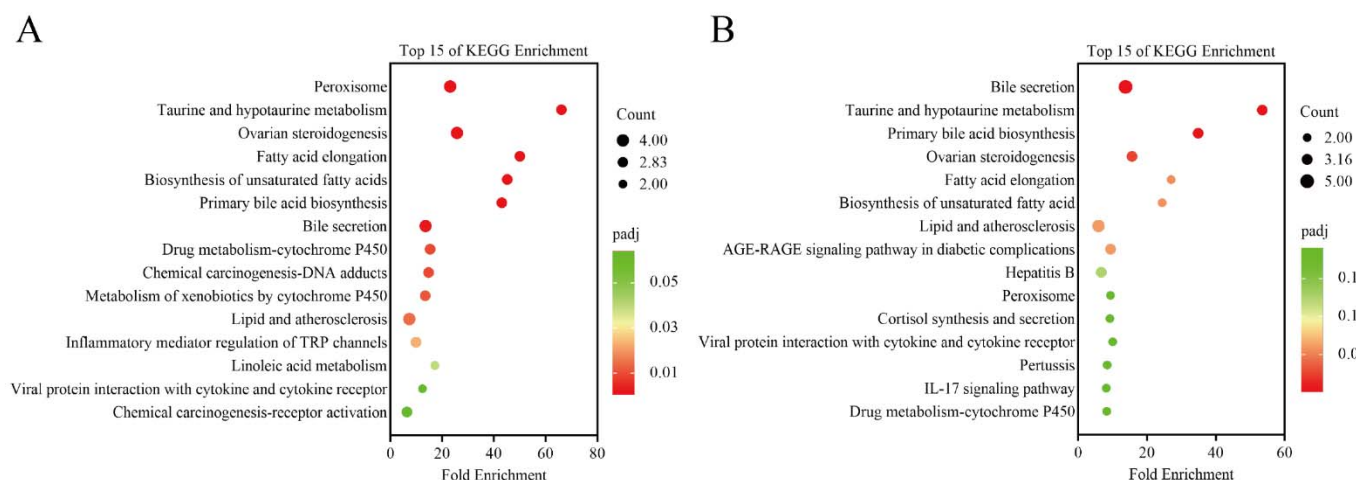


Figure 3 Effects of SPP and SeSPP on pathways involved in DEGs in the liver of T2DM mice. (A) KEGG enrichment analysis of identified DEGs in SPPH vs MC groups; (B) KEGG enrichment analysis of identified DEGs in SeSPPH vs MC groups.

Notably, the significant enrichment of the Bile acid secretion and biosynthesis pathway suggested that SPP and SeSPP may modulate lipid metabolism and glucose homeostasis by regulating bile acid metabolism, thereby enhancing lipid uptake and glucose regulation. Furthermore, the enrichment of Taurine metabolism pathway implied that SPP and SeSPP may exert hepatoprotective effects by modulating taurine-mediated antioxidant and anti-inflammatory activities, which may contribute to their therapeutic potential in managing metabolic disorders. Moreover, the notable enrichment of the fatty acid metabolism and steroidogenesis pathways highlighted the pivotal role of SPP and SeSPP in maintaining lipid metabolism homeostasis. The synergistic regulation of these pathways likely constituted the core molecular mechanism by which SPP and SeSPP alleviated metabolic disorders in T2DM, providing substantial pathway-level evidence that elucidated the underlying mechanisms of their glucose-lowering effects and underscored their therapeutic potential in the management of T2DM.

3.2 Metabolomics analysis of liver tissue

In this study, untargeted metabolomics was employed to analyze the metabolic profile of liver and to evaluate whether the benefits of SPP and SeSPP were attributable to alterations in metabolites. As illustrated in Fig. 4A, a total of 780 metabolites were identified in both ESI+ and ESI− modes, by the HMDB metabolome database (<http://www.hmdb.ca/>). This included 271 lipids and lipid-like molecules (34.74%), 199 organic acids and their derivatives (25.51%), 122 organic oxygen compounds (15.64%), 93 organoheterocyclic compounds (11.92%), 46 benzenoids (5.90%), 26 phenylpropanoids and polyketides (3.33%), 18 organic nitrogen compounds (2.31%), 4 alkaloids and their derivatives (0.51%), and 1 lignan, neolignan, and related compound (0.13%).

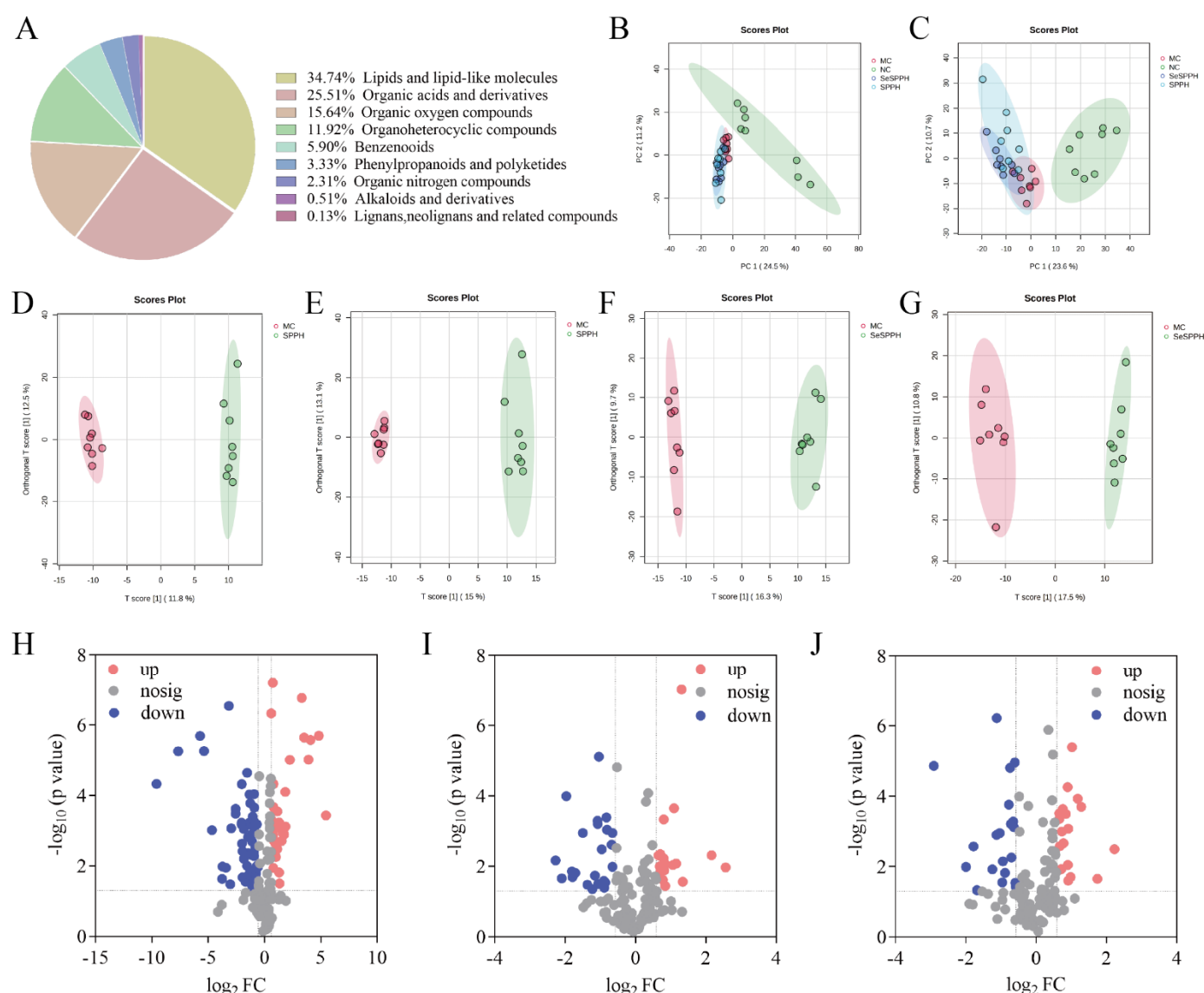


Figure 4 Effects of SPP and SeSPP on the DMs in the liver of T2DM mice. (A) Biochemical categories of all DMs identified; (B-C) PCA analysis of hepatic genes identified between NC, MC, SPPH, and SeSPPH groups in positive and negative modes; (D-E) OPLS-DA analysis of hepatic genes identified between SPPH vs MC groups in positive and negative mode; (F-G) OPLS-DA analysis of hepatic genes identified between SeSPPH vs MC groups in positive and negative mode; (H) Volcanic map of DMs between MC vs NC groups; (I) Volcanic map of DMs between SPPH vs MC groups; (J) Volcanic map of DMs between SeSPPH vs MC groups.

Multidimensional statistical analysis of liver metabolites was conducted through PCA score plots. Figures 4B-C illustrated the PCA score plots for the NC, MC, SPPH, and SeSPPH groups under both positive and negative ion modes. The results indicated that the NC and MC groups exhibited a significant separation trend in both ion modes, suggesting that HFD/STZ induction markedly altered the metabolite composition, leading to considerable disruption of metabolic profiles. OPLS-DA was employed to further investigate the effects of SPP and SeSPP on the metabolic profiles of HFD/STZ-induced T2DM mice. As depicted in Fig. 4D-G, the MC group demonstrated a distinct separation from each treatment group in the OPLS-DA analysis under both the positive and negative ion models. To verify the reliability of the model, we performed a 100-sample substitution test (Figure S2), which revealed that the blue regression lines at point Q2 all intersected the vertical axis below zero. This finding indicated that the established OPLS-DA model possessed

strong predictive ability and stability, effectively differentiating the metabolic differences among the various treatment groups.

Subsequently, the effects of SPP and SeSPP on DMs in HFD/STZ-induced T2DM mice were evaluated using a t-test to identify significant DMs with $FC > 1.5$ and $p\text{-value} < 0.05$. The results were presented in volcano plots (Fig. 4H-J). A total of 96 significant DMs (36 down-regulated and 60 up-regulated) were identified in the MC group compared to the NC group. Additionally, compared to the MC group, 41 significant DMs (17 down-regulated and 24 up-regulated) were found in the SPPH group, while 40 significant DMs (19 down-regulated and 21 up-regulated) were identified in the SeSPPH group.

By comparing with the HMDB database, we successfully identified specific DMs between the groups. The results indicated that 53 DMs were identified in NC_MC group in positive-negative ion mode, and 24 DMs were identified in SPPH_MC group, while another 24 DMs were identified in SeSPPH_MC group. Notably, the DMs were primarily associated with lipids, carbohydrates, and amino acids. In comparison to healthy mice, the liver metabolites of HFD/STZ-induced T2DM mice exhibited a significant up-regulation of 23 metabolites, including Taurocholic acid 3-sulfate, Phenylalanine, Norleucine, and Linoleoylcholine, as well as a significant down-regulation of 30 metabolites such as Dolicholide, Dolichosterone, Tetradecanedioic acid, and D-Lysopine. This suggested that HFD/STZ induction led to a disruption of hepatic metabolites in mice. Furthermore, Parviflorin and Hex-3-enedioylcarnitine were significantly up-regulated, while 3-Methyladipic acid, Phenylalanyltryptophan, and Propionyl-CoA were significantly down-regulated following SPP intervention. Similarly, SeSPP treatment significantly up-regulated metabolites such as D-Glucose-6-Phosphate and 3-Hydroxybutyrylcarnitine, while significantly down-regulated metabolites including Propionyl-CoA and 1-Carboxyethylphenylalanine. Heat map visualization further demonstrated that SPP samples and SeSPP samples were clustered separately, indicating these metabolites were significantly restored after SPP and SeSPP interventions (Fig. 5A-B). Collectively, these findings indicated that SPP and SeSPP can ameliorate HFD/STZ-induced metabolic disorders by modulating the levels of specific metabolites.

Based on the identified DMs, we further utilized the KEGG database to conduct metabolic pathway enrichment analysis, aiming to explore the roles of SPP and SeSPP in regulating potential metabolic pathways. Metabolic pathway prediction analysis was performed by importing the DMs between groups into the MetaboAnalyst 6.0 online platform, employing a topological analysis method in conjunction with the KEGG database. As illustrated in Figures 5C-E, the primary metabolic pathways impacted by HFD/STZ-induced T2DM include D-Amino acid metabolism, Propanoate metabolism, Lysine degradation, Glycerophospholipid metabolism, Valine, leucine, and isoleucine degradation, Cysteine and methionine metabolism, Alanine, aspartate, and glutamate metabolism, as well as Pantothenate and CoA biosynthesis. A total of 6 metabolic pathways were identified based on the differences between the SPPH and MC groups, which were Propanoate metabolism, Pyrimidine metabolism, Folate biosynthesis, Valine, leucine, and isoleucine degradation, Glycolysis/Gluconeogenesis, and Glycine, serine, and threonine metabolism. The

DMs between the SeSPPH group and the MC group were primarily associated with eleven pathways, including Propanoate metabolism, Pyruvate metabolism, Glycolysis/Gluconeogenesis, Pyrimidine metabolism, and TCA cycle. These findings indicated that SPP and SeSPP may mitigate HFD/STZ-induced metabolic disorders by modulating several key metabolic pathways, particularly core pathways related to energy metabolism, amino acid metabolism, and coenzyme biosynthesis.

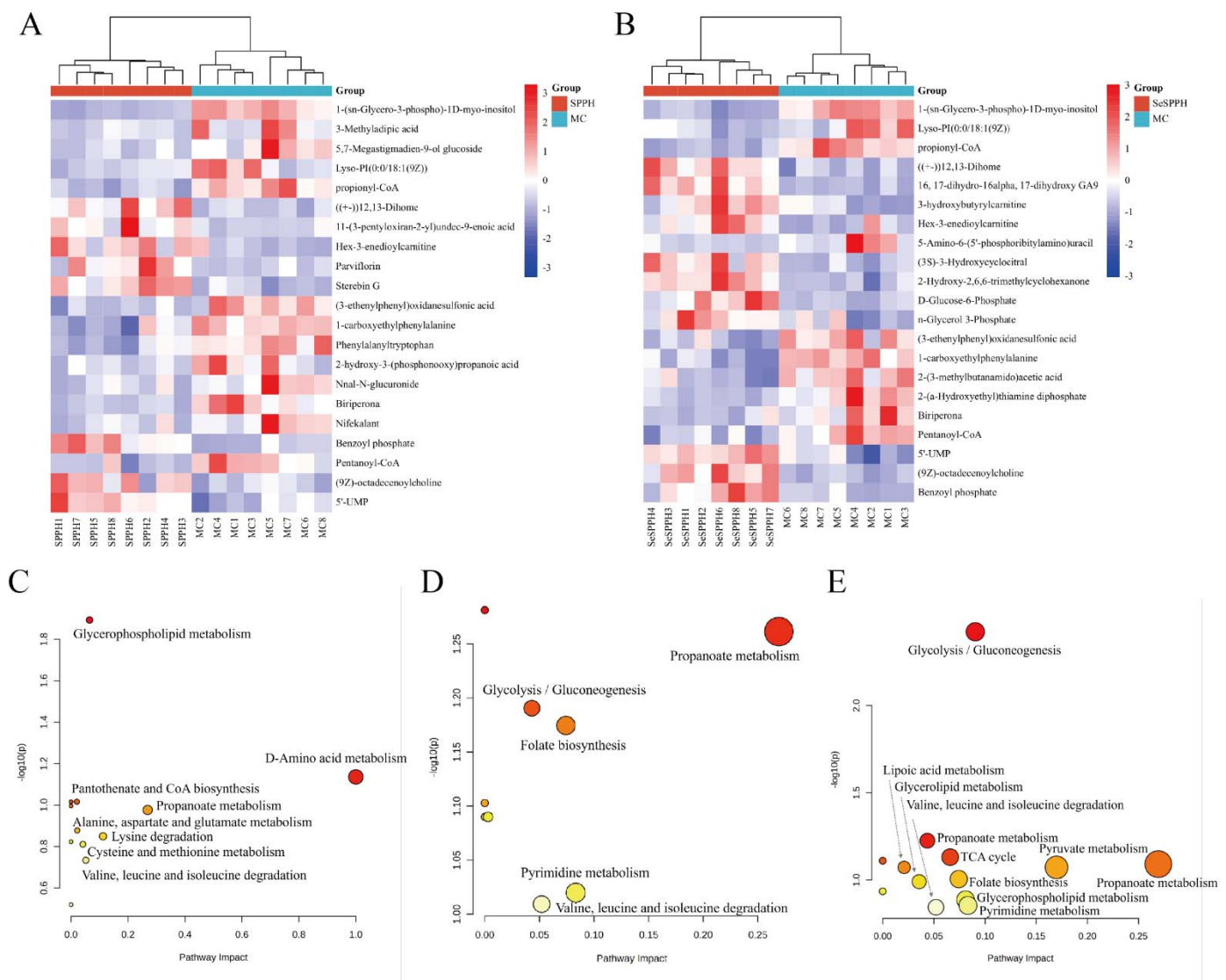


Figure 5 Effects of SPP and SeSPP on the DMs and metabolic pathways in the liver of T2DM mice. (A) Heatmap of DMs between SPPH vs MC groups; (B) Heatmap of DMs between SeSPPH vs MC groups. (C) Pathways of the potential biomarkers between MC vs NC groups; (D) Pathways of the potential biomarkers between SPPH vs MC groups; (E) Pathways of the potential biomarkers between SeSPPH vs MC groups.

3.3 Combined transcriptomics and metabolomics analysis

To systematically explore the metabolic regulatory networks of SPP and SeSPP in the liver of HFD/STZ-induced T2DM mice, we conducted an integrated analysis of DEGs and DMs. Utilizing the KEGG database, we integrated transcriptomics and metabolomics data, and identified 5 significantly related common metabolic pathways in the SPPH_MC group. These pathways include Fatty acid elongation, Xenobiotics metabolism by cytochrome P450, and Central carbon metabolism in cancer, among others (Fig. 6A). In the

SeSPPH_MC group, 4 significantly related common metabolic pathways were identified, including Fatty acid elongation, Amino sugar and nucleotide sugar metabolism, Glycerophospholipid metabolism, and others (Fig. 6B). Notably, DEGs and DMs in the SPPH_MC group were subjected to Spearman correlation analysis (Fig. 6C). Metabolites such as Pentanoyl-CoA, Biriperona, 1-(sn-Glycero-3-phospho)-1D-myo-inositol, and 11-(3-pentylloxiran-2-yl)undec-9-enoic acid exhibited negative correlations with *Slc39a5*, *Acot3*, *Gm37914*, *Lrp4*, and *Acsn2*. Sterebin G, Nnal-N-glucuronide, and Nifekalant exhibited positive correlations with *Acot3* and *Acsn2*. Based on the above analysis, this study identified *Acot3* and *Acsn2* as key regulatory genes in the intervention treatment of T2DM by SPP and SeSPP.

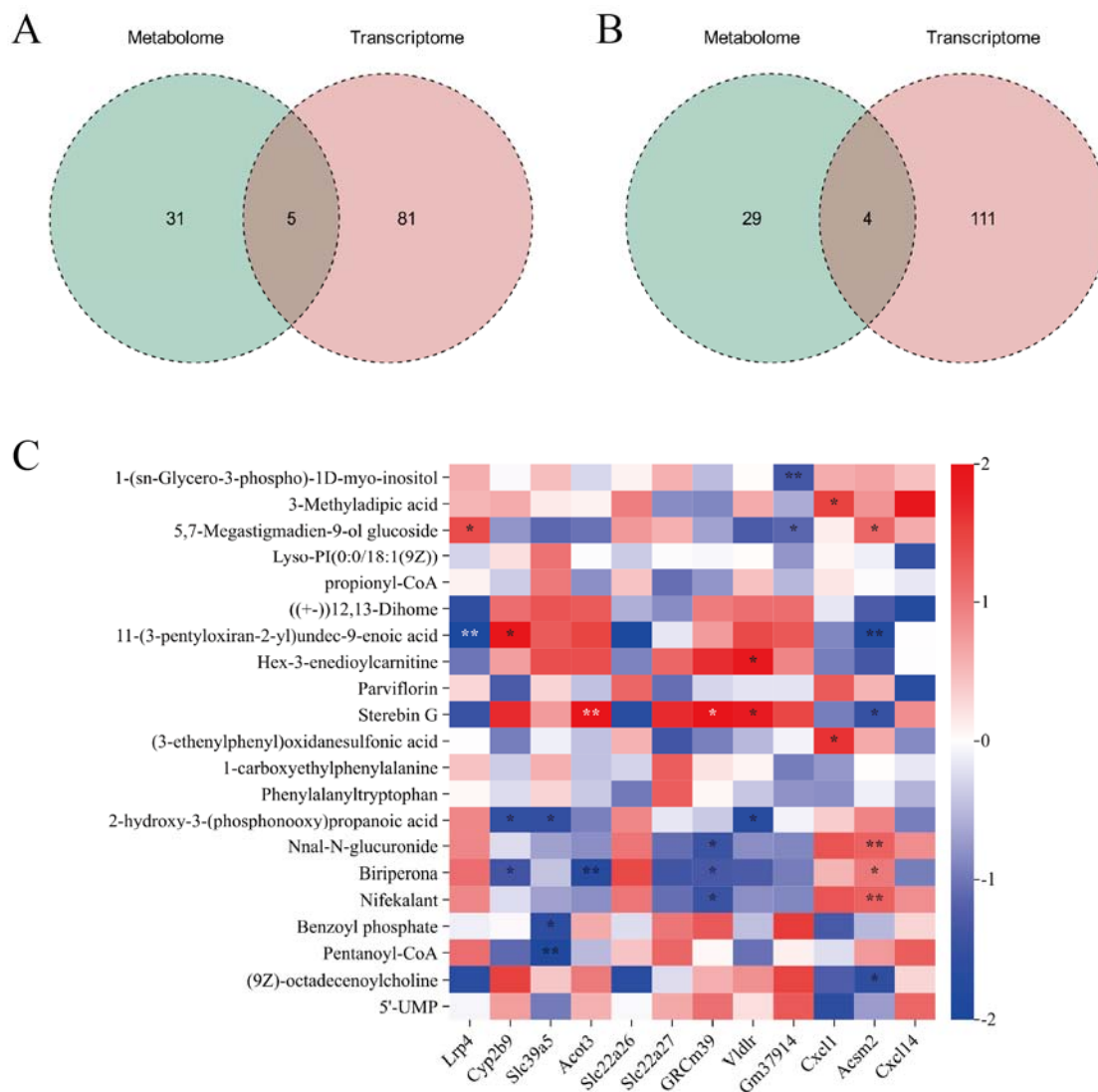


Figure 6 Correlation analysis of DEGs and DMs. (A) Venn diagram demonstrating the correlation of transcriptomics and metabolomics pathways between SPPH and MC groups; (B) Venn diagram demonstrating the correlation of transcriptomics and metabolomics pathways between SeSPPH and MC groups. (C) Heatmap showing Spearman's rank correlation coefficients between selected DEGs and DMs.

3.4 Gut microbiota analysis of fecal

Through 16S rRNA sequencing, we further revealed the effects of SPP and SeSPP on the gut microbiota composition of HFD/STZ-induced T2DM mice. Valid sequences of 46,276, 56,457, 52,892, 51,880, and 57,847 were obtained for the NC, MC, SPPH, and SeSPPH groups, respectively, with corresponding mean

sequence lengths of 440, 441, 440, 437, and 440. The results from the petal plot (Fig. 7A) indicated that the number of OTUs in the MC group was 471, significantly higher than the NC group, which had 432. This observation suggested that HFD/STZ induction markedly altered the gut microbiota diversity in mice. Notably, the number of OTUs in T2DM increased to 486 and 487 following SPP and SeSPP interventions, respectively, indicating that these two polysaccharides can significantly modulate the composition of gut microbiota. The increase in the number of OTUs may be attributed to the ability of SPP and SeSPP to promote the proliferation of specific beneficial microbiota, which may contribute to the amelioration of metabolic disorders through the production of SCFAs or modulation of gut barrier function.

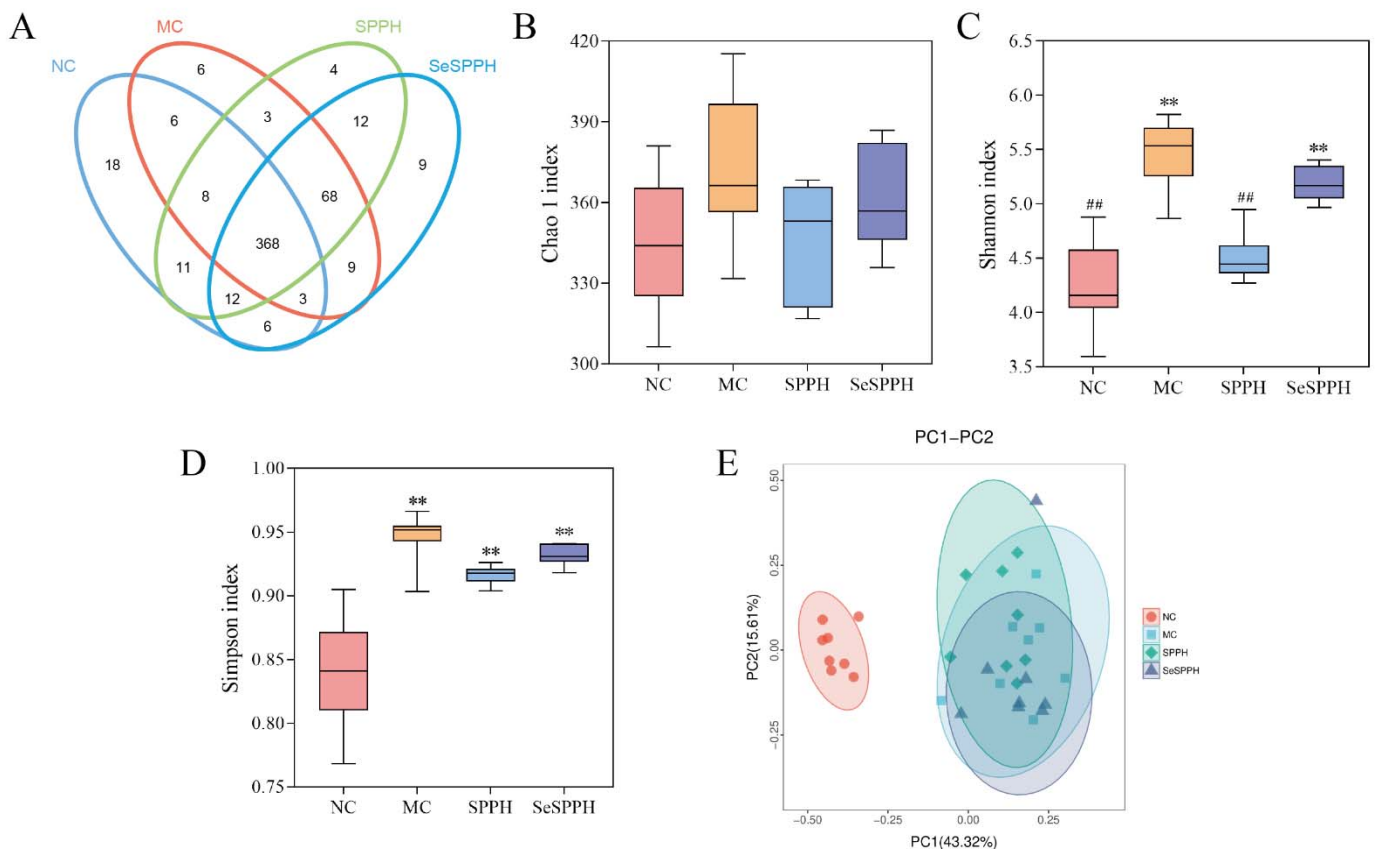


Figure 7 Effects of SPP and SeSPP on the diversity of gut microbiota of T2DM mice. (A) Venn diagram based on operational taxonomic units; (B-D) α -diversity indexes for microbiota in the fecal contents; (E) PCA analysis of gut microbiota identified between NC, MC, SPPH, and SeSPPH groups. Note: * $P < 0.05$, ** $P < 0.01$ vs. NC group; # $P < 0.05$, ## $P < 0.01$ vs. Model group.

In this study, the Chao1, Shannon, and Simpson indexes were employed to assess α -diversity, as illustrated in Fig. 7B-D. The indices for the MC, SPPH, and SeSPPH groups were found to be greater than those of the NC group. These findings suggested that the diversity and abundance of gut microbiota communities in both the model and treated groups were significantly altered compared to the NC group. However, the interventions with SPP and SeSPP did not significantly reverse the diversity and abundance of gut microbiota in T2DM mice. It is proposed that these two polysaccharides may exert their ameliorative effects primarily by modulating the abundance of specific microbial taxa rather than overall diversity. β -diversity analysis evaluated the differences between microbial communities and indicated the disparities

between each pair of samples within the community. The results of the PCoA (Fig. 7E) demonstrated that the NC group clustered in a distinct region from the other groups, indicating that the gut microbiota of HFD/STZ-induced T2DM mice had been disrupted. Additionally, the interventions with SPP and SeSPP exhibited a tendency to cluster closer to the NC group.

Based on the sequencing taxonomic annotation results, we analyzed the changes in the gut microbiota at the phylum level. Figure 8A illustrated the distribution of the top 10 microbiota in terms of relative abundance at the phylum level. At this level, *Firmicutes* and *Bacteroidetes* were predominant across all experimental groups, with their combined relative abundance consistently maintained at approximately 90%, aligning with findings reported in the previous study [26]. Following the intervention with SPP and SeSPP, the abundance of *Actinobacteria* and *Deferribacterota* in mice converged to levels observed in the NC group, indicating that SPP and SeSPP can ameliorate HFD/STZ-induced gut dysbiosis by modulating the abundance of specific bacterial phyla. Notably, *Actinobacteria* may be closely associated with improvements in host metabolism, as this phylum encompasses various strains with prebiotic functions, such as *Bifidobacteria* [27]. Additionally, the SPP and SeSPP interventions effectively reversed the *Firmicutes/Bacteroidetes* (F/B) ratio (Fig. 8B).

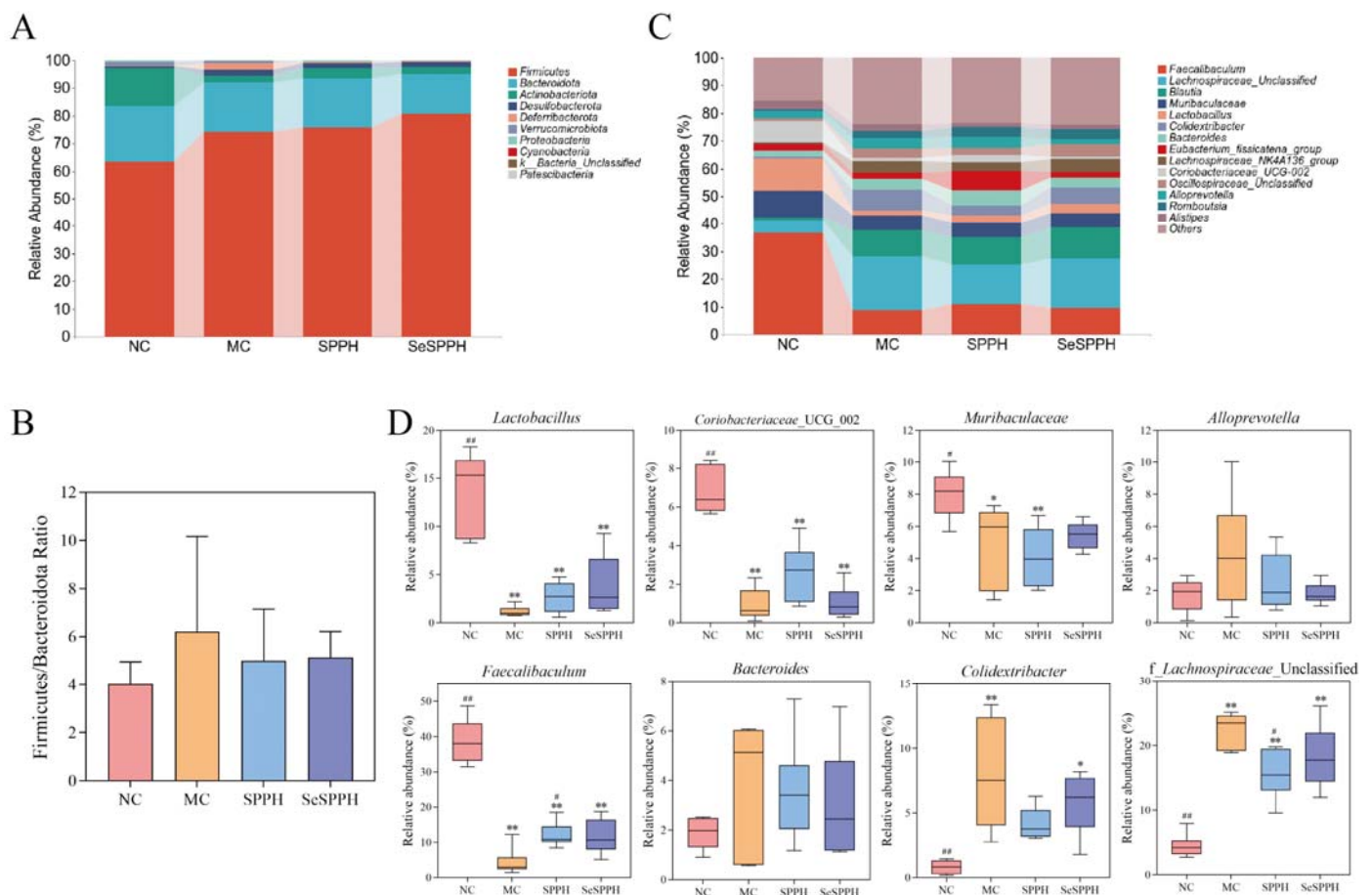


Figure 8 Effects of SPP and SeSPP on the composition of gut microbiota of T2DM mice. (A) Taxonomic composition of community at phylum level; (B) The abundance of *Firmicutes/Bacteroidetes* ratio in different groups; (C) Taxonomic composition of community at genus level; (D) Eight species of microbiota at the genus level. Note: * $P < 0.05$, ** $P < 0.01$ vs. NC group; # $P < 0.05$, ## $P < 0.01$ vs. Model group.

At the genus level, a significant difference in gut microbiota composition was observed between in the NC and MC groups (Fig. 8C). The abundance of *Faecalibaculum*, *Muribaculaceae*, and *Lactobacillus* was

significantly lower of the MC group compared to the NC group, whereas the genera *Lachnospiraceae*, *Blautia*, and *Colidextribacter* exhibited significantly higher abundances. Following the interventions with SPP and SeSPP, there was a notable improvement in the abundance of all genera, with the gut microbiota composition of the SPPH group being more similar to that of the NC group. This suggested that both SPP and SeSPP ameliorated the gut dysbiosis in HFD/STZ-induced T2DM mice, with SPP demonstrating a superior effect compared to SeSPP. Although the gut microbiota compositions of the treated group and the NC group still exhibited some differences at the genus level, the clustering of the treated groups was less effective than that of the NC group. Cluster analysis results indicated that the species composition of the SPPH group was closer to that of the NC group, further suggesting that SPP produced a more pronounced effect on improving HFD/STZ-induced gut microbiota dysbiosis. At the genus level, the abundance of *Faecalibaculum*, *Coriobacteriaceae-UCG-002*, *Lactobacillus*, and *Muribaculaceae* was significantly reduced in the MC group, while the abundance of *f-Lachnospiraceae-Unclassified*, *Alloprevotella*, *Bacteroides*, and *Colidextribacter* increased (Fig. 8D). Notably, *Faecalibaculum* and *Lactobacillus* were recognized as crucial for maintaining gut health [28, 29], whereas *Alloprevotella* was a pathogenic bacterium linked to inflammation and toxin production [30]. The intervention with SPP markedly mitigated the abundance of *f-Lachnospiraceae-Unclassified* and *Faecalibaculum*, while the abundance of other bacteria approached that of the NC group. Although SeSPP did not exhibit significant modulatory effects on specific bacterial genera, it still contributed to an improvement in the gut microbiota composition to some extent. This indicated that SPP and SeSPP can regulate gut microbiota disorders in HFD/STZ-induced T2DM mice by inhibiting the proliferation of harmful bacteria and promoting the growth of beneficial bacteria, with SPP demonstrating a superior regulatory effect.

4. Discussion

In recent years, natural polysaccharides have garnered significant attention in the interventional therapy of T2DM due to their remarkable bioactivities, including antioxidant, anti-inflammatory, and hypoglycemic effects [31, 32]. However, the intake of active polysaccharides, such as SPP and SeSPP, triggers complex biological responses in the host, involving alterations at multiple levels of gene expression and small-molecule metabolism. These interactions collectively form a multidimensional regulatory network that ameliorate T2DM symptoms, by modulating glucose and lipid metabolism, inflammation, and other pertinent physiological processes. Traditional single-omics approaches, which focus solely on specific genes or metabolites signaling pathways, have hindered a comprehensive understanding of the hypoglycemic mechanisms of polysaccharides. In contrast, multi-omics analysis offers robust technical support for systematically investigating the hypoglycemic mechanisms of polysaccharides [33]. Metabolomics can comprehensively detect dynamic changes in various small molecule metabolites *in vivo* and elucidate the regulatory effects of polysaccharides on metabolic networks [34], while transcriptomics can explore the regulatory mechanisms by which polysaccharides influence the expression of T2DM-related genes [17]. More importantly, recent studies have indicated that algae polysaccharides can also exert hypoglycemic effects by

regulating gut microbiota homeostasis, suggesting that the “gut-liver axis” plays a crucial role in polysaccharide intervention in T2DM. Therefore, the multi-omics strategy, which integrates metabolomics, transcriptomics, and microbiomics, presents a novel research paradigm for comprehensively revealing the molecular mechanisms underlying the hypoglycemic effects of polysaccharides through the regulatory network of the “gut-liver axis.” This approach provides a significant theoretical foundation for the development of polysaccharide-based interventional strategies for T2DM.

As a core organ for carbohydrate and lipid metabolism, the liver exhibited significant histopathological alterations during the pathology of diabetes [35]. In this study, liver transcriptomic analysis revealed the multiple regulatory effects of SPP and SeSPP on hepatic metabolic processes. GO functional enrichment analysis indicated that SPP and SeSPP significantly influenced key biological processes, including Unsaturated monocarboxylic acid metabolism, Xenobiotic metabolism, Bile acid metabolism, and Short-chain fatty acid transport. Among them, Steroid metabolism process was unique to SeSPP. KEGG pathway analysis further suggested that these two polysaccharides may ameliorate T2DM by modulating the Peroxisomal pathway, Fatty acid metabolism, Bile acid synthesis, and pathways related to lipid and atherosclerosis. Enhanced fatty acid oxidation was found to elevate the level of acyl-CoA, which in turn activated pyruvate carboxylase via denaturation, thereby promoting gluconeogenesis and endogenous glucose production [36]. Both SPP and SeSPP interventions significantly upregulated the expression of *Acot3* gene, which encoded acyl-CoA thioesterase 3, known for its inhibitory effects on gluconeogenesis, thus positioning it as a potential glucose-lowering target gene [37]. In addition, SPP and SeSPP can significantly down-regulate the expression of the *Acsm2* gene. *Acsm2* encoded mitochondrial acyl-CoA synthetase, a key enzyme in fatty acid metabolism, which promoted fatty acid activation by catalyzing the formation of acyl-CoA [38]. Our results indicated that SPP and SeSPP effectively reduce fatty acid accumulation in the liver of T2DM mice by inhibiting the fatty acid activation pathway mediated by *Acsm2*. Furthermore, SCFAs served as crucial metabolic regulators, participating in the modulation of metabolic diseases through various pathways, including the regulation of glucose-lipid metabolism, maintenance of energy homeostasis, protection of the gut barrier, and inhibition of inflammatory responses [39]. Additionally, SPP and SeSPP interventions markedly enhanced the expression of *Slc22a26* gene, which encoded an organic cation transporter protein. This protein activated SCFAs membrane transporter activity, thereby facilitating the transmembrane transport of SCFAs in metabolic tissues [40]. This mechanism may represent a significant pathway through which polysaccharides exerted their metabolic regulatory effects.

In this study, the novelty of Propionyl-CoA, 12,13-diHOME, and Lyso-PI (0:0/18:1(9Z)) in the context of SPP and SeSPP action was clear in T2DM mice. Among these, Propionyl-CoA, a key marker of fatty acid metabolism disorders, was involved in metabolic regulation through its conversion to succinyl CoA, which was further degraded to succinate and CoA. Clinical studies have demonstrated that plasma succinate levels were significantly elevated by 53% in T2DM patients compared to healthy individuals, suggesting that high circulating succinate levels may persist during chronic metabolic stress states [41]. Notably, SPP and SeSPP

interventions significantly reduced Propionyl-CoA levels, consequently, succinate production, which may be an important mechanism by which they alleviate chronic oxidative stress. 12,13-diHOME, a novel lipid mediator, has been shown to be closely associated with improved metabolic health at elevated levels [42]. Studies have shown that 12,13-diHOME significantly promoted fatty acid uptake and oxidation by activating brown adipose tissue-dependent thermogenesis. Notably, both SPP and SeSPP interventions can effectively enhance the biosynthesis level of 12,13-diHOME, thereby inhibiting the abnormal accumulation of fatty acids in the body. Therefore, intervention strategies aimed at increasing 12,13-diHOME levels hold significant potential for the prevention and treatment of metabolic diseases including T2DM. It was worth noting that Lyso-PI (0:0/18:1(9Z)), a pathogenic lipid molecule closely related to pancreatic β -cell damage, has been reported to be significantly elevated in the pre-diabetic stage of T2DM [43]. This study for the first time found that SPP and SeSPP intervention could significantly inhibit the abnormal accumulation of this lipid molecule. Given the cytotoxic effect of Lyso-PI (0:0/18:1(9Z)), this regulatory effect may protect pancreatic β -cell function by reducing lipotoxic stress, providing a new molecular target for elucidating the hypoglycemic mechanism of SPP and SeSPP.

Based on the DMs identified following SPP and SeSPP treatments, we conducted a systematic analysis of the potential metabolic pathways through which these two polysaccharides exerted their effects in T2DM mice. This analysis aimed to elucidate the mechanisms underlying their hypoglycemic effects. The metabolic pathway enrichment analysis revealed that the DMs are primarily involved in key metabolic pathways, including Fatty acid metabolism, Nucleotide metabolism, Amino acid metabolism and degradation, Carbohydrate metabolism, and Folate biosynthesis. Notably, SCFAs recognized as significant metabolic regulators, have demonstrated substantial hypoglycemic and hypolipidemic effects. Specifically, propionate not only inhibited hepatic lipogenesis and reduced cholesterol levels but also exhibited anti-inflammatory activity [44]. Furthermore, the metabolism of amino acids and nucleotides was closely linked to the development of insulin resistance. Importantly, folate metabolism played a crucial role in maintaining liver health. The study indicated that folate deficiency leads to increased secretion of pro-inflammatory factors from the liver, resulting in disruptions in hepatic lipid metabolism and enhanced fat oxidation and decomposition [45]. These findings suggested that SPP and SeSPP may act synergistically to ameliorate metabolic disorders associated with T2DM through the regulation of fatty acid metabolism and maintenance of folate homeostasis. This provided significant metabolomic evidence for a deeper understanding of their glucose-lowering mechanisms.

Previous work has demonstrated that SPP and SeSPP exhibit significant lipid metabolism regulatory capabilities [25]. The finding was consistent with numerous studies. It has been demonstrated that *Laminaria japonica* polysaccharides can ameliorate insulin resistance by upregulating the expression of insulin receptor substrate 1 and modulating lipid metabolism, as indicated by a decrease in palmitic acid levels and an increase in glutathione levels [17]. Notably, linoleic acid metabolism played a crucial role in blood glucose regulation by promoting lipid degradation and consumption while reducing cholesterol and triacylglycerol synthesis,

thereby effectively lowering blood lipid levels ^[46]. This mechanism aligned closely with the findings of the present study. 12,13-diHOME, an important metabolite of linoleic acid, acted synergistically to alleviate glucose-lipid metabolism disorders through multiple pathways. This study emphasized the positive role of 12,13-diHOME in improving T2DM and suggested that it may exert a metabolic protective effect by regulating the function of brown adipose tissue and promoting the browning of white adipose tissue. In summary, SPP and SeSPP may promote fatty acid uptake and degradation, inhibit lipid accumulation, and thereby alleviate insulin resistance and suppress glucose production caused by T2DM by regulating the expression of *Acot3* and *Acsm2* and the content of metabolites such as propionyl-CoA, 12,13-diHOME and Lyso-PI (0:0/18:1(9Z)). SPP and SeSPP interventions can significantly ameliorate T2DM-induced abnormalities of metabolites and metabolic pathway disorders in liver.

As mentioned earlier, gut microbiota is crucial for T2DM and its complications, with its disruption being a primary cause of gut ecological abnormalities in T2DM. In the present study, we observed an increased abundance of *Firmicutes*, *Desulfobacteriota*, and *Deferribacterota* in the MC group compared to the NC group, while the abundance of *Bacteroidota* decreased, resulting in significant dysbiosis of gut microbiota in T2DM mice. Notably, we elevated the abundance ratio of F/B and found that SPP and SeSPP interventions resulted in a decrease in the F/B ratio. This observation was consistent with the work of Wang et al., which reported that polysaccharides from *Rosa roxburghii* Tratt fruit reversed the F/B ratio, thereby reducing hyperglycemia and hyperlipidemia in T2DM mice ^[47]. The SPP and SeSPP interventions significantly increased the abundance of *Lactobacillus* while decreasing the abundance of *Alloprevotella*, *Colidextribacter*, and *Bacteroides*. Notably, *Lactobacillus* and *Lachnospiraceae_NK4A136_group* were recognized as important probiotic bacteria that enhance immunity and combat metabolic diseases ^[48]. Study indicated that *Lactobacillus* can ameliorate obesity and glucose homeostasis dysregulation induced by HFD ^[48]. Meanwhile, *Lachnospiraceae_NK4A136_group* was identified as a potentially beneficial bacterium linked to obesity ^[49]. Conversely, the abundance of *Alloprevotella* and *Colidextribacter* enterotypes showed a positive correlation with body weight and inflammation levels. Furthermore, *Lactobacillus* may mitigate inflammation and maintain gut homeostasis by promoting the production of SCFAs ^[50]. These findings suggested that the hepatoprotective effects of SPP and SeSPP may be partially attributed to their modulatory effects on gut microbiota dysbiosis in T2DM mice.

While there were numerous connections between host metabolism and gut microbiota, this research represented merely a small fraction of the overall metabolic system. Consequently, the underlying hypoglycemic mechanisms of SPP and SeSPP require further investigation utilizing multi-omics techniques. Although the study identified individual biomarkers including Propionyl-CoA, 12,13-diHOME, and Lyso-PI (0:0/18:1(9Z)), the specific hypoglycemic mechanisms remained ambiguous. Therefore, the above markers will be explored in future to more effectively elucidate the therapeutic potential of SPP and SeSPP.

5. Conclusions

In the study, SPP was isolated and extracted, followed by selenium modification to obtain SeSPP. The intervention of SPP and SeSPP could significantly improve the metabolic disorder of HFD/STZ-induced T2DM mice by regulating the microbiome-metabolome-transcriptome network. Specifically, SPP and SeSPP could reshape the structure of the gut microbiota and significantly increase the relative abundance of beneficial microbiota. The combined analysis of transcriptome and metabolome indicated that these two polysaccharides could positively regulate key metabolic pathways including lipid metabolism, carbohydrate metabolism and signal transduction. Further studies found that SPP and SeSPP could significantly affect the fatty acid metabolism process by regulating key metabolites including Propionyl-CoA, 12,13-diHOME and Lyso-PI (0:0/18:1(9Z)). Correspondingly, the expression levels of key genes *Acot3* and *Acsn2* in the fatty acid metabolism pathway were also significantly regulated by SPP and SeSPP. These results suggested that SPP and SeSPP may mainly exert their hypoglycemic effects by regulating the fatty acid metabolism pathway. Specifically, SPP and SeSPP alleviated insulin resistance and glucose release caused by T2DM by decomposing fatty acids and inhibiting their abnormal accumulation. However, to clarify their exact therapeutic mechanisms, future studies still need to conduct more in-depth verification and analysis of the key bacterial genera, functional genes and metabolic markers related to SPP and SeSPP.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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