Journal of Food and Nutrition Research, 2023, Vol. 11, No. 7, 491-499
Available online at http://pubs.sciepub.com/jfnr/11/7/5
Published by Science and Education Publishing
DOI:10.12691/jfnr-11-7-5

Effect of Polygonatum Sibiricum Aqueous Extract on Gut Microflora of Type 2 Diabetic Mice

Fuding Zhou[1,#], Jinchuan Yu[1,#], Guangjun Wang[2], Ting Wang[1], Zhengxiang Liu[1], Wenjun Chen[1,*]

[1]Department of Nutrition and Food Hygiene, School of Public Health, Anhui Medical University, Hefei, China
[2]School of Public Health, Anhui Medical University, Hefei, China
[#]These authors contributed equally to this work.
*Corresponding author: chenwj71024@163.com

Received May 21, 2023; Revised July 03, 2023; Accepted July 25, 2023

Abstract This study aimed to explore the protective effect of Polygonatum sibiricum aqueous extract (PSAE) on lipid metabolism in vivo. 72 mice were divided randomly into Polygonatum sibiricum polysaccharide (PSP) group, Control group, Model group, Low-, Medium-, and High-dose PSAE (0.5, 1, 2g/kg) groups, 12 mice in each group. In this experiment, 60% high-fat diets were used for 6 weeks. After 3 consecutive doses of 35mg/kg, an intraperitoneal injection of 1% streptozotocin (STZ) was used to induce type 2 diabetic mice (T2DM). Streptozotocin was dissolved in citric acid sodium citrate buffer solution in PH=4.4. Mice were treated with PSAE and PSP by gavage one week before modeling, and the gavage process lasted until the mice were killed. After one week of gavage treatment, the feeding diets of mice in the Model group, PSAE groups, and PSP group were all changed to a 60% high-fat diet. After feeding with a high-fat diet for 6 weeks, the mice were fasted overnight for 3 consecutive days and injected with 1% STZ intraperitoneally. The mice in the Control group were injected with the same volume of solvent as the same surgical control. The results showed that, compared to mice in the Model group, the levels of serum total cholesterol, triglyceride, low-density lipoprotein cholesterol, high-density lipoprotein cholesterol, and liver triglyceride level were significantly decreased in the high-dose PSAE group. The composition and diversity of the gut microbiota were considerably altered by PSAE. In particular, high-dose PSAE lowered the relative abundance of the phyla Firmicutes and Bacteroidetes while increasing the relative abundance of the phylum Proteobacteria. At the genus level, high-dose PSAE decreased the relative abundance of Bacteroides, while significantly increasing the relative abundance of Parabacteroides and Alistipes. Besides, PSAE alleviated insulin resistance in type 2 diabetic mice. These results imply that PSAE may be a potential functional food for T2DM intervention by regulating gut microbiota and against lipid metabolism disorders.

Keywords: *type 2 diabetic, Polygonatum sibiricum aqueous extract, gut microflora*

Cite This Article: Fuding Zhou, Jinchuan Yu, Guangjun Wang, Ting Wang, Zhengxiang Liu, and Wenjun Chen, "Effect of Polygonatum Sibiricum Aqueous Extract on Gut Microflora of Type 2 Diabetic Mice." Journal of Food and Nutrition Research, vol. 11, no. 7 (2023): 491-499. doi: 10.12691/jfnr-11-7-5.

1. Introduction

Type 2 Diabetes Mellitus (T2DM) is a complex metabolic disease dominated by hyperglycemia and insulin resistance. Chronic hyperglycemia in diabetes causes many complications, such as kidney, liver, intestinal tract, nerve, blood vessels, and heart damage [1]. According to the ninth edition of the global diabetes map published by the International Diabetes Federation in 2019, the worldwide population of adults with diabetes has reached 463 million. It is projected that by 2045, the number of individuals affected by diabetes could rise to 700 million, indicating a significant increase. This upward trend in diabetes prevalence poses a growing economic burden on a global scale [2]. More than 90% of all diabetes patients worldwide have type 2 diabetes [3]. While the pathogenesis of diabetes remains incompletely understood, type 2 diabetes mellitus (T2DM) is primarily attributed to insulin resistance (IR) and dysfunction of the pancreatic islet β cells, leading to impaired insulin secretion [4]. It is mainly characterized by insulin resistance, when the body has insulin resistance, the body's islets β compensate for insulin resistance by enhancing insulin secretion, to maintain the blood glucose level in the body [5]. However, prolonged insulin resistance diminishes the body's insulin sensitivity, causing the biological effect of a given amount of insulin to be lower than normal. This impairment hampers the ability of insulin to facilitate glucose absorption and utilization. To control the stability of blood glucose, the body will continue to enhance islets β cells secrete insulin through feedback regulation, which causes hyperinsulinemia. Hyperinsulinemia will reduce the expression of insulin receptor genes and reduce the synthesis of receptor protein, thus hindering the normal transmission of insulin signals [6,7,8]. There is increasing evidence that T2DM is closed related to genetic mutations

[9], islet oocyte loss [10], IR, and changes in gut microbiota [11,12]. Emerging evidence has demonstrated that alterations in the gut microbiota composition and function influence IR and T2DM.

The natural product Polygonatum sibiricum (PS) (also called Huangjing), is well-known as a traditional medicinal herb and functional food in China [13,14], belonging to the Liliaceae Family, which is distributed throughout the temperate Northern Hemisphere such as China, Japan, Korea, India, Russia, Europe, and North America, China especially has abundant resources of PS [15,16,17]. PS has been reported to have many pharmacological applications and biological activities, such as antioxidant activity, anti-aging activity, anti-osteoporosis, neuroprotective, immunity enhancement, anti-diabetic, anti-fatigue, and anti-cancer effects [18,19,20]. The abundant biological activities have been attributed to the presence of multiple components, PS is ascribed to contain multiple bioactive compounds such as Polygonatum sibiricum polysaccharides (PSP), steroid saponins, flavones, homoisoflavanone, alkaloids, lignins, triterpenoid saponins, phenolic compound, etc [21,22,23,24,25]. PSP are believed to be one of the most important active compounds of PS. Moreover, natural phytochemicals such as polysaccharides, saponins, and flavonoids in Polygonatum sibiricum have obvious regulatory effects on glucose and lipid metabolism [26,27].

Due to its health-promoting properties, PS has gained significant popularity as an ingredient or supplement in the food industry. In a previous study conducted by our team, we revealed that PSAE effectively alleviates ethanol-induced liver injury in mice through the regulation of the Nrf2/ARE pathway [28]. However, the protective effect of PSAE on lipid metabolism in type 2 diabetic mice remains unknown. In this study, we used a high-fat diet combined with a low-dose streptozotocin mouse model to explore the protective effect of PSAE on mice with type 2 diabetes.

2. Methods

2.1. Materials

PSAE (No. 20170308) was purchased from Chizhou Jingtian Food Co., Ltd. (Anhui, China). Streptozotocin and Tween-20 (CAS 9005-64-5) were purchased from Beijing solebao Technology Co., Ltd. (Beijing, China). Insulin kit (021100101) was purchased from Shenzhen Xinye Biomedical Engineering Co., Ltd. (Shenzhen, China) Triglyceride assay kit (auz6359) and total cholesterol assay kit (auz6673) were purchased from Beckman Kurt Experimental System Co., Ltd. (Jiangsu, China). Low density lipoprotein cholesterol assay kit (19052306) and high density lipoprotein cholesterol assay kit (19051602) were purchased from Beijing Lidman Biochemical Co., Ltd. (Beijing, China). The fecal genomic DNA extraction kit used in the experiment was purchased from Shanghai Ouyi Biomedical Technology Co., Ltd. (Shanghai, China).

2.2. Preparation of Polygonatum Sibiricum Aqueous Extract

We prepared an aqueous extract of Polygonatum sibiricum (PSAE) by processing fresh polygonatum sibiricum using the methodology previously established by our research group. The bioactive compounds believed to be present in P. sibiricum include polysaccharides, flavonoids, and saponins. To determine the contents of polysaccharides, saponins, and flavonoids in PSAE, we utilized UV spectrophotometry, following the standards specified in the Chinese Pharmacopoeia. The corresponding results are presented in Table 1.

Table 1. The contents of polysaccharides, saponins, and flavonoids in PSAE

Ingredients	g/100g
polysaccharides	34.1
saponins	6.1
flavonoids	0.1

2.3. Animal Experiment

The 4-week-old C57BL mice were obtained from Zhejiang weitonglihua Experimental Animal Technology Co., Ltd. Throughout the experiment, the mice were provided with a suitable feeding environment, maintaining a controlled temperature of 20-25°C and humidity of 50±5%. A 12-hour light-dark cycle was also implemented. The Animal Experiment Ethics Committee of Anhui Medical University approved conducting the study. After a one-week acclimatization period, the mice were randomly divided into different groups: Control group, Model group, PSAE low dose group (LPSAE) receiving gavage of 0.5g/kg PSAE, PSAE medium dose group (MPSAE) receiving gavage of 1g/kg PSAE, PSAE high dose group (HPSAE) receiving gavage of 2g/kg PSAE, and a positive group administered with Polygonatum sibiricum polysaccharides (PSP). Each group consisted of 12 mice. In this experiment, a 60% high-fat diet was administered to the mice for 6 weeks. Following three consecutive doses of 35mg/kg, type 2 diabetes was induced in the mice through intraperitoneal injection of 1% streptozotocin (STZ). The STZ was dissolved in a citric acid sodium citrate buffer solution with a pH of 4.4. The treatment with PSAE and PSP via gavage started one week before modeling and continued until the mice were euthanized. After one week of gavage treatment, the mice in the Model group, PSAE dose group, and PSP group had their diets changed to a 60% high-fat diet. Following 6 weeks of high-fat diet feeding, the mice were fasted overnight for three consecutive days before receiving an intraperitoneal injection of 1% STZ. The mice in the Control group received the same volume of solvent as the surgical control. Throughout the experiment, the mice's weight was regularly recorded weekly, and their overall activity levels were observed in each group.

2.4. Determination of Biochemical Indexes in Mouse Serum and Liver

After one week of the last intraperitoneal injection of STZ, the mice were fasted overnight and water-free for 12 hours, then collected blood from their eyeballs and extracted the liver tissue. The total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were determined according to the instructions using the corresponding kit respectively.

2.5. Histological Analysis

The livers of mice from each group were extracted, washed with normal saline, and then placed in a 4% paraformaldehyde solution for fixation, with a minimum duration of 24 hours. Subsequently, the tissues were removed, dehydrated, and subjected to transparent treatment in a fume hood. The processed tissues were then embedded and sliced for Periodic Acid-Schiff (PAS) staining. To prepare the working solution for oil red O staining, the stock solution and diluent were mixed in a 5:2 ratio, and the mixture was filtered using low-speed filter paper. The fixed tissues were embedded with an optimal cutting temperature (OTC) compound and sectioned using a cryostat at a thickness of 10 μm. The sections were allowed to sit at room temperature for 5 minutes. Then, the sections were placed in a staining dish containing the prepared working solution for 15 minutes, followed by removal from the solution and rinsing with pure water for 10-20 seconds. Subsequently, the sections were placed in a re-dyeing solution in another dish, kept for 5 minutes, and then washed with pure water for 30-60 seconds. Reagent water-based sealing agent was dropped onto the sections, and a coverslip was slowly placed over the sections to seal them. The tissue sections were observed using a high-power microscope, and the deposition of lipids was recorded and captured using a pathological image analysis system.

2.6. Gut Microbiota Analysis

One week after the last intraperitoneal injection of STZ, fresh intestinal contents samples of mice in each group were collected after collecting blood and liver samples and stored in a refrigerator at -80°C. Total genomic DNA was extracted using the MagPure Soil DNA LQ Kit following the manufacturer's instructions. The quality and quantity of DNA was verified with NanoDrop and agarose gel. Extracted DNA was diluted to a concentration of 1 ng/μl and stored at -20°C until further processing. The diluted DNA was used as a template for PCR amplification of bacterial 16S rRNA genes with the barcoded primers and Takara Ex Taq (Takara). For bacterial diversity analysis, V3-V4 variable regions of 16S rRNA genes were amplified with universal primers 343 F (5'- TACGGRAGGCAGCAG -3') and 798 R (5'- AGGGTATCTAATCCT-3'). PCR amplification was performed in a total volume of 30 μl, which contained 15 μl of 2×Gflex PCR Buffer, 1 μl of 5 pmol/μl primer F, 1 μl of 5 pmol/μl primer R, 50ng of Template DNA, 0.6μl of Tks Gflex DNA Polymerase (1.25U/μl). Here are the thermal cycling conditions: initial denaturation for 5 minutes at 94°C, and then by 26 cycles for 30S at 94°C, 30S at 56°C, and 20S at 72°C, with a final extension of 5 minutes at 72°C. Amplicon quality was visualized using gel electrophoresis, purified with AMPure XP beads (Agencourt), and amplified for another round of PCR. Next, a second round of PCR was performed in a 30 μL reaction, which contained 15 μl of 2×Gflex PCR Buffer, 0.6 μL of Tks Gflex DNA Polymerase (1.25U/μl), 1 μl of Adapter I5, 1 μl of Adapter I7, and 50ng of PCR products from the first step. The following are the conditions for thermal cycling: initial denaturation at 94°C for 5 min, followed by 26 cycles at 94°C for 30S, 56°C for 30S, and 72°C for 20S, with a final extension of 5 minutes at 72°C. After being purified with the AMPure XP beads again, the final amplicon was quantified using a Qubit dsDNA assay kit. Equal amounts of purified amplicon were pooled for subsequent sequencing.

2.7. Bioinformatics and Statistical Analysis

After preprocessing the sequencing data to obtain high-quality sequences, the sequences were classified into multiple operational taxonomic units (OTUs) using search software, considering sequence similarity as the criterion. OTUs were defined with a threshold of 97% sequence similarity. The primer sequences were removed, and clustering was performed on the clean reads to generate OTUs using search software, applying a 97% similarity cutoff. From each OTU, a representative read was selected utilizing the QIIME package. The representative reads were then annotated by performing a BLAST search against the Silva database (Version 123) using the RDP classifier, with a confidence threshold of 70%. Based on the clustering results, alpha diversity, and beta diversity analyses were conducted, providing information on the species composition at all levels of the samples from the annotation results. All the results were reported as the mean ± standard deviation (x ± s), and statistical analysis was conducted using SPSS software. The differences among multiple groups were determined using the t-test method.

3. Results

3.1. Effect of PSAE on Body Weight and Liver Weight in Mice

The weight of mice in each group increased with the increase in feeding time. Due to the feeding of a high-fat diet, the weight gain rate of the Model group, PSAE dose groups, and PSP group were significantly higher than that of the Control group. However, compared with the Model group, the weight gain rate of mice in PSAE dose groups was slower and dose-dependent, while the weight growth rate of mice in the HPSAE group and PSP group was close to that in the Control group, as shown in Figure 1a,1b. The liver weight of mice in each group is shown in Figure 1c [29]. Compared with the Control group, the liver weight of mice in the Model group, LPSAE group, MPSAE group, and PSP group increased significantly ($P < 0.01$, $P < 0.05$), but there was no

significant difference between the PSAEH group and Control group (P > 0.05); Compared with the Model group, the liver weight of mice in the PSAE dose groups and PSP group showed a certain downward trend and showed a dose dependence. Among them, the liver weight of mice in LPSAE group and MPSAE group had no significant decrease (P > 0.05), while the liver weight of mice in HPSAE group and PSP group had a significant decrease (P < 0.01, P < 0.05); There was no significant difference in liver weight between HPSAE group and PSP group (P > 0.05). Based on the comparative results, PSAE could slow down the weight and liver gain in mice.

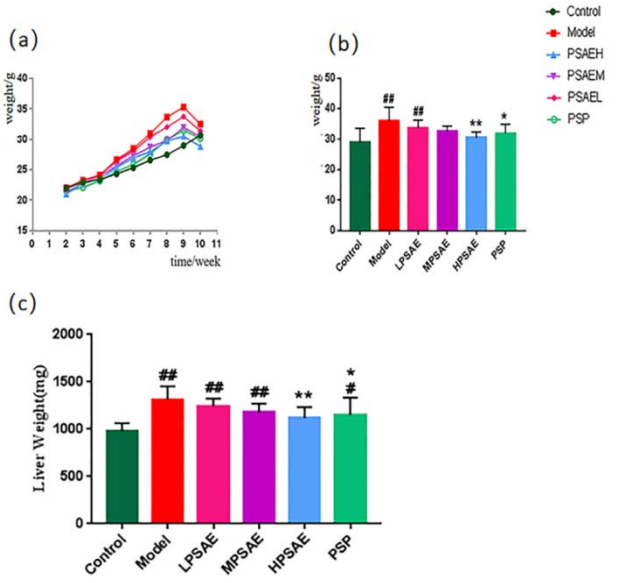

Figure 1. The effect of PSAE on body weight and liver weight in mice. (a) Weekly body weight of mice. (b) The mice body weight at the 9th week. (c) The mice liver weight at the 9th week. ##p < .01 versus the Control group; #p < .05 versus the Control group; **p < .01 versus the Model group; *p < .05 versus the Model group

3.2. Effect of PSAE on Blood Lipid Metabolism in Mice

The level of TC, TG, HDL-C, and LDL-C in the serum were determined to investigate the effects of PSAE on blood lipid metabolism in vivo (Table 2) [29]. Compared with the Control group, the levels of TG, TC, HDL-C, and LDL-C in the Model group increased significantly, and the levels of the TG and TC in the LPSAE group and MPSAE group increased significantly (P < 0.01, P < 0.05), while the levels of TG and TC in HPSAE group and PSP group increased to some extent, but there was no statistical significance (P > 0.05). The level of HDL-C in the Model group and PSAE dose groups increased significantly (P < 0.01, P < 0.05). The level of HDL-C in the PSP group increased, but there was no statistical significance (P > 0.05). The level of LDL-C in the Model group and PSAEL group increased significantly (P < 0.01, P < 0.05). There was no significant difference in the level of LDL-C in the MPSAE group, HPSAE group, and PSP group compared with the Control group. Compared with the Model group, after the intragastric intervention of PSAE, the indexes of TG, TC, HDL-C, and LDL-C of mice in the MPSAE group and HPSAE group decreased to a certain extent. The decrease level of mice in the MPSAE group was not statistically significant (P > 0.05), and the decrease of various indexes of mice in the HPSAE group was statistically significant (P < 0.01, P < 0.05); The changing trend of all indexes in the PSP group was the same as that in the HPSAE group, and there was no statistical difference compared with that in the Control group (P > 0.05). Compared with the Model group, all indexes in the PSP group showed a statistically significant decrease (P < 0.01). There was no significant difference in all indexes between the PSP group and the HPSAE group (P > 0.05). Those results showed that there was a disorder of blood lipid metabolism in T2DM mice. Dyslipidemia is mainly manifested as hyperlipidemia, and hypertriglyceridemia and hypercholesterolemia are the most common. HDL-C is an anti-atherosclerotic protein and a protective factor against coronary heart disease. The anti-atherosclerotic effect of HDL-C is mainly manifested in promoting the outflow of cellular cholesterol to the liver so that cholesterol is discharged from the bile duct by transforming it into bile acids. It has been reported that hyperlipidemia is related to the occurrence and development of cardiovascular and cerebrovascular diseases in diabetic patients. Dyslipidemia is an important risk factor for atherosclerosis and coronary heart disease. Therefore, detecting the levels of TG, TC, HDL-C, and LDL-C were commonly indexed to reflect the degree of dyslipidemia in T2DM mice. In this study, after treatment with HPSAE, the levels of TG, TC, HDL-C, and LDL-C significant decrease compared with the type 2 diabetic Model group. Figure 2 shows the comparison of TG levels in the liver tissue of mice in each group. Compared with the Control group [29], the TG levels in the liver of mice in Model group and LPSAE group were significantly higher (P < 0.01, P < 0.05), and there was no significant difference in TG levels in the liver of mice in MPSAE group, HPSAE group, and PSP group compared with the Control group (P > 0.05); Compared with the Model group, the liver TG level of PSAE dose groups showed a downward trend.

Table 2. Effect of PSAE on TG, TC, HDL-C, LDL-C in mice

Group	TG	TC	HDL-C	LDL-C
Control	0.9717±0.09	3.6633±0.72	3.0142±0.55	0.2825±0.08
Model	1.4425±0.48$^{\#\#}$	5.1958±0.10$^{\#\#}$	4.3417±0.48$^{\#\#}$	0.4350±0.16$^{\#\#}$
HPSAE	1.0800±0.18**	4.1883±0.54**	3.6392±0.53$^{\#,\ **}$	0.3217±0.12*
MPSAE	1.2350±0.28$^{\#}$	4.9958±1.05$^{\#}$	4.0775±0.84$^{\#\#}$	0.3667±0.08
LPSAE	1.3767±0.48$^{\#\#}$	5.2767±0.75$^{\#\#}$	4.3092±0.38$^{\#\#}$	0.3758±0.11$^{\#}$
PSP	1.0250±0.11**	4.1317±0.85**	3.4917±0.68**	0.2800±0.09**

Note: Mean ± SD was used for the above data. Through one-way ANOVA among the six groups, compared with the control group, ##p<0.01, #p<0.05; Compared with the model group, **p<0.05, *p<0.01; n=12.

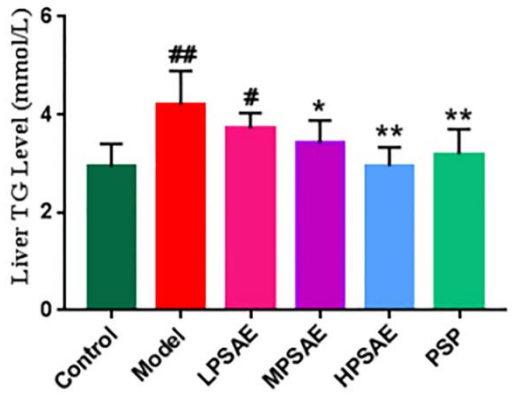

Figure 2. The effect of PSAE on liver triglyceride (TG) level of mice with type 2 diabetic. ##p < .01 versus the Control group; #p < .05 versus the Control group; **p < .01 versus the Model group; *p < .05 versus the Model group

3.3. Effect of PSAE on the Lipid Deposition in the Liver of Mice with Type 2 Diabetes

The results of oil red O staining in the liver of mice in each group are shown in Figure 3 [29]. Compared with the Control group, the red lipid deposition in the oil red O staining section of the liver of mice in the Model group is significantly increased, and red lipid droplets are infiltrated around the cells. There is also red lipid deposition in the results of oil red O staining in the liver of mice in the LPSAE group, MPSAE group, HPSAE group, and PSP group; Compared with the Model group, the lipid deposition of PSAE dose groups showed significant improvement, and this improvement effect strengthened with the increase of dose. The liver tissue morphology of the HPSAE group mice was closer to that of the Control group, and the positive control PSP group mice showed a similar protective effect to that of the HPSAE group. These results showed that PSAE and PSP could reduce the level of liver lipids in mice with type 2 diabetes.

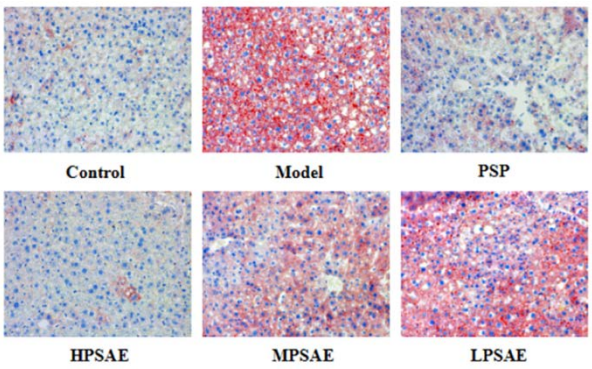

Figure 3. The effect of PSAE on liver lipid accumulation: Oil Red O staining, the red staining is hepatic lipid droplets

3.4. Effect of PSAE on the Structure of Dyslipidemia Mice's Gut Microbiota

3.4.1. Alpha Diversity Analysis

The abundance and diversity of each group sample species can be reflected by alpha diversity, and different indicators have been identified. For general species, its abundance metrics included the Chao1 richness estimator. The Shannon-Wiener diversity index and Simpson diversity index be used to reflect the diversity of the flora. Indexes of Chao1, Shannon, and Simpson were calculated by statistical analysis based on the obtained OTU. Compared with the Control group, the index of Chao1 decreased dramatically in group Model group, PSAEH, PSAEM, and PSPL, while no significant change in groups PSAEL and PSPH (Figure 4a). Besides, the index of Shannon and Simpson in group Model group, PSAEM, PSAEL, PSPH, and PSPL decreased significantly, but no significant difference in group PSAEH was observed (Figure 4b,4c). The index of Chao1, Shannon, and Simpson indexes were no significant change in the group Model. These results indicated that the richness and uniformity of gut microbiota in groups PSAEM, PSAEL, PSPH, and PSPL were dramatically improved. The sample Shannon-Wiene (Figure 5a) and Rank Abundance (Figure 5b) Curve were as follows. The results suggested that the sequencing data were reasonable and could reflect the vast majority of microbial diversity information and the bacterial community structure and diversity in the samples.

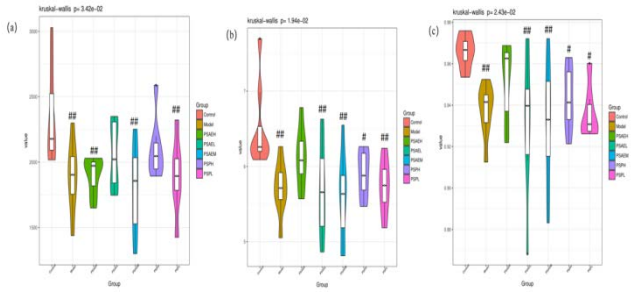

Figure 4. Diversity and richness indexes of intestinal flora in mice from each group. (a)Index of Chao1. (b)Index of Shannon and Simpson. ##p < .01 versus the Control group; #p < .05 versus the Control group

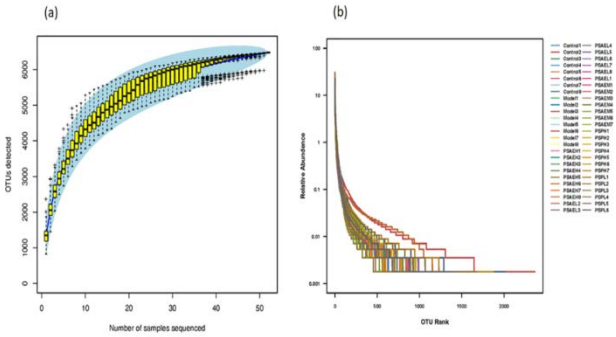

Figure 5. The sample Shannon-Wiene (a) and Rank Abundance (b) Curve

3.4.2. Beta Diversity Analysis

Principal Coordinate Analysis (PCoA) was conducted as the primary index for beta diversity analysis to assess the variation in species diversity among the samples. Notably, the PSAE groups (PSAEH, PSAEM, PSAEL) exhibited complete separation from the other experimental groups. This clear separation indicates a significant dissimilarity in the bacterial flora structure between the PSAE group and the Control group, Model group, and PSPH group. Therefore, our findings confirm that

dyslipidemia has a significant impact on the gut microbiota structure in mice (Figure 6).

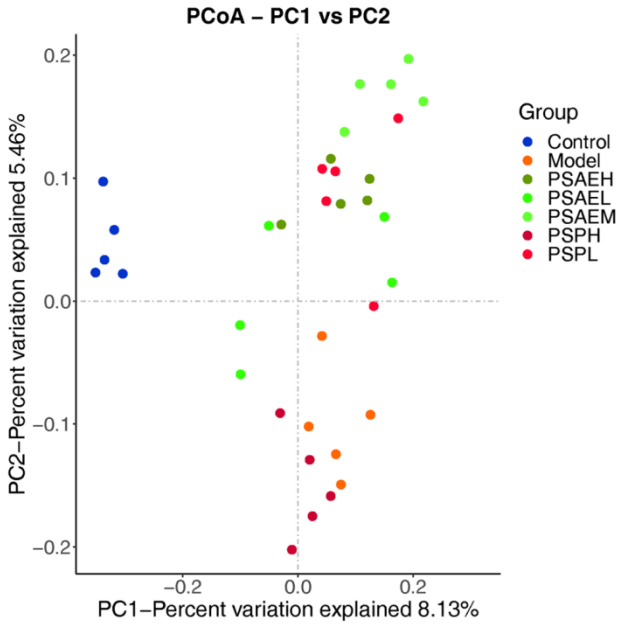

Figure 6. Relative abundance of the bacterial communities at the phylum level

3.4.3. Analysis of the Species Composition of the Gut Microbiota

We analyzed and compared the differences in each group's gut microbiota species structure at phylum and genus levels. The results showed that Bacteroidetes, Firmicutes, and Proteobacteria were the primary in each group's gut microbiota species structure at phylum (Figure 7). Compared with the Control group, the relative abundance of Proteobacteria significantly increased, and the relative abundance of Bacteroidetes and Firmicutes in the Model group decreased, which suggested that the ratio of Bacteroidetes, Firmicutes, and Proteobacteria in mice's intestinal contents after dyslipidemia. Compared with the Model group, the relative abundance of Bacteroidetes and Firmicutes decreased in the PSAEH, PSAEL, and PSPH groups, whereas the relative abundance of Proteobacteria increased. Besides, the relative abundance of Firmicutes and Proteobacteria decreased in the PSAEM and PSPL groups, whereas the relative abundance of Bacteroidetes increased. More importantly, a significant difference was observed between the PSAE groups and the PSP group. The ratio of Firmicutes, Bacteroidetes, and Proteobacteria was an essential marker to represent the status of the gut microbiome. The findings indicated that PSAE and PSP can regulate the relative content and diversity of microorganisms at the phylum level. As described in Figure 8, the top 30 genera in the mouse intestinal flora were analyzed. Among these genera, in the Model group, the relative abundance of Parabacteroides, Bilophila, and Blautia was lower than that in the Control group, whereas that of Bacteroides, Alloprevotella, and Alistipes was higher. Compared with the Model group, the relative abundance of Bilophila, Bacteroidetes and Alloprevotella decreased, whereas the relative abundance of Parabacteroides, and Alistipes increased in the PSAEH, the relative abundance of Bacteroidetes, Parabacteroides and Alistipes increased, whereas the relative abundance of Alloprevotella, Oscillibacter, Lachnoclostridium decreased in the PSAEM and PSPL groups. Besides, the relative abundance of Parabacteroides, Bacteroidetes, and Bilophila decreased, whereas the relative abundance of Alistipes and Helicobacter increased in the PSAEL and PSPH groups. Thus, PSAE and PSP regulate the relative content and diversity of microorganisms at the genus level. The results showed that PSAE and PSP can regulate the relative content and diversity of microorganisms at the genus level.

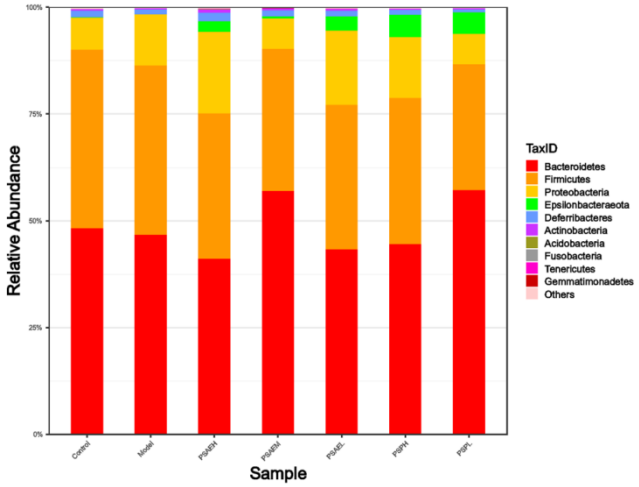

Figure 7. Relative abundance of the bacterial communities at the phylum level

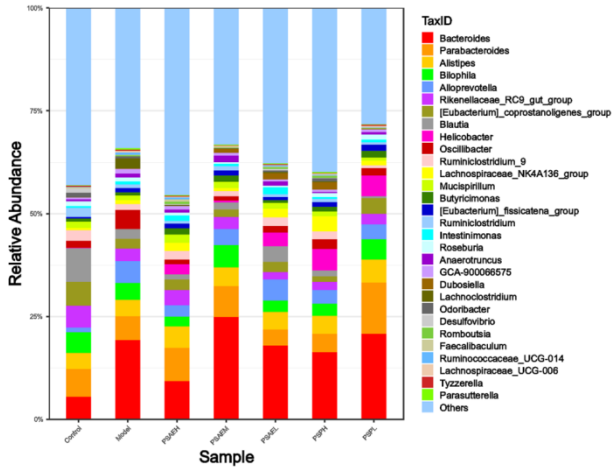

Figure 8. Relative abundance of the bacterial communities at the genus level

3.4.4. Significant Difference Analysis among the Groups

The Line Discriminant Analysis Effect Size (LEfSe) method aims to identify statistically different colony species amongst different groups. Figure 9a shows the bar chart of the taxon LDA distribution of the species with the greatest difference between the Model group and PSAEM group, and the corresponding evolutionary cladistic diagram is shown in Figure 9b. These results suggested that differences were observed in 72 species between the two groups, and statistical differences in the relative abundance of Bacteroidales, Bacteroidetes, Bacteroidia, Butyricimonas, Erysipelotrichia, Erysipelotrichales,

Erysipelotrichaceae, Atopostipes, Anaerotruncus, Tateyamaria, Carnobacteriaceae, uncultured, et al were observed in the PSAEM group. Besides, in Model group, distinct difference in the relative abundance of Lachnospiraceae_FCS020_group, Lactobacillus, Proteus, Lactobacillaceae, Faecalibacterium, Lactococcus, Lactobacillales, uncultured, Ruminococcaceae_UCG_005, Ruminococcaceae_UCG_009, Mycobacteriaceae, Mycobacterium, et al were observed. The relative abundance of Lachnoclostridium and Oscillibacter increased in the model group mice's intestinal flora. The relative abundance of the Bacteroidetes in the gut microbiota of mice increased, which benefited the intervention of medium-dose PSAE, and this result was consistent with the above-mentioned species composition analysis.

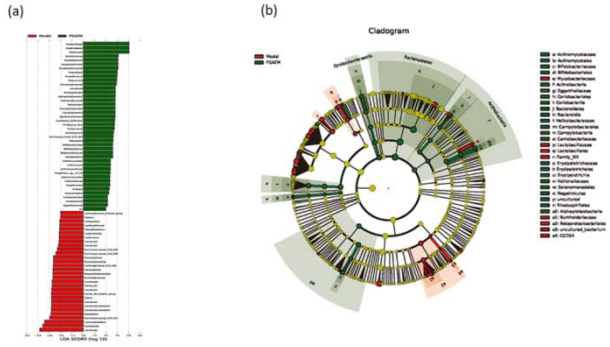

Figure 9. Distribution of LDA value (a) and LEfSe analysis of the evolutionary branch (b)

3.4.5. Prediction of KEGG Functional Genes

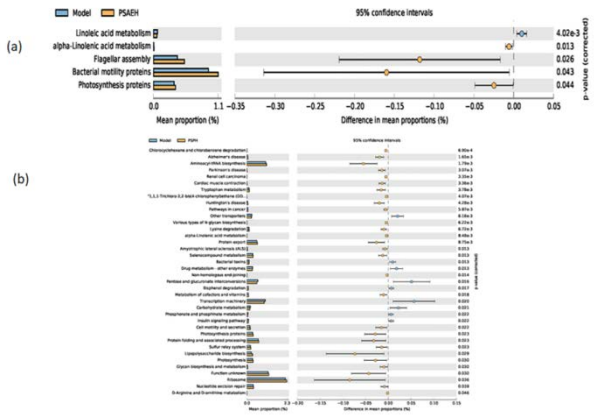

Figure 10. Differential analysis of the KEGG metabolic pathways. (a) Comparing M with PSAEH. (b) Comparing M with PSPH

Metabolic pathway analysis using the KEGG database was employed to identify differences and alterations in functional gene metabolic pathways among the different sample groups. When comparing the Model group with the PSAEH group, five significant differences were observed in the metabolic pathways of the gut microbiota (Figure 100a). Notable variations were found in linoleic acid metabolism, alpha-linolenic acid metabolism, flagellar assembly, bacterial motility proteins, and photosynthesis proteins. Similarly, when comparing the Model group with the PSPH group, significant differences were observed in the metabolic pathways of the gut microbiota, with five metabolic classes showing notable variations (Figure 100b). These differences encompassed ribosome, aminoacyl-tRNA biosynthesis, transcription machinery, protein folding and associated processing, pentose and glucuronate interconversions, and protein export. Based on these results, it can be inferred that the changes in the flora structure induced by Polygonatum sibiricum aqueous extract may impact linoleic acid metabolism, alpha-linolenic acid metabolism, and bacterial motility proteins. Additionally, the changes in the flora structure induced by Polygonatum sibiricum polysaccharides may affect ribosome, aminoacyl-tRNA biosynthesis, transcription machinery, protein folding and associated processing, pentose and glucuronate interconversions, and protein export.

4. Discussion

Traditional Chinese medicine was used to control the type and quantity of dominant bacteria and alter the gut microbiota's general function, which in turn prevented the body from absorbing and using chemicals and negatively impacted human health. Polysaccharides widely exist in natural plants, which are difficult to directly digest and absorbed by the human body but have a positive regulatory effect on the intestinal flora. They could control how the gut microbiome functions to treat various disorders. For example, a study found that the mice fed with a monomeric polysaccharide from Polygonatum sibiricum (PSP-1)’s 16s rRNA results showed that the PSP-1 reconstructed the gut microbiota composition, including reducing the relative abundance of Helicobacter and increasing Ackermann’s muciniphila. It indicates that PSP-1 improves cognitive functions in a model of Alzheimer's disease by reshaping the gut microbiota [30]. According to the research [31], a polysaccharide from bamboo (Phyllostachys edulis) shoot significantly reduced colonic pathological damage, inhibited the activation of inflammatory signaling pathways, and increased Prevotella, Alitipes, Anaerostipes, Odoribacter, Bifidobacterium, Butyricimonas, and Lactobacillus levels to alleviate the dextran sulfate sodium (DSS)-induced colitis mouse model. Besides, the structure of gut microbiota was dramatically changed by APS, and 13 bacteria (such as c_Bacteroidia, p_Bacteroidetes, and g_Allpprevotella) could serve as biomarkers for APS-improved osteoporosis. Five genera (uncultured_bacterium_f_Ruminococcaceae, Alloprevotella, Ruminococcaceae_UCG-014, Blautia, and Lactobacillus) were inferred as the key bacteria in APS-improved osteoporosis [32].

According to linked research studies, the gut microbiota will vary in accordance with the body's age and metabolic illnesses [33,34,35]. According to this study [36], the TG levels in the metformin group and NSSP low-dose group decreased significantly ($P < 0.01$), while the TG levels in the NSSP high-dose group and NSSP medium-dose group decreased significantly ($P < 0.001$) compared with the STZ model group. At the phylum level, the TG levels in the NSSP high-dose group positively correlated with the abundance of Firmicutes and negatively correlated with Lachnospiraceae_NK4A136_group and f_Lachnospiraceae_Unclassified compared with the model group in the genus level. The SOD levels in the

metformin group, NSSP high-dose group and NSSP and medium-dose group were significantly increased compared with STZ model and positively correlated with the abundance of Lachnospiraceae_NK4A136_group and f_Lachnospiraceae_Unclassified and negatively correlated with the abundance of Firmicutes in the NSSP high-dose group. These modifications showed a relationship between the gut microbiota's composition and the in vivo state of blood lipid metabolism.

At the phylum level in this study, the predominant bacterial phyla found in the flora structure of each group were Bacteroidetes, Firmicutes, and Proteobacteria. Compared to the Control group, a significant increase in the relative abundance of Proteobacteria was observed in the Model group, while the relative abundance of Bacteroidetes and Firmicutes decreased. These findings indicate that dyslipidemia led to a change in the ratio of Bacteroidetes, Firmicutes, and Proteobacteria in the intestinal contents of the mice. This alteration aligns with the impact on in vivo lipid metabolism activities. At the genus level, a notable decrease in the relative abundance of beneficial bacteria (such as Parabacteroides, Bilophila, and Blautia) was observed in the gut microbiota of the Model group. Conversely, the abundance of harmful bacteria, specifically Bacteroides, Alloprevotella, and Alistipes, showed a significant increase. However, PSAE treatment reversed some of these modifications. The intervention with PSAEH, PSAEM, and PSAEL groups led to a reversal in the relative abundance of harmful bacteria (Bacteroides and Alloprevotella). Interestingly, previous studies have reported an increase in the relative abundance of Alloprevotella in mice with Alzheimer's disease and those fed a high-fat diet [37,38]. These findings collectively highlight the impact of dyslipidemia and dietary interventions on the relative abundance of specific bacterial taxa, including both beneficial and harmful bacteria, within the gut microbiota.

Dyslipidemia is mainly manifested as hyperlipidemia, and hypertriglyceridemia and hypercholesterolemia are the most common, which leads to the increased activities of TG, TC, LDL-C, HDL-C, and other biomarker of dyslipidemia in the body. A considerable rise in harmful bacteria is caused by hyperlipidemia and hypertriglyceridemia, and a decrease in the number of good bacteria may result in an imbalance of the intestinal flora.

The addition of PSAE to the diet may help to reduce dyslipidemia and repair damaged gut flora. However, a greater study is necessary to fully understand their underlying mechanisms of action, which appear to be complicated.

5. Conclusion

This study provides evidence that PSAE offers protective effects against blood lipid metabolism disorders. PSAE was shown to decrease the abundance of Bacteroidetes, Bilophila, and Alloprevotella bacteria, while increasing the abundance of Parabacteroides and Alistipes bacteria in T2DM mice. These changes in bacterial composition contributed to a reduction in blood triglyceride (TG) levels and other biomarkers, thereby alleviating dyslipidemia and insulin resistance. From the perspective of the gut microbiota, this study systematically elucidated the hypoglycemic mechanism of PSAE. The findings present a new possibility for effectively preventing and treating diabetes through dietary interventions and also enhance the market value of PSAE.

Acknowledgements

This study was supported by Anhui Academician Workstation of Polygonatum cyrtonema Hua in Jiuhua Mountain Keeping in Good Health Industry Research Institute in Qingyang (JHHJYSGZZ19001).

Statement of Competing Interests

The authors have no competing interests.

References

[1] Zhao, C., Yang, C., Wai, S. T. C., Zhang, Y., M, P. P., Paoli, P., Wu, Y., San Cheang, W., Liu, B., Carpéné, C., Xiao, J., Cao, H., "Regulation of glucose metabolism by bioactive phytochemicals for the management of type 2 diabetes mellitus," *Crit Rev Food Sci Nutr*, 59(6). 830-847. 2019.

[2] Saeedi, P., Petersohn, I., Salpea, P., Malanda, B., Karuranga, S., Unwin, N., Colagiuri, S., Guariguata, L., Motala, A. A., Ogurtsova, K., Shaw, J. E., Bright, D., Williams, R., "Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9(th) edition," *Diabetes Res Clin Pract*, 157. 107843. Nov. 2019.

[3] Zhang, L., Chu, J., Hao, W., Zhang, J., Li, H., Yang, C., Yang, J., Chen, X., Wang, H., "Gut Microbiota and Type 2 Diabetes Mellitus: Association, Mechanism, and Translational Applications," *Mediators Inflamm*, 5110276. Aug. 2021.

[4] Hu, X., Wang, S., Xu, J., Wang, D. B., Chen, Y., Yang, G. Z., "Triterpenoid saponins from Stauntonia chinensis ameliorate insulin resistance via the AMP-activated protein kinase and IR/IRS-1/PI3K/Akt pathways in insulin-resistant HepG2 cells," *Int J Mol Sci*, 15(6). 10446-10458. Jun. 2014.

[5] Wang, J., He, Y., Yu, D., Jin, L., Gong, X., Zhang, B., "Perilla oil regulates intestinal microbiota and alleviates insulin resistance through the PI3K/AKT signaling pathway in type-2 diabetic KKAy mice," *Food Chem Toxicol*, 135. 110965. Jan. 2020.

[6] Chen, T., Zhang, Y., Liu, Y., Zhu, D., Yu, J., Li, G., Sun, Z., Wang, W., Jiang, H., Hong, Z., "MiR-27a promotes insulin resistance and mediates glucose metabolism by targeting PPAR-γ-mediated PI3K/AKT signaling," *Aging (Albany NY)*, 11(18). 7510-7524. Sep. 2019.

[7] Chen, S. H., Liu, X. N., Peng, Y., "MicroRNA-351 eases insulin resistance and liver gluconeogenesis via the PI3K/AKT pathway by inhibiting FLOT2 in mice of gestational diabetes mellitus," *J Cell Mol Med*, 23(9). 5895-5906. Sep. 2019.

[8] Huang, X., Liu, G., Guo, J., Su, Z., "The PI3K/AKT pathway in obesity and type 2 diabetes," *Int J Biol Sci*, 14(11). 1483-1496. Aug. 2018.

[9] McCarthy, M. I., "Genetics of T2DM in 2016: Biological and translational insights from T2DM genetics," *Nat Rev Endocrinol*, 13(2). 71-72. Feb. 2017.

[10] Weng, J. P., Hu, G., "Diabetes: Leveraging the Tipping Point of the Diabetes Pandemic," *Diabetes*, 66(6). 1461-1463. Jun. 2017.

[11] Sampath Kumar, A., Maiya, A. G., Shastry, B. A., Vaishali, K., Ravishankar, N., Hazari, A., Gundmi, S., Jadhav, R., "Exercise and insulin resistance in type 2 diabetes mellitus: A systematic review and meta-analysis," *Ann Phys Rehabil Med*, 62(2). 98-103. Mar. 2019.

[12] Tanase, D. M., Gosav, E. M., Neculae, E., Costea, C. F., Ciocoiu, M., Hurjui, L. L., Tarniceriu, C. C., Maranduca, M. A., Lacatusu,

C. M., Floria, M., Serban, I. L., "Role of Gut Microbiota on Onset and Progression of Microvascular Complications of Type 2 Diabetes (T2DM)," *Nutrients*, 12(12). Dec. 2020.

[13] Zhang, H., Cao, Y., Chen, L., Wang, J., Tian, Q., Wang, N., Liu, Z., Li, J., Wang, N., Wang, X., Sun, P., Wang, L., "A polysaccharide from Polygonatum sibiricum attenuates amyloid-β-induced neurotoxicity in PC12 cells," *Carbohydr Polym*, 117. 879-886. Mar. 2015.

[14] Yelithao, K., Surayot, U., Lee, J. H., You, S., "RAW264.7 Cell Activating Glucomannans Extracted from Rhizome of Polygonatum sibiricum," *Prev Nutr Food Sci*, 21(3). 245-254. Sep. 2016.

[15] Zhao, P., Zhao, C., Li, X., Gao, Q., Huang, L., Xiao, P., Gao, W., "The genus Polygonatum: A review of ethnopharmacology, phytochemistry and pharmacology," *J Ethnopharmacol*, 214. 274-291. Mar. 2018.

[16] Liu, X., Yi, H., Yao, L., Ma, H., Zhang, J., Wang, Z., "Advances in plants of Polygonatum and discussion of its development prospects," *Chin. Pharm*, 52. 530-534. 2017.

[17] Li, L., Tian, L., Ren, Z., Long, Z., "Research progress on the structural analysis and functional activity of polysaccharides," *Chin. J. Exp. Tradit. Med. Fromul*, 21(15). 231-234. 2015.

[18] Liu, N., Dong, Z., Zhu, X., Xu, H., Zhao, Z., "Characterization and protective effect of Polygonatum sibiricum polysaccharide against cyclophosphamide-induced immunosuppression in Balb/c mice," *Int J Biol Macromol*, 107(Pt A). 796-802. Feb. 2018.

[19] Zhao, X., Li, J., "Chemical constituents of the genus Polygonatum and their role in medicinal treatment," *Nat Prod Commun*, 10(4). 683-688. Apr. 2015.

[20] Shi, X., Li, P., Wei, Y., Li, B., Wang, D., Xu, T., "Research progress on extraction and purifification technologies and pharmacological effects of Polygonatum polysaccharide: A review," *Guid. J. Tradit. Chin. Med. Pharm*, 21. 103-105. 2015.

[21] Zhang, H. Y., Hu, W. C., Ma, G. X., Zhu, N. L., Sun, X. B., Wu, H. F., Xu, X. D., "A new steroidal saponin from Polygonatum sibiricum," *J Asian Nat Prod Res*, 20(6). 586-592. Jun. 2018.

[22] Zong, S., Zeng, G., Zou, B., Li, K., Fang, Y., Lu, L., Xiao, D., Zhang, Z., "Effects of Polygonatum sibiricum polysaccharide on the osteogenic differentiation of bone mesenchymal stem cells in mice," *Int J Clin Exp Pathol*, 8(6). 6169-6180. Jun. 2015.

[23] Chen, H., Li, Y. J., Li, X. F., Sun, Y. J., Li, H. W., Su, F. Y., Cao, Y. G., Zhang, Y. L., Zheng, X. K., Feng, W. S., "Homoisoflavanones with estrogenic activity from the rhizomes of Polygonatum sibiricum," *J Asian Nat Prod Res*, 20(1). 92-100. Jan. 2018.

[24] Hu, C. Y., Xu, D. P., Wu, Y. M., Ou, S. Y., "Triterpenoid saponins from the rhizome of Polygonatum sibiricum," *J Asian Nat Prod Res*, 12(9). 801-808. Sep. 2010.

[25] Zhai, L., Wang, X., "Syringaresinol-di-O-β-D-glucoside, a phenolic compound from Polygonatum sibiricum, exhibits an antidiabetic and antioxidative effect on a streptozotocin‐induced mouse model of diabetes," *Mol Med Rep*, 18(6). 5511-5519. Dec. 2018.

[26] Kato, A., Miura, T., "Hypoglycemic action of the rhizomes of Polygonatum officinale in normal and diabetic mice," *Planta Med*, 60(3). 201-203. Jun. 1994.

[27] Kato, A., Miura, T., "Hypoglycemic activity of polygonati rhizoma in normal and diabetic mice," *Biol Pharm Bull*, 16(11). 1118-1120. Nov. 1993.

[28] Wang, G., Fu, Y., Li, J., Li, Y., Zhao, Q., Hu, A., Xu, C., Shao, D., Chen, W., "Aqueous extract of Polygonatum sibiricum ameliorates ethanol-induced mice liver injury via regulation of the Nrf2/ARE pathway," *J Food Biochem*, 45(1). e13537. Jan. 2021.

[29] Wang, G., Liu, Z., Liang, D., Yu, J., Wang, T., Zhou, F., Chen, W., "Aqueous extract of Polygonatum sibiricum ameliorates glucose and lipid metabolism via PI3K/AKT signaling pathway in high-fat diet and streptozotocin-induced diabetic mice," *J Food Biochem*, 46(12). e14402. Dec. 2022.

[30] Luo, S., Zhang, X., Huang, S., Feng, X., Zhang, X., Xiang, D., "A monomeric polysaccharide from Polygonatum sibiricum improves cognitive functions in a model of Alzheimer's disease by reshaping the gut microbiota," *Int J Biol Macromol*, 213. 404-415. Jul. 2022.

[31] Li, Q., Wu, W., Fang, X., Chen, H., Han, Y., Liu, R., Niu, B., Gao, H., "Structural characterization of a polysaccharide from bamboo (Phyllostachys edulis) shoot and its prevention effect on colitis mouse," *Food Chem*, 387. 132807. Sep. 2022.

[32] Liu, J., Liu, J., Liu, L., Zhang, G., Zhou, A., Peng, X., "The gut microbiota alteration and the key bacteria in Astragalus polysaccharides (APS)-improved osteoporosis," *Food Res Int*, 138(Pt B). 109811. Dec. 2020.

[33] Liang, F., Lu, X., Deng, Z., Zhong, H. J., Zhang, W., Li, Q., Zhou, H. H., Liou, Y. L., He, X. X., "Effect of Washed Microbiota Transplantation on Patients With Dyslipidemia in South China," *Front Endocrinol (Lausanne)*, 13. 827107. Apr. 2022.

[34] Ling, Z., Liu, X., Cheng, Y., Yan, X., Wu, S., "Gut microbiota and aging," *Crit Rev Food Sci Nutr*, 62(13). 3509-3534. 2022.

[35] Conway, J., N, A. D., "Ageing of the gut microbiome: Potential influences on immune senescence and inflammageing," *Ageing Res Rev*, 68. 101323. Jul. 2021.

[36] Dong, J., Liang, Q., Niu, Y., Jiang, S., Zhou, L., Wang, J., Ma, C., Kang, W., "Effects of Nigella sativa seed polysaccharides on type 2 diabetic mice and gut microbiota," *Int J Biol Macromol*, 159. 725-738. Sep. 2020.

[37] Zhao, D., Cao, J., Jin, H., Shan, Y., Fang, J., Liu, F., "Beneficial impacts of fermented celery (Apium graveolens L.) juice on obesity prevention and gut microbiota modulation in high-fat diet fed mice," *Food Funct*, 12(19). 9151-9164. Oct. 2021.

[38] Liu, J., Ye, T., Zhang, Y., Zhang, R., Kong, Y., Zhang, Y., Sun, J., "Protective Effect of Ginkgolide B against Cognitive Impairment in Mice via Regulation of Gut Microbiota," *J Agric Food Chem*, 69(41). 12230-12240. Oct. 2021.