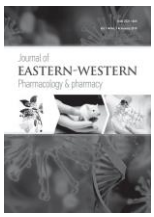

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RESEARCH ON THE MECHANISM OF DIGEDA-4'S ANTIPYRETIC EFFECT ON INTESTINAL FLORA BASED ON MONGOLIAN MEDICINE ELIMINATION OF XILA DISEASE

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KEYWORDS

Mongolian medicine, Digeda-4, Xila disease, Intestinal flora, Lipopolysaccharide

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ABSTRACT

Background: Through research, the correlation between Xila disease and intestinal flora is analyzed. Using the diversity analysis of intestinal flora to explain the modern scientific connotation of the theory of Mongolian medicine to eliminate Xila disease. Use modern research methods to further explain the antipyretic and liver protection mechanism of Digeda-4.

Methods: Xijie et al. reported that rats were given sunflower oil to induce Xila disease. Xila disease is classified as a "heat" transmitted disease. Therefore, animal experiments often use the injection of "*lipopolysaccharide*" and "10% yeast" as fever models. 16s rRNA sequencing technology was used to analyze the diversity of fecal flora in rats to observe the effect of the pylori-ligated liver injury model on the diversity of intestinal flora.

Results: The effect of Digeda-4 antipyretic for Xila disease on the body temperature of rats was compared with that of the normal group. The body temperature of the rats in the experimental group increased significantly ($P<0.05$); compared with the experimental group, the body temperature of the rats in the drug-administered group increased. The body temperature increased significantly in the 1st, 3rd, and 6th hour of drug administered group ($P<0.05$). A total of 22 species were identified that were significantly correlated with body temperature (1 was positively correlated with body temperature, and 2 were negatively correlated with body temperature). The positively correlated strain is Rikenellceae_ RC9_gut_group; the negatively correlated strains are Anaerovibrio and Unclassified. Among them, two

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bacterial species, Rikenellceae_RC9_gut_group and Anaerovibrio, are closely related to inflammatory factors. Compared with the normal group, the relative abundance of Rikenbacteriaceae_RC9 intestinal group in the experimental group was significantly increased (P<0.05), and the relative abundance of Unclassified was significantly decreased (P<0.05); the relative abundance of Anaerovibrio The abundance increased significantly (P<0. 05).

1. INTRODUCTION

Xila is one of the "three causes of disease - Heyi, Xila, and Badagan" of Mongolian medicine. Xila is a disease in which, due to some external and internal conditions, xila loses its relative balance, increases, decreases, or worsens and poisons the body. Among them, other diseases are due to overeating hot, sour, salty, and greasy foods that are not easy to digest; excessive use of "four fires" (that is, food, daily life, medicine, and external products are all heat) can cause Xila's disease.

Symptoms of Xila lesions mainly show the characteristic symptoms of Xila excess. For example, the clinical symptoms include high fever, easy hunger and thirst, easy suppuration, rapid onset, headache, dry nose, yellowish face and greasy appearance, strong smell of sweat, and abdominal pain. Soft, prone to diarrhea, excessive sweating, red-yellow urine, strong odor, etc. Although the disease spreads throughout the body and shows its unique symptoms, it mainly damages the liver, gallbladder, large intestine, blood, sweat, eyes, skin, and other parts and organs. Analyzing from the perspective of the organs, the liver is located in the right upper abdomen, under the transverse diaphragm. It is the general position of "Xila", the home of "Discolored Xila (one of the five types of Xila)", and it is also the path of "disease Xila". The large intestine is also the general location of "Xila" and the path for "diseased Xila". Xila has seven properties, including heat and sharpness, including lightness, odor, diarrhea, dampness, and greasiness. Therefore, Xila disease belongs to the category of "febrile disease", and it is treated with the principle of removing fever of the blood focusing on febrile disease in the treatment. The Digeda-4 is one of the drugs commonly used for eliminating Xila's disease[1]. The main method used is purgative therapy, which uses drugs to stimulate the digestive tract, facilitate defecation, cleanse the gastrointestinal tract, eliminate stagnation, and promote digestive function. Digeda-4 Decoction, is composed of 4 medicinal herbs such as Listigma (also known as Digeda), Huhuanglian, Gardenia, and Qumai Listiflora is the main drug; HuHuanglian and Gardenia are complementary drugs, Qumai as adjuvant drug[2]. It has the functions of eliminating "Xila", cooling blood, regulating badness, and returning essence. It is mainly used for various diseases such as blood heat fighting, liver and gallbladder heat, sore throat, thirst, and irritability[3]. According to modern research results, it is shown that Sternia japonica contains swertomacroside, which has the effect of protecting the liver[4]; Huhuanglian and Gardenia contain cycloene glucoside, which has the effect of purging[5]; Large intestine and other pharmacological effects. This paper reviewed Digeda-4 Decoction from the aspects of historical description, prescription principle, chemical components, pharmacology, clinical application, and quality control.

2. MATERIALS AND METHODS

2.1. Reagents

Pusion Hot start flex 2X Master Mix (Shanghai Yitao Biological Instrument Co., Ltd. M0536L), DL2000 DNA Maker (Takara 3427A), Gene color (Beijing Jinboyi GBY-1), Qubit dsDNA HS Assay Kit (500 times) (Invitrogen, Life technologies Q32854), Biowest Agarose G-10 (BIOWEST 111860), Sangon 50×TAE Buffer (Shanghai Sangon B548101-500), AMPure XT beads (Beckman A63880)[11].

Include normal temperature centrifuge (Eppendorf Centrifuge 5424), refrigerated centrifuge (Microfuge R 22R Centrifuge), vortex shaker Hualida (WH-861 Vortex Shaker), three-temperature and three-control constant temperature water bath (Shanghai Boxun Industrial Co., Ltd. DK -8D), PCR instrument (Hangzhou Langi Scientific Instrument Co., Ltd. A200 gene amplification instrument), electrophoresis

instrument (Tanon Tianneng R EPS300), gel imager (Tanon-2500), ultra-low temperature freezing storage box (Zhongke Meiling Low Temperature Technology Co., Ltd. DW-HL388), fully automatic sample rapid grinder-96L (Shanghai Jingxin JXFSTPRP-96)[12].

2.2. Experimental drugs

Digeda-4 flavor soup: It is a dark brown powder, bitter, and slightly astringent. Usage and dosage: Oral, 3-5g per time for adults, 1-3 times a day. Based on a body weight of 70kg, the maximum daily dosage for adults is 0.25 g\kg\d. Prescription composition: Listigma 250g, Huhuanglian 250g, Gardenia 250g, Qumai 250g. Functions and Indications: It has the effects of removing "Xila" heat, cooling blood, removing dregs and returning essence. It is mainly used to treat blood-heat conflict, liver and gallbladder heat, thirst and irritability, and sore throat[6]. During the experiment, distilled water was used to prepare 3.9 g\kg\d for the high-dose group, 2.6 g\kg\d for the medium-dose group, and 1.3 g\kg\d for the low-dose group, respectively equivalent to 10 and 5 times the clinical dosage for adults. The dosage volume is 10ml\kg.

2.3. Animals

SPF grade SD male rats, 60 rats, body weight (180-220g), provided by Liaoning Changsheng Biotechnology Co., Ltd. (Production License Number: SCXK (Liao)-2020-0001). The laboratory experiment started 5 days before the formal experiment and ended in the animal room of the Mongolian Medicine College of Inner Mongolia University for Nationalities (SPF level, room temperature 22°C-24°C, relative humidity 50-60%, day and night light and dark alternating time: 10-12h)[7].

2.4. Method

2.4.1. Xijie et al. reported that rats were given sunflower oil to induce Xila disease

Xila disease is classified as a "heat" sexually transmitted disease. Therefore, animal experiments often use injections of "*lipopolysaccharide*" and "10% yeast" as fever models. Take 60 SD rats, weighing 180-220g, male, divided into normal group, experimental group, positive control group, and Digeda-4 (high, medium, and low dose) groups, with 10 rats in each group. After adapting to the experimental environment for 2-4 days, the remaining rats except the normal group were given high-concentration spicy vegetables by gavage once a day for a total of 14 days. After the last gavage, the remaining rats except the model were given *lipopolysaccharide* (LPS). Starting from the 5th day of modeling, each group was given intragastric administration of 0.5% CMC-Na. The normal and model groups were given intragastric administration of 0.5% CMC-Na. Each administration group was intragastrically administered with the corresponding dose of Digoda-4 0.5% CMC-Na suspension, 1 dose per day times, giving a total of 9d. After the last dose, anesthetized after taking blood from the abdominal aorta (separate the serum, and store at -80°C), release the liver (one part is fixed in 10% formaldehyde, the other part is quick-frozen and stored at -80° for later use), and the hypothalamus is released (stored at -80°C).), collect feces (stored at -80°)[8].

16s rRNA sequencing technology was used to analyze the diversity of fecal flora in rats to observe the effect of the pylori-ligated liver injury model on the diversity of intestinal flora.

2.4.2. Rat feces DNA 16S rRNA sequencing and quality control After the last administration, fingertip massage was used to promote defecation in the rats. Fresh feces from rats in each group were collected, placed in 2 mL sterile cryopreservation tubes, and immediately Freeze in liquid nitrogen, then transferred to a -80°C refrigerator for storage and use. Use a DNA extraction kit to extract the genomic DNA of the sample, and then use agarose gel electrophoresis to detect the purity and concentration of the DNA. Using the diluted genomic DNA as a template, use bacterial 16S V3+V4 region primers: 338F: 5'- ACTCCTACGGAGGCAGCA-3'806R: 5'- GGACTACHVGGGTWTCTAAT-3' for PCR purification and amplification. The CTAB method

was selected to extract total microbiome DNA from microbiome samples from various sources, and the DNA extraction quality was detected by agarose gel electrophoresis, and a UV spectrophotometer was used to quantify the DNA. The purified PCR products were evaluated using an Agilent 2100 bioanalyzer (Agilent, USA) and Illumina (Kapa Biosciences, Woburn, MA, USA) library quantification kit. The qualified library concentration should be above 2nM. After gradient dilution of each qualified on-machine sequencing library (Index sequence cannot be repeated), mix in corresponding proportions according to the required sequencing amount, and denature with NaOH into single strands for on-machine sequencing; use NovaSeq 6000 sequencer for 2×250bp For paired-end sequencing, the corresponding reagent is NovaSeq 6000 SP Reagent Kit (500 cycles)[9].

2.4.3. Diversity analysis of rat intestinal flora.

Use the obtained ASV (feature) feature sequence and ASV (feature) abundance table to conduct alpha diversity analysis and beta diversity analysis. Among them, alpha diversity analysis mainly evaluates the diversity within the habitat through seven major indices: observed_ species, shannon, simpson, chao1, goods_coverage, and pielou_e. Beta diversity analysis usually starts by calculating the distance matrix between environmental samples, which contains the distance between any two samples, mainly through principal component analysis (PCA), principal coordinates analysis (PCoA), Clustering analysis (UPGMA), non-multidimensional scaling (NMDS), analysis of similarities (ANOSIM), multivariate analysis of variance (PermANOVA, also known as Adonis) and other methods to observe the differences between samples difference. Use QIIME software to perform Beta diversity analysis to compare the degree of dependence of different samples in terms of species diversity. According to the ASV (feature) sequence file, the SILVA (Release 138, https://www.arbsilva.de/documentation/release138/) database is used to annotate species with the NT-16S database, and each species is classified according to the ASV (feature) abundance table. The abundance in each sample was calculated[10]. The confidence threshold for annotations is: 0.7

2.4.4. Statistical analysis

Fisher's exact test is used to compare the differences between samples without biological replicates; Mann-Whitney U test is used to compare the differences between two groups of samples with biological replicates; Kruskal-Wallis test is used to compare samples with biological replicates. Comparison between multiple groups[13]. Screening threshold: $p<0.05$

3. RESULTS

3.1. The effect of Digeda-4 antipyretic for Xila disease

The body temperature of rats was compared with the normal group. The body temperature of the rats in the experimental group increased significantly ($P<0.05$); compared with the experimental group, the rats in the drug administrated group The increase in body temperature was reduced, and the increase in body temperature at the 1st, 3rd, and 6th hour of drug administrated group was significantly reduced ($P<0.05$).

Table 1. Effect of Digeda-4 antipyretic for Xila disease on body temperature in rats (x ± s, n = 10)

Group	Before modeling	1h	3h	4h	6h
ZZ	34.57	37.30 ± 2.73	37.30 ± 0.00	37.30 ± 0.01	
MZ	36.48	37.32 ± 0.84 ∗	37.83 ± 0.52 ∗	37.93 ± 0.10	36.69 ± 1.25 ∗
YZ	36.34	37.59 ± 1.29 ∗	37.68 ± 0.10	37.68 ± 0.01	37.24 ± 0.44
DGZ	36.42	37.48 ± 1.07 ∗	37.69 ± 0.21	37.78 ± 0.08	37.81 ± 0.08
DZZ	35.95	37.79 ± 1.84 ∗	37.59 ± 0.20	37.74 ± 0.15	38.55 ± 0.81 ∗
DDZ	35.95	37.41 ± 1.46 ∗	37.61 ± 0.19	37.71 ± 0.10	38.31 ± 0.60

Compared with the experimental group ∗ P<0. 05 (the same below).

3.2. Effect of Digeda-4 on antipyretic intestinal flora in patients with Xila disease

ASV distribution Venn diagram: According to the obtained ASV abundance table, the Venn diagram visually presents the total number of ASVs of 1.336.641 and the unique ASV number of 5175 for each sample/group. The next step of the experiment is the number of ASVs and the ASV feature sequence after denoising by QIIME2. The result is 5175 ASV numbers. As can be seen in Figure 1, the number of unique ASVs in the normal group, experimental group, and drug-administrated group is 5175. The number of unique ASVs in each group/sample are: normal group (700), experimental group (1177), drug administrated group (1259). The number of ASVs shared by the two groups/samples of the normal group and the experimental group is 267, the number of ASVs shared by the two groups/samples of the normal group and the drug administrated group is 223, and the number of ASVs shared by the two groups/samples of the experimental group and the drug administrated group 457, the number of ASVs shared by the three groups/samples is 1092. Note: Each circle in venn Figure 1 represents a sample/group. The number of overlaps between circles and circles represents the number of ASVs common to the two groups/samples. The non-overlapping portion represents the number of ASVs unique to each group/sample.

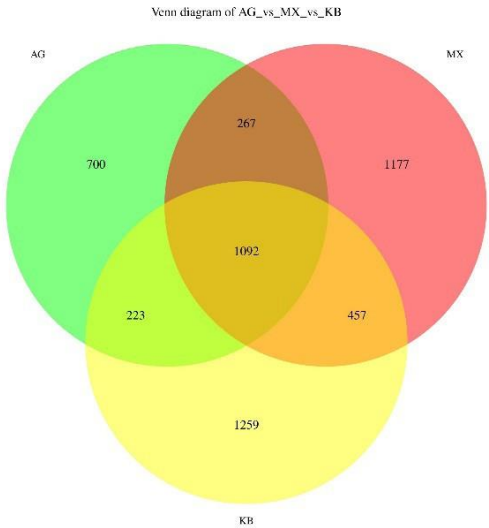

Figure 1. Venn diagram AG-normal group，MX-experimental group，KB-drug administrated group

3.3. Alpha diversity analysis

Alpha diversity refers to the diversity within a specific environment or ecosystem and is mainly used to reflect species richness and evenness as well as sequencing depth. Alpha diversity mainly reflects richness and evenness through indices such as Chao1, Observed species, Goods_coverage, Shannon, Simpson, and pielou-e. The dilution curve (rarefaction curve) simulates the resampling process, observes the trend of species changes, and estimates the species richness in the environment. By drawing dilution curves, counting the abundance of ASV, and comparing the dilution curves of different samples, we can visually display the differences in species diversity between samples. Whether the amount of sequencing data is sufficient can also be estimated from the dilution curve, which is a reference indicator. If the curve rises sharply, it indicates that the amount of sequencing data has not reached the optimal level; if the curve tends to be flat, it indicates that the amount of sequencing data has been saturated. On the contrary, the sequencing data of the table sample is sufficient and data analysis can be performed. Use Mothur software and R language tools to plot the Shannon index (an index that reflects the microbial diversity in the sample) based on the sequencing amount of each sample at different sequencing depths. The Shannon index is derived from information entropy. The larger the Shannon index, the greater the uncertainty. The results are shown in Figures 2 below: Each curve represents a sample and the richness and diversity of each sample, marked with different colors. The larger the Shannon index, the greater the uncertainty[14]. The greater the uncertainty, the greater the uncertainty. The more unknown factors in the community, the higher the diversity.

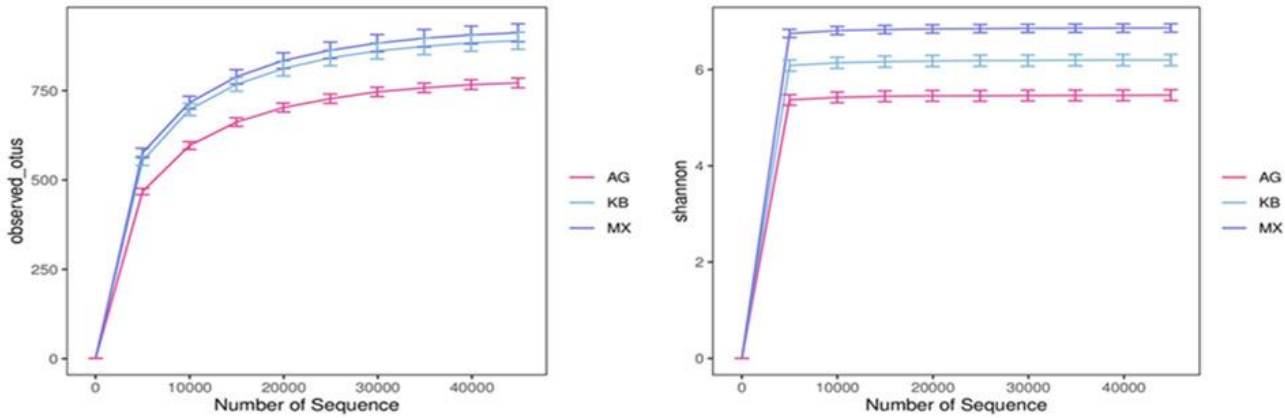

Figure 2. Dilution curve (rarefaction curve), Shannon Index curve (Shannon Index), AG-normal group, MX-experimental group, KB-drug administrated group.

3.4. Beta diversity analysis

Beta diversity refers to species differences between different environmental communities. Beta diversity and alpha diversity together constitute the overall diversity or the biological heterogeneity of a certain environmental community. Beta diversity analysis mainly uses four algorithms, including binary jaccard, bray curtis, weighted unifrag, and unweighted unifrag, to calculate the distance between samples to obtain the Beta value between samples. The calculation of the Beta diversity index PCoA is shown in the figure. PCoA1 as the first principal component can explain 22.6% of the difference between groups, and PCoA2 as the second principal component can explain 16.05% of the difference between groups. ANOSIM For similarity analysis between groups, the calculation results show R= 0. 10, P= 0. 001.

Note: Different colors represent different groups. The closer the samples are, the more similar the microbial composition structure between the samples is and the smaller the difference. The percentages on the horizontal and vertical coordinates represent the degree to which the first and second axes explain the sample differences.

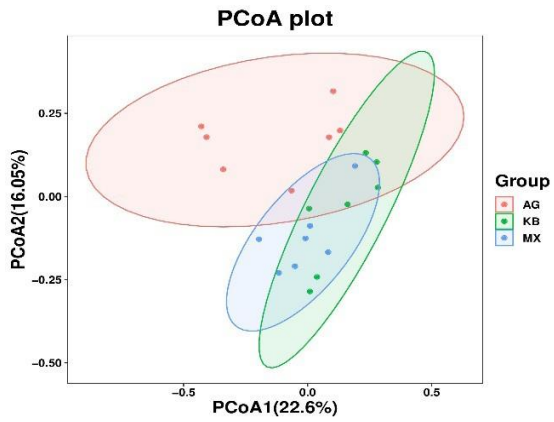

Figure 3. PCoA 2D (weighted_unifrac_pcoa graphic from left to right), AG-normal group, MX-experimental group, KB-drug administrated group

3.4.A total of 22 species were identified that were significantly correlated with body temperature (1 was positively correlated with body temperature, 2 were negatively correlated with body temperature).

The positively correlated strains are Rikenellceae_RC9_gut_group; the negatively correlated strains are Anaerovibrio and Unclassified. Among them, two bacterial species, Rikenellceae_RC9_gut_group and Anaerovibrio, are closely related to inflammatory factors. Compared with the normal group, the relative abundance of Rikenbacteriaceae_RC9 intestinal group in the model group was significantly increased ($P<0.05$), and the relative abundance of Unclassified was significantly decreased ($P<0.05$); the relative abundance of Anaerovibrio The abundance increased significantly ($P<0.05$). Digeda-4 antipyretic effect of Sheila disease on the intestinal flora of the rat model is based on the species relative abundance table, and the community composition data of the top 30 relative abundances at each taxonomic level are based on the abundance distribution of taxa or similarities between samples. The degree is clustered, taxa and samples are sorted according to the clustering results, and presented through heat maps. Through clustering, high-abundance and low-abundance taxa can be distinguished, and the similarity and difference in the composition of multiple samples at each taxonomic level can be reflected by color gradients and similarity levels. Note: In Figure 4, the rows represent species, the columns represent environmental indicators, the * mark represents p-value <0.05, red represents positive correlation, and blue represents negative correlation. The darker the color, the stronger the correlation.

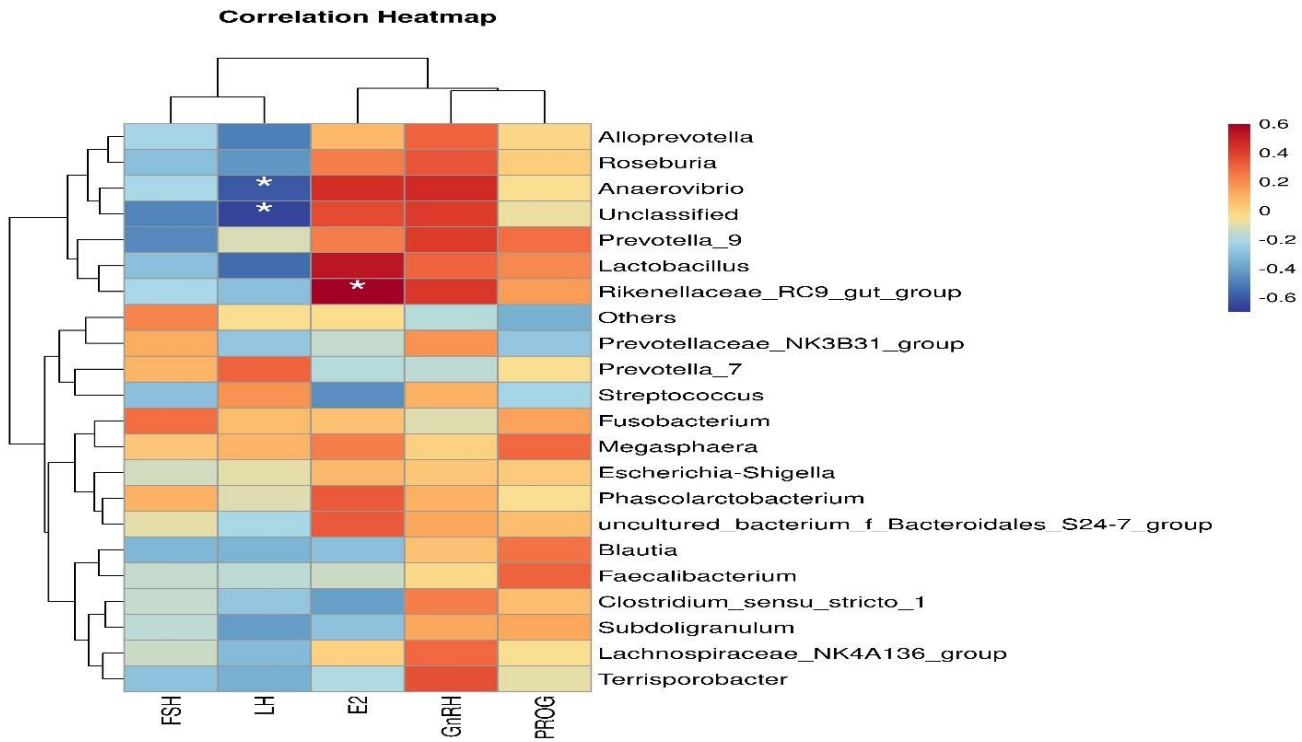

Figure 4. Correlation clustering heat map of bacterial groups and indicators

3.5. Species analysis

The ASV characteristic sequence is the original file for our species classification. To analyze the species composition more accurately, SILVA (Release 138, https://www.arb-silva.de/documentation/release-138/) and NT-16S database are used. Species classification and subsequent analysis to ensure complete and accurate annotation results. Annotation threshold: Confidence greater than 0.7. Based on the ASV annotation results and the ASV abundance table of each sample, species abundance tables at the kingdom, phylum, class, order, family, genus, and species levels were obtained, and species composition analyses of different samples (groups) were conducted based on species abundance tables at different levels. Different colors correspond to different species at the same level. At the same time, species composition and expression within the group and species expression trends between groups can also be observed. This table represents the species annotation results at different levels of kingdom, phylum, class, order, family, genus, and species corresponding to each feature value. Effect of Digeda-4 Antipyretic for Xila Disease on Species Diversity (Phylum) Taxonomic Level Species Abundance Values of Rat Model Intestinal Microbiota. The phylum taxonomic flora detected 5 main bacterial phyla, Firmicutes), namely Bacteroidota, Proteobacteria, Verrucomicriobota, Desultobacterota, etc. Effect of Digeda-4 antipyretic for Xila disease on the species diversity (genus) taxonomic level species abundance value of rat model intestinal flora. Five major bacterial genera were detected, including Lactobacillus and its close relatives (Ligilalactobacillus), namely Ligilalactobacillus. Muribaculaceae, HT002, g_ Clostrida_UCG_014_unclassified, Monoglobus, etc.

3.6. Stacked bar chart

According to the species abundance table and species annotation table, the TOP30 species classifications are selected by default, and the relative abundance of each sample/group is displayed in different forms. The histogram is displayed in the form of a stacked histogram to facilitate a more intuitive comparison of sample abundance. At each level, you can intuitively see the expression of dominant bacterial species and the changing trends in different treatments.

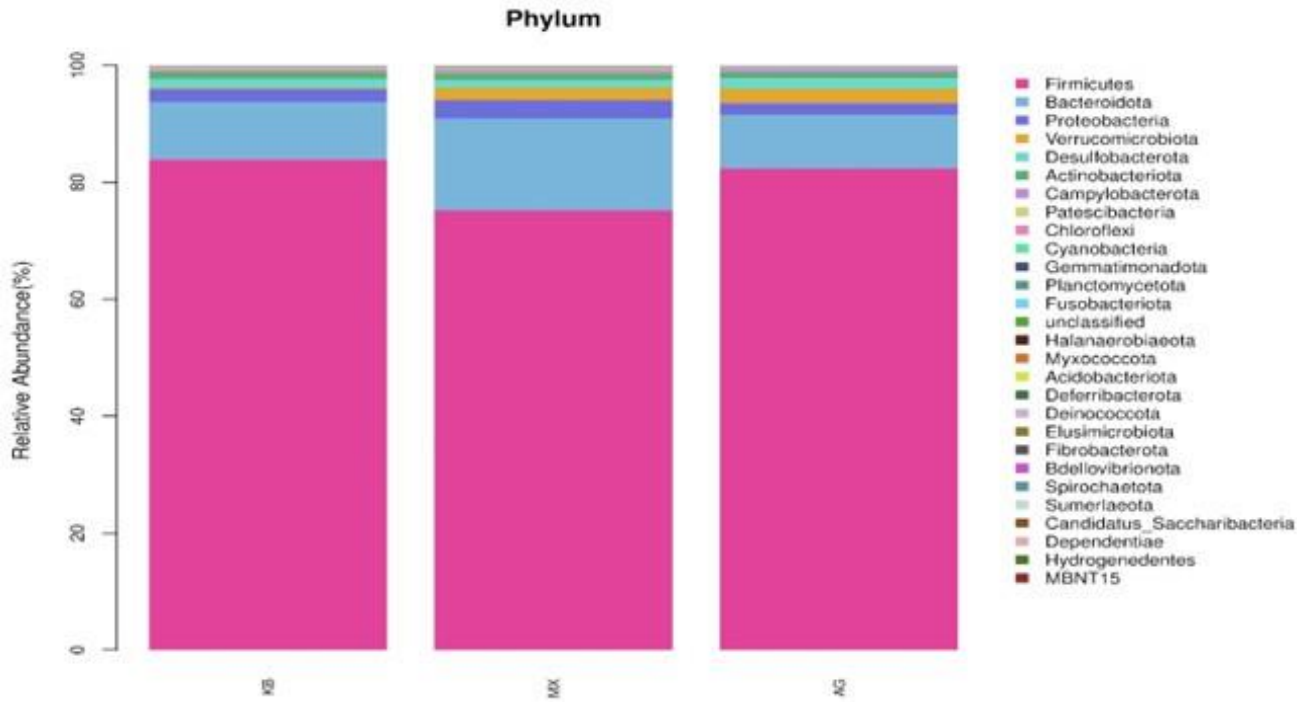

Figure 5. Grouped stacked column chart

3.7. Cluster diagram (cluster)

Although the species composition column stack chart can visually see the species abundance of different samples or groups, the similarity between specific samples cannot be directly observed. To study the differences and similarities between different samples in more detail, based on the histogram, cluster analysis can also be performed on the samples according to the species composition distance of the samples. This analysis uses the Bray-Curtis distance (the most commonly used distance index in systematic clustering methods, which is mainly used to describe the degree of similarity between samples. The size of the distance is the basis for conducting the sample.

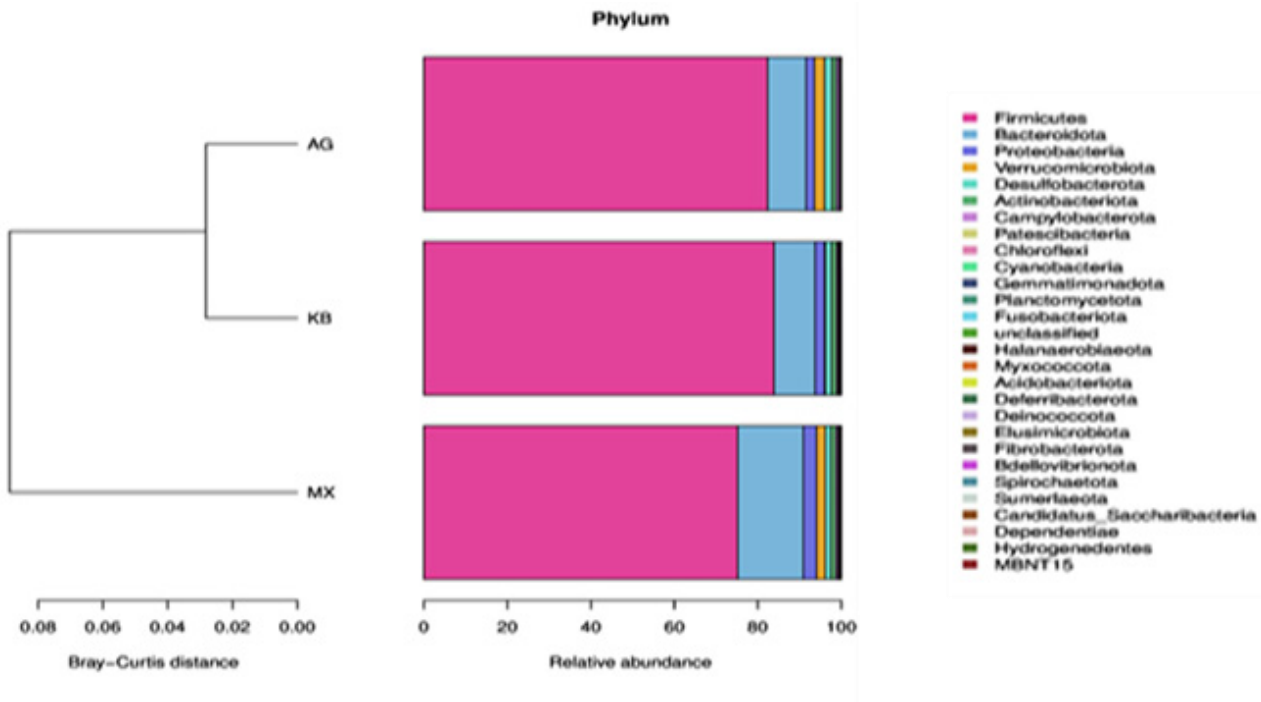

Figure 6. Group cluster analysis

3.8. Significant difference analysis

The main research purposes of 16S rDNA sequencing analysis are as follows: to study the microbial composition of a certain group (such as the intestinal flora of healthy people), to find out the commonalities of microorganisms in this group, and to find out the impact of microorganisms on the group (such studies usually have a large sample size). Very large); study the differences in microbial composition between samples treated with different treatments (such as disease and health, soil with differentfertilizers), etc.According to the sample species abundance table, Fisher's exact test (suitable for comparison of differences between samples without biological replicates), Mann-Whitney U test (suitable for comparison of differences between two groups of samples with biological replicates), and Kruskal-Wallis test (suitable for comparisons of samples with biological replicates) were used. Comparison between multiple groups of biological replicate samples) to test for species differences. We will select a suitable analysis method to analyze the significant differences between groups based on your experimental design. Explanation of the results: Difference analysis was performed on all species at each level, and the top 30 species with the highest abundance among species with p values less than 0.05 were selected to draw a histogram. The abscissa 11 in the figure represents differential species (arranged from left to right according to abundance), and the ordinate is relative abundance. The abundance of each differential species in different groups can be intuitively judged through the height of the column.

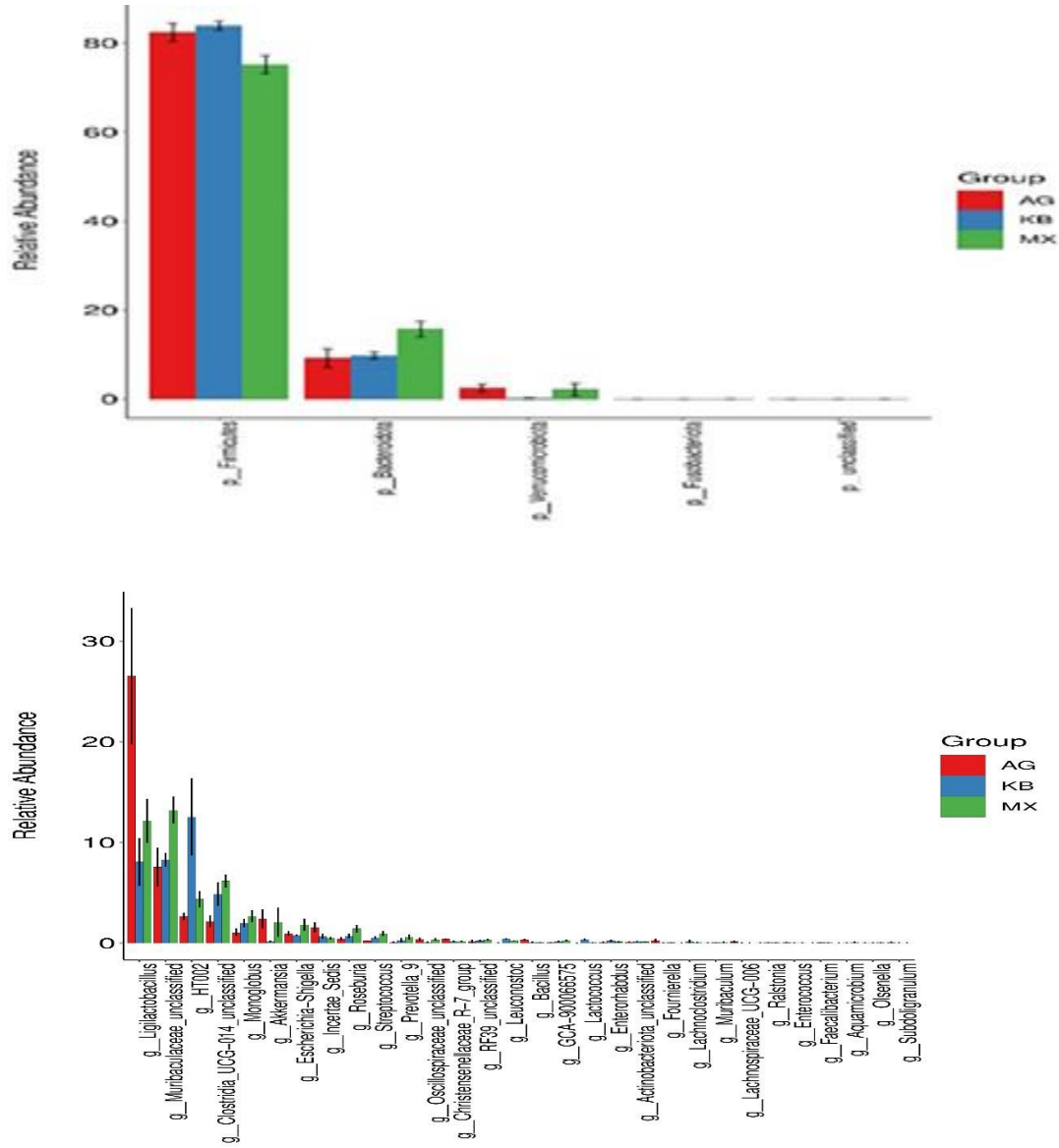

Figure 7. barplot difference analysis (taking the genus level as an example)

3.9. Difference analysis LEfSe

The main purpose of LEfSe (LDA Effect Size) analysis is to compare two or more groups and find species (biomarkers) with significant differences in abundance between different groups. It has been widely used. The LEfSe analysis drawing more intuitively displays the differential species at all levels between groups and is also the most frequently used display chart of differential species. Note: The figure shows species whose LDA score is greater than the set value (default is 5.0). The length of the figure represents the impact size of different species (LDA score), and different colors represent species in different groups. Note: Different circle layers radiate from the inside to the outside respectively representing the seven classification levels of order, family, genus and species.

Each node represents a species classification at this level. The higher the abundance of the species, the larger the node. If the node color is yellow, it means that the species has no significant difference in the comparison group. If the node color is red, it means that the species has a significant difference in the comparison group, and the species has a higher abundance in the red group.[16] Others The colors are similar. The phyla with significant differences are marked directly in the figure, and the species nodes with other levels of differences are identified by letters, specifically representing the species.

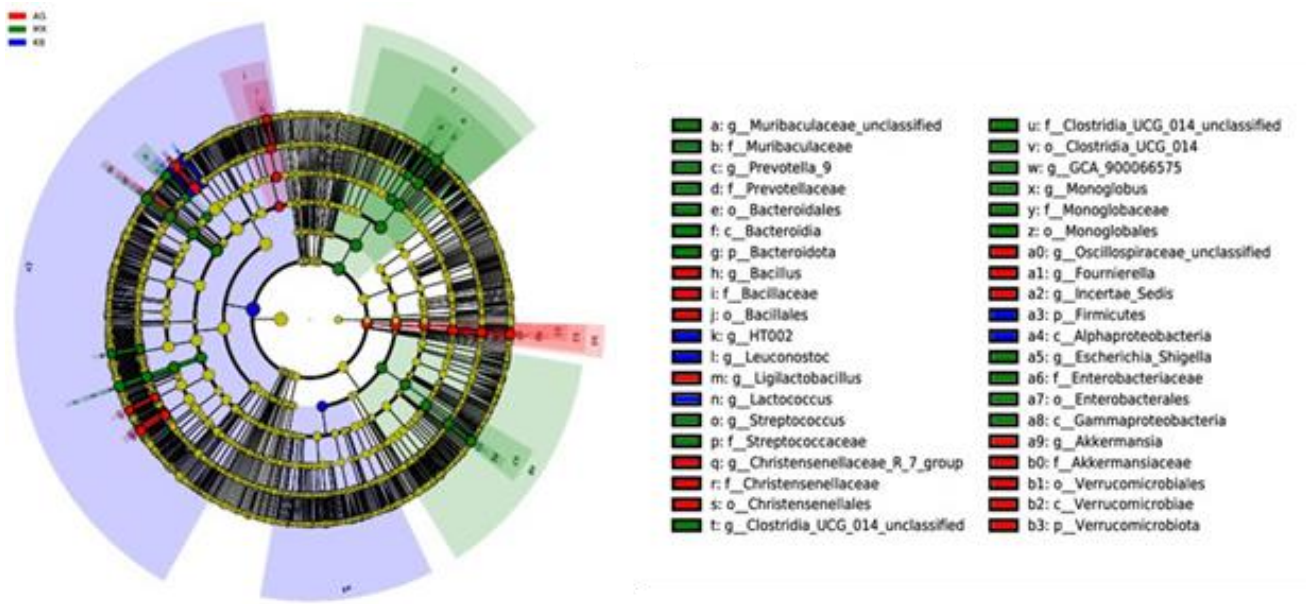

Figure 8. LEfSe difference analysis

Figure 9. LDA value distribution

4. DISCUSSION

Digeda-4 Decoction, is composed of 4 medicinal herbs such as Listigma (also known as Digeda), Huhuanglian, Gardenia, and Qumai Listiflora is the main drug; HuHuanglian and Gardenia are complementary drugs, Qumai as adjuvant drug.[2] It has the functions of eliminating "Xila", cooling blood, regulating badness, and returning essence. It is mainly used for various diseases such as blood heat fighting, liver and gallbladder heat, sore throat, thirst, and irritability.[3] According to modern research results, it is shown that Sternia japonica contains swertomacroside, which has the effect of protecting the liver, Huhuanglian and Gardenia contain cycloene glucoside, which has the effect of purging the Large intestine and other pharmacological effects.[4,5] This paper reviewed Digeda-4 Decoction from the aspects of historical description, prescription principle, chemical components, pharmacology, clinical application, and quality control. A total of 22 species were identified that were significantly correlated with body temperature (1 species was positively correlated with body temperature, and 2 species were negatively correlated with body temperature). The positively correlated strain is Rikenellceae_RC9_gut_group; the negatively correlated strains are Anaerovibrio and Unclassified.[17] Digeda-4 antipyretic effect of Xila disease on the intestinal flora of the rat model is based on the species relative abundance table, and the community composition data of the top 30 relative abundances at each taxonomic level are based on the abundance distribution of taxa or similarities between samples. The degree is clustered, taxa and samples are sorted according to the clustering results, and presented through heat maps. Through clustering, high-abundance and low-abundance taxa can be distinguished, and the similarity and difference in the composition of multiple samples at each taxonomic level can be reflected by color gradients and similarity levels.[18] Effect of Digeda-4 Antipyretic for Xila Disease on Species Diversity (Phylum) Taxonomic Level Species Abundance Values of rat model Intestinal microbiota. The phylum taxonomic flora detected 5 main bacterial names Firmicutes, Bacteroidota, Proteobacteria, Verrucomicriobota, Desultobacterota, etc. Effect of Digeda-4 antipyretic for Xila disease on the species diversity (Genus) taxonomic level species abundance value of rat model intestinal flora. Five major bacterial genera were detected, including Lactobacillus and its close relatives (Ligilalactobacillus), namely Ligilalactobacillus. Muribaculaceae, HT002, g_ Clostrida_UCG_014_unclassified, Monoglobus, etc. Firmicutes (Phylum Firmicutes) refers to the cell wall containing a high amount of peptidoglycan, about 50%-80%, the cell wall thickness is 10-50nm, the Gram stain reaction is positive, and the bacteria are globular, rod-shaped or irregular rod-shaped shaped, filamentous or branched filamentous, etc., reproduce by binary fission, and a few can produce endospores (called spores) or exospores (called conidia), all of which are chemotrophic and have no phototrophy. Firmicutes include a variety of Gram-positive bacteria. Obesity

is related to the distribution of intestinal bacteria. The presence of more Firmicutes than Bacteroidetes in the intestine leads to more efficient absorption of calories from food, leading to obesity. Jeffrey Gordon of the University of Washington School of Medicine and others discovered that obese people have a completely different type of bacteria swimming in their intestines than those of slim people.[19] Proteobacteria is the largest phylum of bacteria, including many pathogenic bacteria, such as Escherichia coli, Salmonella, Vibrio cholerae, Helicobacter pylori and other well-known species. The phylum Proteobacteria is divided into five categories (usually as five classes) based on rRNA sequences, named with the Greek letters α, β, γ, δ, and ε. Proteobacteria (6 photos) δ-Proteobacteria include basically aerobic fruiting body-forming myxobacteria and some strictly anaerobic species, such as sulfate-reducing bacteria (Desulfovibrio, Desulfobacter) , Desulfococcus, Desulfonema, etc.) and sulfur-reducing bacteria (such as Desulfuromonas), as well as anaerobic bacteria with other physiological characteristics, such as those that reduce trivalent Geobacter and the symbiotic genera Pelobacter and Syntrophus.[20] In 2017 the Epsilonproteobacteria were reclassified into a new phylum - Epsilonbacteraeota. Inflammatory bowel disease (IBD) is a chronic inflammatory gastrointestinal disease, and an imbalance of intestinal flora is a key factor in its development. Gastrodia elata (G.elata) is an orchid plant known for its nutritional and medicinal values. Research shows that Gastrodia polysaccharide (GBP) has anti-inflammatory properties and may improve IBD. However, the improving effect of GBP on intestinal microbiota metabolism remains unknown. Therefore, we aimed to examine the therapeutic potential of Gastrodia elata extract and GBP on dextran sulfate sodium (DSS)-induced IBD mice. GBP caused a significant increase in the relative abundance of bacteria with potential anti-inflammatory effects, such as Ligilactobacillus and Alloprevotella, and a decrease in the levels of bacteria associated with pro-inflammatory responses, such as Bacteroidetes and Escherichia coli[21]. In summary, the antipyretic effect of Digeda-4 on Xila disease is better on the diversity of intestinal flora in the rat model. Different administration models have different effects on the intestinal flora of rats. The antipyretic mechanism of Digeda-4 in Xila disease has laid a preliminary research foundation. In the future, further research on the antipyretic mechanism of Digeda-4 will be carried out. The antipyretic effect of Digeda-4 on Xila disease provides more scientific, systematic data and useful theoretical reference for the suitability of future clinical applications for the species diversity of intestinal flora in the rat model.

5. CONCLUSION

It can effectively reduce the symptoms of rats and effectively reduce the fever and body temperature of rats. Its mechanism of action may be related to its reduction in the intestinal Rikenellceae bacteriaceae_ RC9 intestinal group and the increase in the abundance of intestinal Anaerovibrio bacteria.

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