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ORIGINAL PAPER

Effect of *Senecio scandens* ethanol extract on gut microbiota composition in mice

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Abstract

The gut microbiota inhabits the animal intestinal tract, and dysbiosis of the gut microbiota may result in disease. *Senecio scandens* has pharmaceutical antibacterial activities and is regarded as a broad-spectrum antibiotic in traditional Chinese medicine. Extracts of *S. scandens* are reported to show strong antimicrobial activity, and quercetin significantly decreases some species in the caecal microflora. However, the bactericidal effects of the extracts on the gut microbiota remain obscure. Here, we supplied ethanol extract of *S. scandens*, which might possibly be used as an alternative for chemical antibiotics, to mice to investigate the state of the intestinal microbiota. Our studies included a control group, low-, moderate-, and high-dose ethanol extract groups, and cefixime capsule group. The ethanol extract groups did not present reduced diversity or differences in the gut microbiota balance. There were significant differences between the ethanol extract and cefixime capsule groups in terms of the gut microbiota. The control and ethanol extract groups contained similar bacteria, which suggested that the ethanol extract has no inhibitory effect on the gut microbiota in vivo. Bifidobacteriales and *Lactobacillus acidophilus* were significantly increased in the high-dose group. Both secretory immunoglobulin A and mucin 2 concentrations increased as the dose of ethanol extract increased. The functional prediction differences between the control and ethanol extract groups decreased with increasing extract doses, which indicated that the low-dose and high-dose extract treatments might regulate different pathways and functions of the gut microbiota. The results also highlighted the prevention of bacterial drug resistance in the ethanol extract groups.

Keywords *Senecio scandens* · Antibacterial activity · Gut microbiota · Secretory IgA · MUC2

Introduction

The gut microbiota inhabits the animal intestinal tract, mainly within the colon (Van der Lugt et al. 2018). It is favourable to inoculate pups or infants with gut microbes for the development of the immune system (Nyangahu et al. 2018). The gut microbiota also contributes to metabolism and digestion (Alfa et al. 2018). However, gut microbiota dysbiosis may induce various diseases (Petersen and Round 2014), such as obesity (Yao et al. 2019), type I diabetes (Leiva-Gea et al. 2018), multiple sclerosis (Cantarel et al. 2015), and inflammatory bowel diseases (Matsuoka and Kanai 2015). Thus, gut microbiota dysbiosis results in intestinal or extraintestinal disorders and damages the colon or other organs (Carding et al. 2015). Gut microbial dysbiosis is attributed to many different factors, including antibiotics, inapposite dietary components, and psychological and physical stress (Myers 2004).

Gut microbiota homeostasis is regulated by many factors, two of which are secretory immunoglobulin A and mucin 2 (Li et al. 2020; Liu et al. 2020). Secretory immunoglobulin A (sIgA) is a polymeric immunoglobulin A and is primarily generated by the animal gut (Li et al. 2020). SIgA acts as a

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first-line barrier in preventing the epithelium from pathogen microbes in the gut lumen (Pabst 2012). SIgA is reported to play an important role in the regulation of gut microbiota homeostasis (Li et al. 2020). Mucin 2 (MUC2) is another important protein that protects the gut barrier (Liu et al. 2020). MUC2 produced by goblet cells is a primary mucus network component in the gut, and the mucus network separates the intestinal bacteria and the epithelial cells (Boltin et al. 2013). MUC2 also regulates microbiome homeostasis and prevents diseases (Liu et al. 2020).

Antibiotics are widely used in humans and in farm livestock, and they are generally considered to be reliable treatments for many diseases. Antibiotics have also been used as additives in animal feed for many years (Langeveld et al. 2014) because they can save lives, reduce suffering, and increase livestock yield. However, antibiotic resistance has resulted in substantial challenges for humans and has become an issue of increasing concern. Many studies have suggested that the emergence of multiresistant bacteria (also referred to as superbugs) owing to antibiotic overuse causes serious clinical problems (Martin et al. 2015; Pham et al. 2018). Antibiotic use is also a risk factor for dysbiosis in the gut microbiota. It has been reported that antibiotics exert major effects on the gut microbiota (Sun et al. 2019a). Short-term antibiotic treatment could cause the gut microbiota to enter a state of long-term dysbiosis (Lange et al. 2016; Weber et al. 2017). However, some antibiotics are still used as basic medicines, such as cefixime (Williams and Berkley 2018). Cefixime is used as an anti-inflammatory drug and for the treatment of infectious diarrhoea. Studies have shown that cefixime presents a high efficiency in the treatment of shigellosis or diarrhoea (Helvaci et al. 1998; Lee et al. 2010; Mohammed et al. 2018). Therefore, new drugs need to be developed as alternatives to antibiotics.

The traditional Chinese medicine *Senecio scandens* shows high efficacy in the treatment of infectious diarrhoea in traditional Chinese medicine (Wang et al. 2013). Previous studies have shown that feeding *S. scandens* to piglets can increase blood immunoglobulin G (IgG), immunoglobulin A (IgA), and immunoglobulin M (IgM) concentrations and reduce diarrhoea (Lu et al. 2014). Studies on *S. scandens* may contribute to the development of feed supplements or new therapeutic approaches for inflammatory or infectious diarrhoea. The pharmacological activities of *S. scandens* include heat clearing, the improvement of eyesight (Anonymity 2010), antioxidant effects, free-radical scavenging, and antibacterial, antiviral, and antitumour activities (Meng et al. 2010). *S. scandens* is used to treat bacterial skin disease (Hu 1998) and acute inflammatory bowel disease (Bao and Kong 2011) and as an animal feed supplement that is an alternative to antibiotics based on its antibacterial activities (Sun et al. 2019b).

Extracts of *S. scandens* contain flavonoids, alkaloids, phenolic acids, a volatile fraction, jacaranones, lactones, and other compounds (Wang et al. 2013). A study reported that flavonoids extracted from *S. scandens* exhibited strong suppressive effects on *Staphylococcus aureus*, *Escherichia coli*, *Salmonella enteritidis*, *Bacillus anthracis*, and *Streptococcus hemolyticus*. The minimal inhibitory concentrations for these bacteria were 0.04 g/L, 0.10 g/L, 0.08 g/L, 0.10 g/L, and 0.06 g/L, respectively (Chen et al. 1999). Quercetin (flavonoid) and chlorogenic acid (phenolic acids) are the main constituents of *S. scandens* extracts and are usually tested as a reference for the quality of medical material (Wang et al. 2013). These two substances have been reported to exhibit strong antimicrobial activity, and quercetin causes significant decreases of the caecal microflora in some species (Naveed et al. 2018; Wang et al. 2018). Consequently, some studies have revealed that *S. scandens* shows strong efficacy in opposing bacteria, and *S. scandens* is regarded as exhibiting broad-spectrum antibacterial activities (Liu et al. 2007; Rao et al. 2013; Li et al. 2018). A review summarizing the antibacterial activities of *S. scandens* suggested that an *S. scandens* water decoction showed remarkable antibiotic activity against *S. aureus*, *E. coli*, *Dysenteric bacilli*, *Bacillus paratyphosus*, *Pseudomonas aeruginosa*, and *E. coli* R plasmids (Wang et al. 2013), and an in vivo study showed that an extract from *S. scandens* exerted an inhibitory effect on *S. aureus* (Yang et al. 2010). Studies have shown that a 60% ethanol extract of *S. scandens* significantly inhibits *Streptococcus pneumonia*, *S. aureus*, and *E. coli* in vitro (Rao et al. 2013).

However, the effects of ethanol extracts of *S. scandens* on gut microbiota dysbiosis when administered as an oral drug or feed additive are not clear. Here, we used a 60% ethanol extract of *S. scandens* with the objective of determining (i) whether *S. scandens* damages colon tissue or promotes the secretion of sIgA and MUC2 and (ii) whether *S. scandens* exerts a dose-dependent effect on the gut microbiota similar to cefixime.

Materials and methods

Ethanol extract

The aboveground parts of *S. scandens* were collected in September 2018 based on the optimum time of harvest (Lin and Ye 2003). The sample was identified by Dr Zhiwei Wang, and a voucher specimen (No. 20180925) was deposited at the Institute of Laboratory Animal Science at Guizhou University of Traditional Chinese Medicine, Guizhou, China. Then, the sample was dried in the shade at room temperature and crushed into a powder. Approximately 9 kg of powder was exhaustively extracted with 60% ethanol (40

L) by maceration for one week, followed by filtering of the solution.

The whole extraction procedure was repeated three times. All filtrates were pooled together. After filtration, the ethanol extract was evaporated to dryness under reduced pressure in a rotary evaporator at temperatures between 50 and 60 °C.

Animals

All animal experimental procedures were conducted in accordance with the guidelines for ethical review of animal welfare in China (GB/T 35892-2018, the State Standard of the People's Republic of China), and the protocol was approved by the animal care welfare committee of Guizhou University of Traditional Chinese Medicine (No. 20190010). Thirty Institute of Cancer Research (ICR) mice (15 males and 15 females) were purchased from Chongqing Tengxin Biotechnological Limited Company (Chongqing, China). The animals were raised in specific stainless steel mesh-covered cages separated by gender and were acclimated for one week before the experiment. The environment was controlled, and an artificial dark:light cycle of 12:12 h (daytime: 7:00 am to 7:00 pm) was maintained. All mice were provided with sufficient sterilized water and standard full-nutrition compound food.

The animals were divided into 5 groups: (1) a control group; (2) a low-dose group administered 184 mg/kg ethanol extract of *S. scandens*; (3) a moderate-dose group administered 368 mg/kg extract; (4) a high-dose group administered 735 mg/kg extract; and (5) a cefixime capsule group. Each group contained three males and three females that were assigned randomly. We selected the ethanol extract concentrations according to the LD_{50} (with a dose of 2206 mg/kg) recorded following intraperitoneal injection (Li et al. 2008). The oral doses of the ethanol extract were 1/12 LD_{50} (184 mg/kg; low dose), 1/6 LD_{50} (368 mg/kg; moderate dose) and 1/3 LD_{50} (735 mg/kg; high dose). The dried extract was resuspended in a 2% Tween-80 normal saline solution before gavage, and the drug concentrations were 18.4 g/L, 36.8 g/L, and 73.5 g/L, respectively. The control group received an equal volume of 2% Tween-80. The cefixime capsule group received an oral dose of 12.33 times the dose administered to humans (Reagan-Shaw et al. 2008). All groups received an oral dose once a day at 10:00 am, and the whole experiment lasted for 15 days.

Histological analysis

The gut microbiota mainly inhabits the colon, and gut microbiota dysbiosis results in a proportional incidence of colitis (Carding et al. 2015). Pathological sections of colon tissue were used to inspect the effect of the gut microbiota on intestinal tissues. The changes in colon tissues were evaluated by haematoxylin & eosin staining. The mice were sacrificed by the administration of an overdose of sodium pentobarbital. To avoid the erroneous cutting of tissues in the rectum or adjacent to the cecum, we selected colon tissues sampled from the middle portion of the colon (~ 1 cm), and the tissues were then stored in a 4% paraformaldehyde solution in water for over 24 h. Then, the tissues were dehydrated, embedded in paraffin, sliced into paraffin sections, and stained with haematoxylin & eosin.

Measurement of intestinal secretory immunoglobulin A (sIgA) and mucin 2 (MUC2)

To detect the secretion state of sIgA and MUC2 in the gut after gavage with ethanol extract of *S. scandens*, we measured the concentrations of sIgA and MUC2 in the lumen of the colon using mouse-specific ELISA kits (Shanghai Enzyme-linked Biotechnology Co., Ltd.). Each sample was measured three times. The experimental procedures were carried out in accordance with the manufacturer's instructions.

Gut microbiota DNA extraction, 16S rRNA gene amplification, sequencing, and sequence analysis

Stool samples were collected from mice on the last day of our experiment. Approximately 0.3–0.5 g faeces samples were collected into 2 ml sterile centrifuge tubes and stored in a − 70° refrigerator. A Fast DNA stool kit (Qiagen, CA, USA) was used to extract the total DNA genome, whose concentration was measured with a spectrophotometer (Nanodrop 2000, Thermo Scientific, USA).

Bacterial hypervariable regions of V3 to V4 were used as biomarkers to detect the gut microbiome structure. The barcode-indexed primers 338F (5′-TAC GGG AGG CAG CAG-3′) and 806R (5′-GGA CTA CCA GGG TAT CTA AT-3′) were used to amplify the hypervariable region (Yao et al. 2019). Then, a gel DNA purification kit was used to purify the amplicons. The purified amplicons were quantified with a QuantiFluor-ST system (Promega, USA), followed by normalization to equimolar concentrations. The purified amplicons were sequenced with an Illumina MiSeq sequencer based on paired-end sequencing.

FLASH (Magoč and Salzberg 2011) and Trimmomatic (Bolger et al. 2014) software were used to identify correct overlaps and extend them. We used the Usearch platform to select operational taxonomic units (OTUs) following the recommended procedure and defined a taxonomic group by the 16S rRNA sequences clustered at 97% similarity (Edgar 2013). The QIIME platform was utilized for subsequent analysis (Caporaso et al. 2010). The clustered taxonomic groups were identified by alignment with the Greengenes database (DeSantis et al. 2006).

The cumulative curve of the gut microbiome was plotted to test whether our samples included most of the taxonomic groups within the microbiome. Rarefaction curves and Shannon curves were plotted to test whether the sequencing depth was sufficient. The linear discriminant analysis effect size (LEfSe) method was used to identify significantly different microbial species among different experimental groups (Segata et al. 2011). Principal coordinate analysis (PCoA) was used to determine whether these groups had different microbiome compositions. A hierarchical clustering tree of all samples was established via the UPGMA (unweighted pair-group method with arithmetic mean) approach. PICRUSt was used to predict metagenome functions in accordance with metabolic pathways (Langille et al. 2013). Then, the functional predictions were categorized into KEGG pathways (Kanehisa and Goto 2000), and the differences in the pathways were compared with STAMP (Parks et al. 2014).

Results

Histological morphology

The histological analysis of all groups showed that the structure of the colon tissue was intact, and there were no differences among the experimental groups (Online Resource Fig S1). The results indicated that the ethanol extract did not damage the colon tissue of the mice.

sIgA and MUC2 measurement

The results showed that the control group presented the lowest sIgA and MUC2 concentrations, and the cefixime capsule group exhibited the highest concentration (Fig. 1). The concentrations of sIgA and MUC2 successively increased from the low-dose ethanol extract group to the high-dose ethanol extract group. The *t* test is a hypothesis testing method based on the Students' *t* distribution (Boslaugh 2012). An

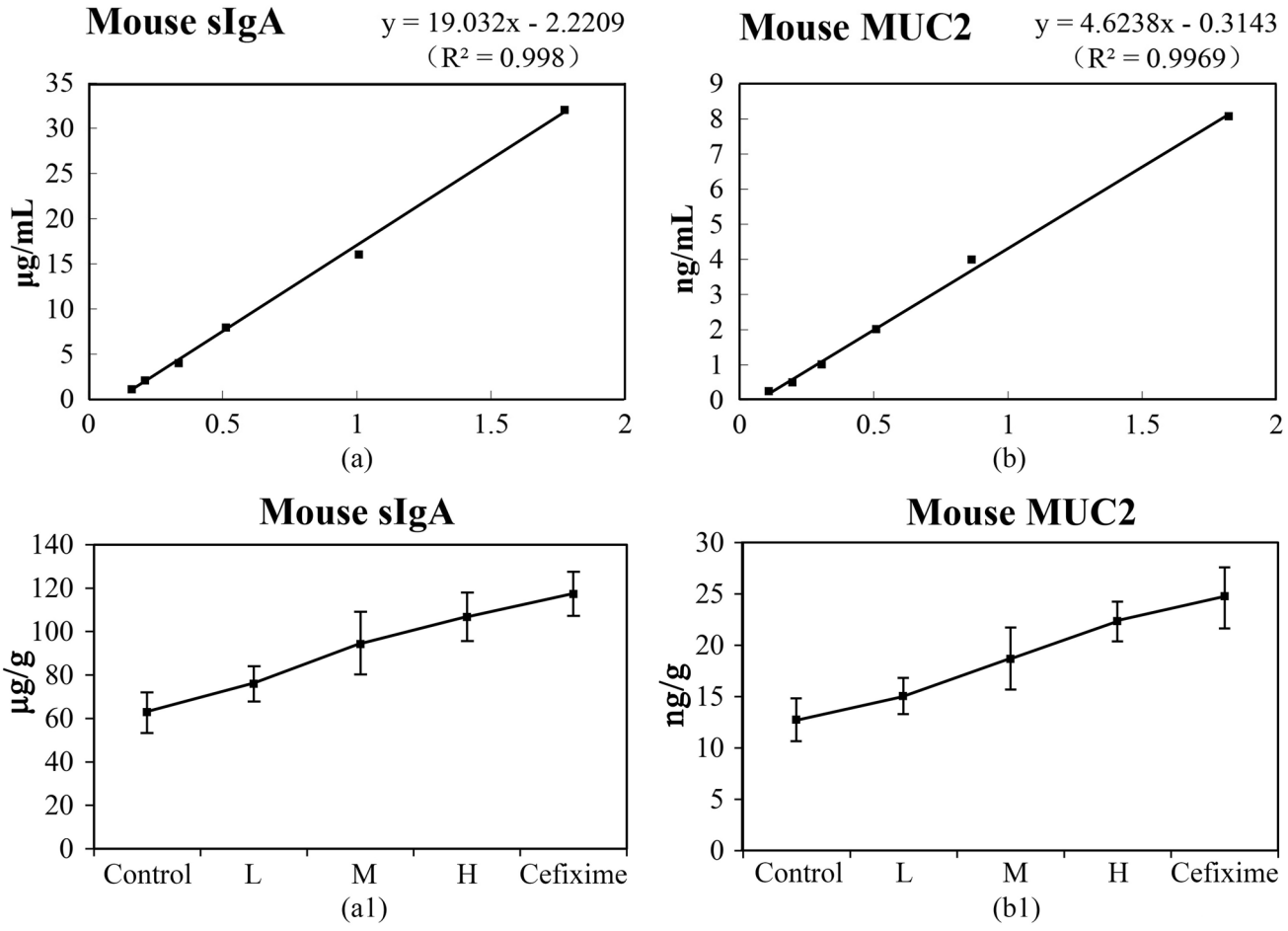


Fig. 1 The concentrations of sIgA and MUC2 in colon tissues. **a**, **b** Are standard curves; a1 and b1 are the concentration curves of sIgA and MUC2, respectively. Control, control group; L, low-dose group; M, moderate-dose group; H, high-dose group; Cefixime, cefixime capsule group

independent-samples *t* test showed that the concentration of sIgA in the control group was significantly lower than that in all the other groups (low-dose group, $p=0.049$; moderate-dose group, $p=0.000$; high-dose group, $p=0.000$). The sIgA concentration in the cefixime capsule group was not significantly different from those in the moderate-dose group ($p=0.104$) and the high-dose group ($p=0.721$). Similarly, the independent-samples *t*-test suggested that the MUC2 concentration in the control group was significantly lower than those in the moderate-dose group ($p=0.000$), the high-dose group ($p=0.000$) and the cefixime capsule group ($p=0.000$). The MUC2 concentration in the cefixime capsule group was not significantly different from those in the moderate-dose group ($p=0.061$), and the high-dose group ($p=0.791$).

Gut microbiome diversity

The cumulative curve showed that almost all bacteria in the samples were detected (Online Resource Fig S2). Good's coverage was used to estimate the sampling completeness of the analyses, and our results showed that Good's coverage was 99.98 ± 0.07% (Tables 1, 2). The rarefaction curves (Online Resource Fig S3) and the Shannon curves (Online Resource Fig S4) were nearly parallel to the sampling axis with increased sampling sequences, which suggested that the OTU number and the Shannon index would not increase with the sequence number. These results suggested that the analyses presented a sufficient sequencing depth and accuracy.

To detect the difference in the gut microbiota between males and females, we analysed the metagenomics results for the gut microbiota between the two sexes. The paired-samples *t* test suggested that there were no significant differences in alpha diversity between the two sexes in each group ($p>0.05$; Table 1). The results showed that there was nearly the same number of OTUs between males and females in all samples (Fig S5). The beta diversity analysis also showed that there were no significant differences between males and females. We observed that samples from different sexes were scattered among each other in the PCoA (Fig. 2a) and that the tree branches of the different sexes crossed (Fig. 2b). We also did not observe significant differences between the two sexes in the heatmap analysis (Fig S6).

The results showed that the alpha diversity of the cefixime group was low compared to that of the other groups (Table 2). The OTU number in the cefixime group was much smaller than those in the other groups (Table 2; Fig. 3). There were only 222 shared OTUs among all the groups, with nearly twice the number of shared OTUs (407 OTUs)

Table 1 Comparison of alpha diversity between males and females

Sex	Groups	OTUs	Ace	Chao	Shannon	Simpson	Coverage
Male	Control	358.00 ± 9.54	368.33 ± 12.74	372.23 ± 15.80	3.50 ± 0.245	0.08 ± 0.024	0.9999 ± 0.000
	Low	372.33 ± 1.53	379.80 ± 3.56	385.39 ± 11.04	3.96 ± 0.208	0.06 ± 0.034	0.9999 ± 0.000
	Moderate	354.00 ± 13.23	364.44 ± 11.05	366.68 ± 8.08	3.79 ± 0.41	0.07 ± 0.051	0.9998 ± 0.000
	High	355.33 ± 16.80	369.39 ± 17.23	372.86 ± 19.50	3.34 ± 0.22	0.11 ± 0.019	0.9998 ± 0.000
	Cefixime	102.00 ± 30.79	231.87 ± 86.28	174.59 ± 36.68	0.92 ± 0.087	0.57 ± 0.076	0.9998 ± 0.000
Female	Control	368.00 ± 10.82	376.28 ± 9.76	377.15 ± 12.47	3.52 ± 0.50	0.097 ± 0.074	0.9999 ± 0.000
	Low	359.33 ± 20.43	372.12 ± 16.37	377.47 ± 25.98	3.98 ± 0.49	0.046 ± 0.025	0.9998 ± 0.000
	Moderate	358.00 ± 11.00	367.42 ± 11.09	378.61 ± 19.10	4.05 ± 0.37	0.043 ± 0.023	0.9998 ± 0.000
	High	359.33 ± 13.58	373.20 ± 11.74	373.26 ± 14.56	3.70 ± 0.38	0.059 ± 0.024	0.9998 ± 0.000
	Cefixime	80.33 ± 14.01	197.98 ± 34.80	133.91 ± 8.60	0.86 ± 1.16	0.67 ± 0.44	0.9998 ± 0.000

Control control group, *low* low-dose group, *moderate* moderate-dose group, *high* high-dose group, *cefixime* cefixime capsule group

Table 2 Alpha diversity and Good's coverage of the gut microbiota among different experimental groups

Groups	OTUs	Ace	Chao	Shannon	Simpson	Coverage
Control	363.00 ± 10.64	372.55 ± 10.94	374.72 ± 13.00	3.51 ± 0.35	0.088 ± 0.050	0.9999 ± 0.00
Low	365.83 ± 14.78	375.96 ± 11.39	381.44 ± 18.37	3.97 ± 0.34	0.052 ± 0.028	0.9999 ± 0.00
Moderate	356.00 ± 11.10	365.93 ± 10.03	372.65 ± 14.66	3.92 ± 0.38	0.057 ± 0.039	0.9998 ± 0.00
High	357.33 ± 13.84	371.29 ± 13.35	373.06 ± 15.39	3.51 ± 0.34	0.083 ± 0.033	0.9998 ± 0.00
Cefixime	91.17 ± 24.47**	214.92 ± 61.70**	154.25 ± 32.62**	0.89 ± 0.74**	0.62 ± 0.29**	0.9998 ± 0.00

**$p<0.01$, significant differences compared with other groups. *Control* control group, *Low* low-dose group, *Moderate* moderate-dose group, *High* high-dose group, *Cefixime* cefixime capsule group

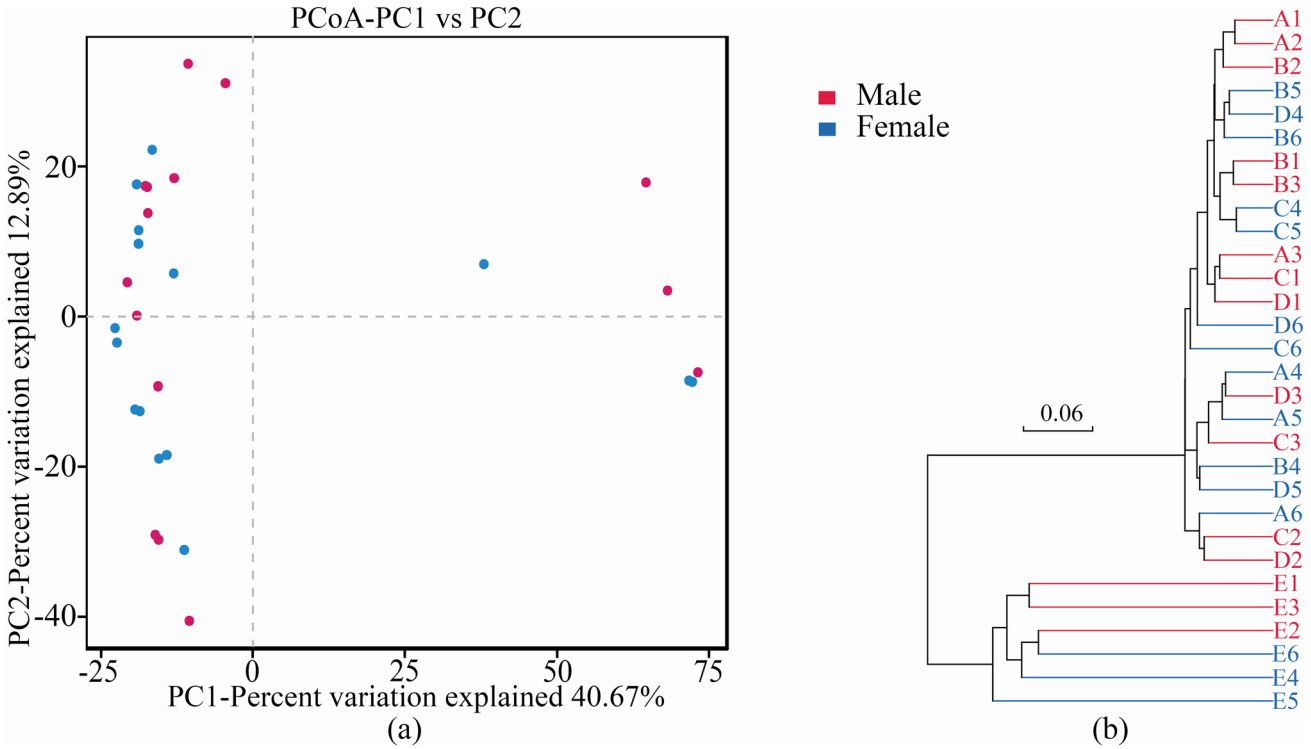


Fig. 2 Gut microbiota composition of male and female mice. **a** PCoA (bray_curtis method) of the gut microbiota community; **b** hierarchical clustering tree (unweighted_unifrac method) of all samples

among the control, low-dose, moderate-dose, and high-dose groups (Fig. 3). These results suggested that the numbers of gut microbiota species were similar among these groups, except for the cefixime group.

PCoA indicated that the gut microbiota composition of the cefixime group was different from that of the other groups (Fig. 4a). The tree branches of the samples from the control and the low-, moderate-, and high-dose groups crossed with each other, but not with that of the cefixime group (Fig. 4b). These results also suggested that the gut microbiota composition was similar among the control group and the ethanol extract groups. Heatmap analysis also showed that the cefixime group had a different gut microbiota composition from the other groups (Online Resource Fig S7).

LEfSe is a popular method for the identification of biomarkers between different metagenome datasets (Segata et al. 2011). LEfSe analysis showed that there were some significant bacterial differences among all of the groups (Fig. 5; LDA scores > 4, Online Resource Fig S8). There were large numbers of microbiota in Bifidobacteriales and *Lactobacillus* in the high-dose group, and there were some species belonging to *Enterococcus* and Clostridiales in the cefixime group.

Functional predictions indicated that the ethanol extract groups presented little difference from the control group based on pathways in the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Fig. 6). The functional predictions suggested that the cefixime capsule group exhibited many differences from the control group in terms of metabolic pathways. Interestingly, we found that functional predictions between the control and the high-dose groups were less different than the comparisons between the low- to moderate-dose groups and the control group, which suggested that the high-dose group might be a better choice as an additive or drug than a low or moderate ethanol extract concentration. The results also indicated a significant drug resistance pathway in the cefixime capsule group. These results all showed that the ethanol extract groups presented little change in the gut microbiota compared to the cefixime group.

Discussion and conclusion

S. scandens is a common traditional Chinese medicine with many potential pharmaceutical activities, such as antibacterial (Rao et al. 2013), anti-inflammatory (Yao et al. 2016), and antitumour (Dou et al. 2017) activities. Here, we investigated the influence of *S. scandens* on the gut microbiota and secreted IgA and MUC2 in the guts of mice.

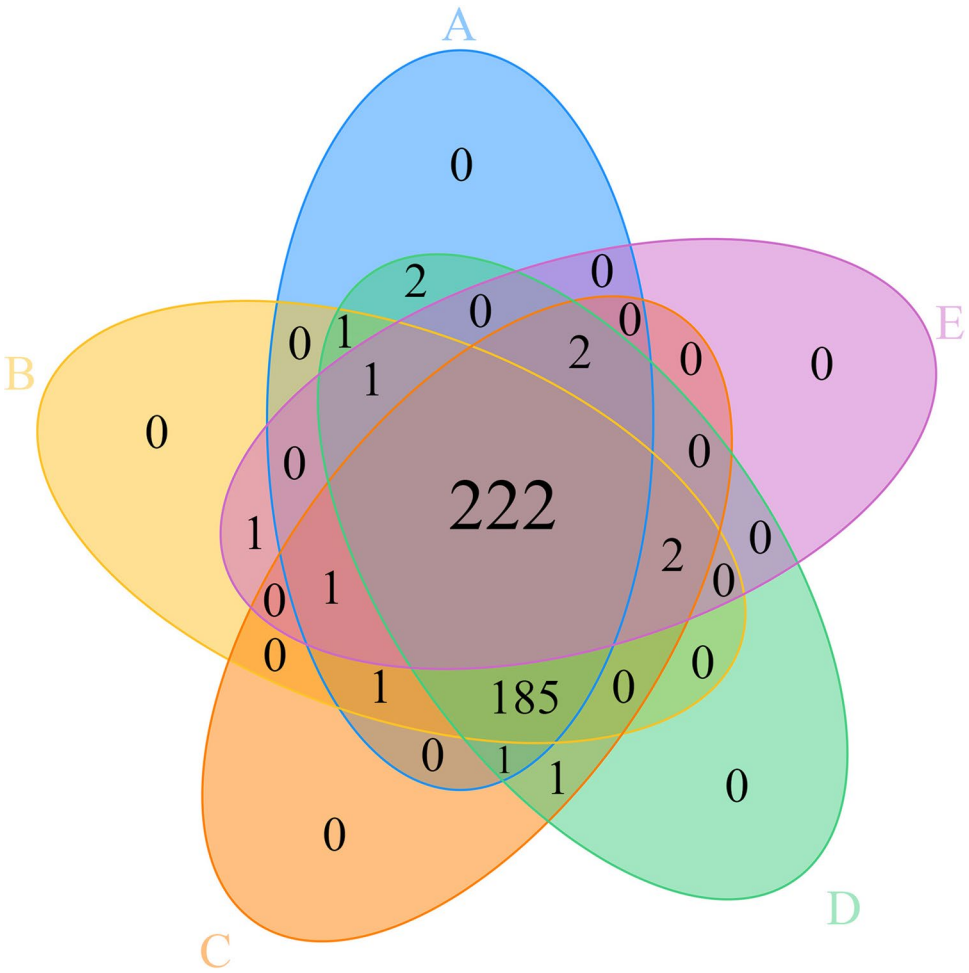


Fig. 3 Venn diagram of shared OTUs among all groups. **a** Control group; **b** low-dose group; **c** moderate-dose group; **d**, high-dose group; **e**, cefixime capsule group

Many factors influence the gut microbiota; for example, gender is reported to have a strong influence on the gut microbiota (Kim et al. 2019). However, some studies have drawn the opposite conclusion (Lay et al. 2005), and some research has shown that other factors (e.g., genetics) are more influential than the effect of gender (Kovacs et al. 2011). Our results suggested that there were no significant differences between male and female mice (Table 1; Fig S5; Fig. 2). However, the numbers of samples in the male and female subgroups were small, and this conclusion needs to be verified in future research.

Because no significant differences were observed between male and female mice, we analysed all male and female mice of each group together in the subsequent analyses. We observed that all of the groups shared only 222 OTUs, but the control group, the low-dose group, the moderate-dose group, and the high-dose group shared 407 OTUs (Fig. 3). Our results showed that there were no significant differences among the control group and the other ethanol extract groups (low-, moderate-, and high-dose groups). The OTUs in the control group and the ethanol extract groups exhibited obvious differences compared with those in the cefixime capsule group. The cefixime capsule group showed similarity to the results of other studies in which antibiotics have been found to cause gut microbiota dysbiosis and reduce microbiota diversity (Zaura et al. 2015). In contrast, the ethanol extract groups did not show a reduction of OTUs (alpha diversity), which suggested that the ethanol extract did not reduce the number of gut microbiota species.

The results of PCoA (Fig. 4a) and the UPGMA tree (Fig. 4b) showed that there were significant differences between the ethanol extract groups and the cefixime capsule group. The gut microbiota of the control group and the ethanol extract groups contained many bacteria from Muribaculaceae, Lactobacillus, and Dubosiella, while the cefixime capsule group contained many members of Robinsoniella. These results collectively suggested that there were nearly no differences in the gut microbiota between the control group and the ethanol extract groups. The ethanol extract of *S. scandens* has been reported to exhibit antibacterial activity in vitro (Rao et al. 2013). However, our study revealed that the ethanol extract of *S. scandens* did not alter the gut microbiota similarly to cefixime in vivo.

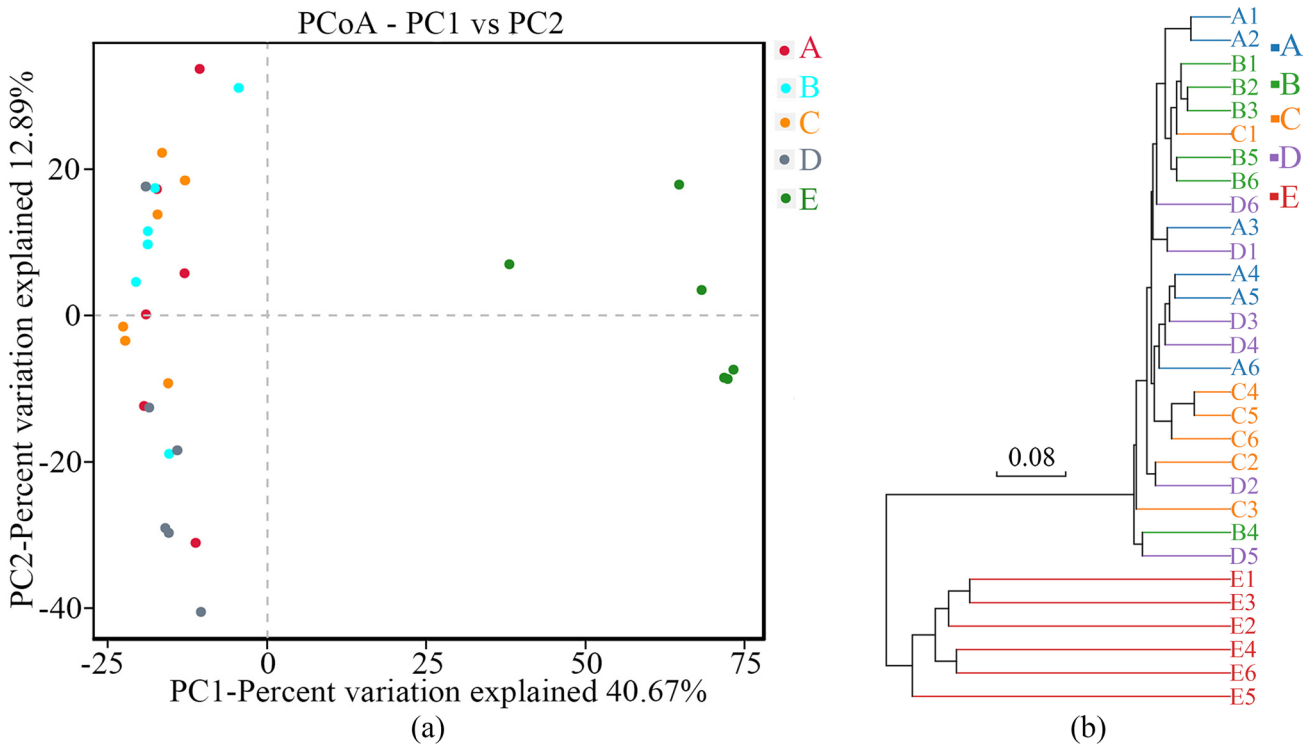


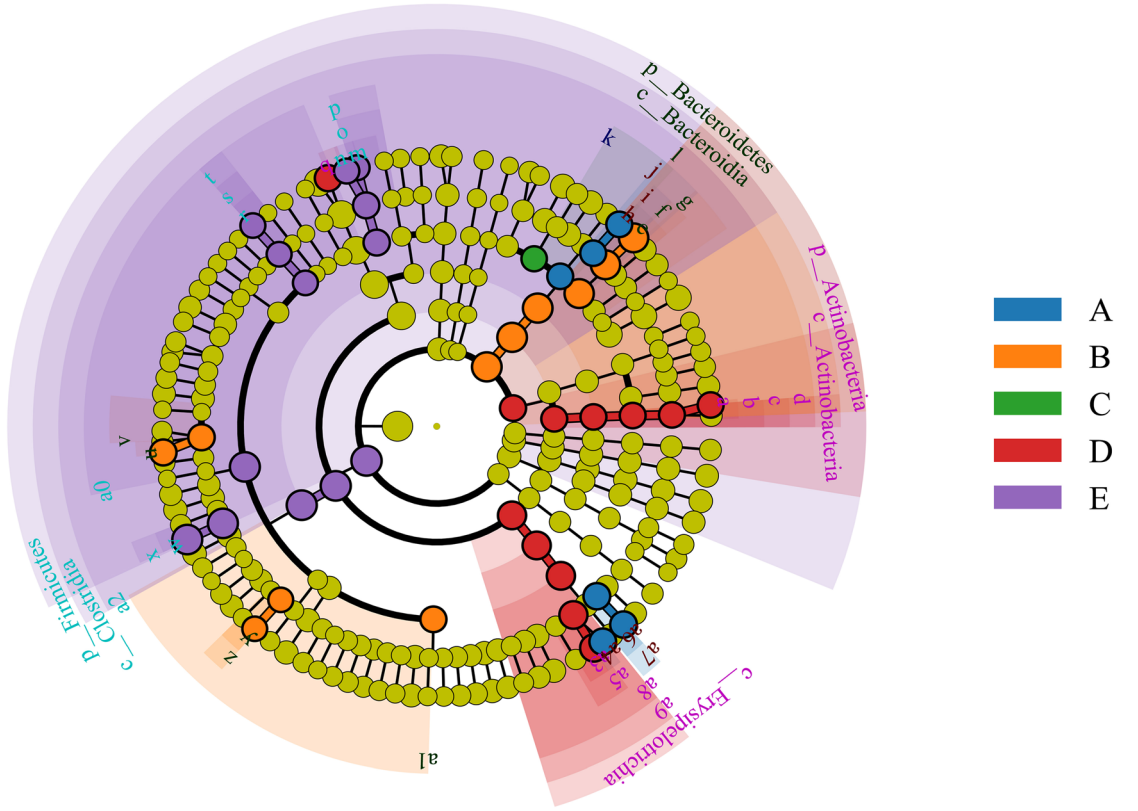
Fig. 4 Gut microbiota composition of all groups. **a** PCoA (bray_curtis method) of the gut microbiota community; **b** Hierarchical clustering tree (binary_jaccard) of all samples. **a** Control group; **b** low-dose group; **c** moderate-dose group; **d** high-dose group; **e** cefixime capsule group

Our results suggested that the sIgA level in the colon lumen increased with the increase in the ethanol extract dose (Fig. 1). The mechanism underlying this phenomenon is obscure. Some studies suggest that probiotics upregulate sIgA concentrations (Yang et al. 2009; Ren et al. 2015; Kusumo et al. 2019; Xiao et al. 2019). However, our studies showed that some species (*Bifidobacterium* spp. and *Lactobacillus acidophilus*) that are considered probiotic agents were only significantly increased in the high-dose group (Fig. 5), which suggested that there may be another factor that promoted the sIgA increase. Previous studies have suggested that some other traditional Chinese medicines can also promote sIgA secretion. For example, Chinese date promotes sIgA secretion in the mucosa of the respiratory tract (Xu 2013), and *Cucumaria frondosa* has an upregulating effect on the secretion of intestinal sIgA in mice (Zuo et al. 2012).

Similar to sIgA, MUC2 was also increased in the ethanol extract groups with increasing ethanol extract dose (Fig. 1). It is also difficult to elucidate the underlying mechanism in this case. Some studies have suggested that probiotics such as *Lactobacillus casei* are able to induce MUC2 gene expression (Mattar et al. 2002) and do not secrete proteases that cleave MUC2 (Subramani et al. 2010). However, our results showed that some probiotic species (*Bifidobacterium* spp. and *L. acidophilus*) were only significantly increased in the high-dose group, and we did not find significant increases in these species in the groups receiving low and moderate ethanol extract doses compared to the control group. Studies suggest that some other traditional Chinese medicines are able to induce MUC2 expression. For example, MUC2 expression increases when DSS mice are treated with *Abelmoschus manihot* (Zhang et al. 2019), and MUC2 is also upregulated when DSS rats are treated with Kuijieling decoction (Li et al. 2010).

Interestingly, our results showed that the differences in functional prediction between the control group and the ethanol extract groups decreased with increasing ethanol extract dose (Fig. 6). This phenomenon was similar to the results of other studies. Studies have shown that a low dose or high dose of JinQi Jiangtang tablets may affect insulin resistance through different pathways (Cao et al. 2019). Our results also suggested that a low dose or high dose of the ethanol extract may regulate metabolism through different gut microbiota pathways.

The results showed that there was a significant drug resistance pathway in the cefixime capsule group, but not in the ethanol extract groups (Fig. 6). Cefixime is an antibiotic. Many studies have demonstrated that the overuse or abuse of antibiotics results in bacterial drug resistance (Martin et al. 2015; Pham et al. 2018). In previous studies showing that



a: s_uncultured_bacterium_g_Bifidobacterium
b: g_Bifidobacterium
c: f_Bifidobacteriaceae
d:o_Bifidobacteriales
e:s_uncultured_bacterium_f_Muribaculaceae
f:g_uncultured_bacterium_f_Muribaculaceae
g:f_Muribaculaceae
h:s_uncultured_bacterium_g_Alloprevotella
i:g_Alloprevotella
j:f_Prevotellaceae
k:f_Rikenellaceae
l:o_Bacteroidales
m:s_*Enterococcus_faecalis*
n:s_*Enterococcus_faecium*
o:g_Enterococcus
p:f_Enterococcaceae
q:s_*Lactobacillus_acidophilus*
r: s_uncultered_bacterium_f_Clostridiales_vadinBB60
s:g_uncultured_bacterium_f_Clostridiales_vadinBB60
t:f_Clostridiales_vadinBB60
u: s_uncultered_bacterium_g_Lachnospiraceae_NK4A136
v:g_Lachnospiraceae_NK4A136
w:s_Robinsoniella_sp_RHS
x:g_Robinsoniella
y:s_uncultured_bacterium_f_Lachnospiraceae
z:g_uncultured_bacterium_f_Lachnospiraceae
a0:f_Lachnospiraceae
a1:f_Ruminococcaceae
a2:o_Clostridiales
a3:s_Firmicutes_bacterium_M10_2
a4:s_uncultured_bacterium_g_Dubosiella
a5:g_Dubosiella
a6:s_uncultured_bacterium_g_Faecalibaculum
a7:g_Faecalibaculum
a8:f_Erysipelotrichaceae
a9:o_Erysipelotrichales

Fig. 5 Different microebiota structures of all groups analysed by LEfSe (LDA scores > 4). The coloured dots (except the yellow dots) represent the different microbiota categories among all groups. The dot diameter is proportional to the relative abundance. Blue indicates significant bacterial abundance in the control group, orange indicates the low-dose group, green indicates the moderate-dose group, red indicates the high-dose group, and purple indicates the cefixime group. **a** Control group; **b** low-dose group; **c** moderate-dose group; **d** high-dose group; **e** cefixime capsule group

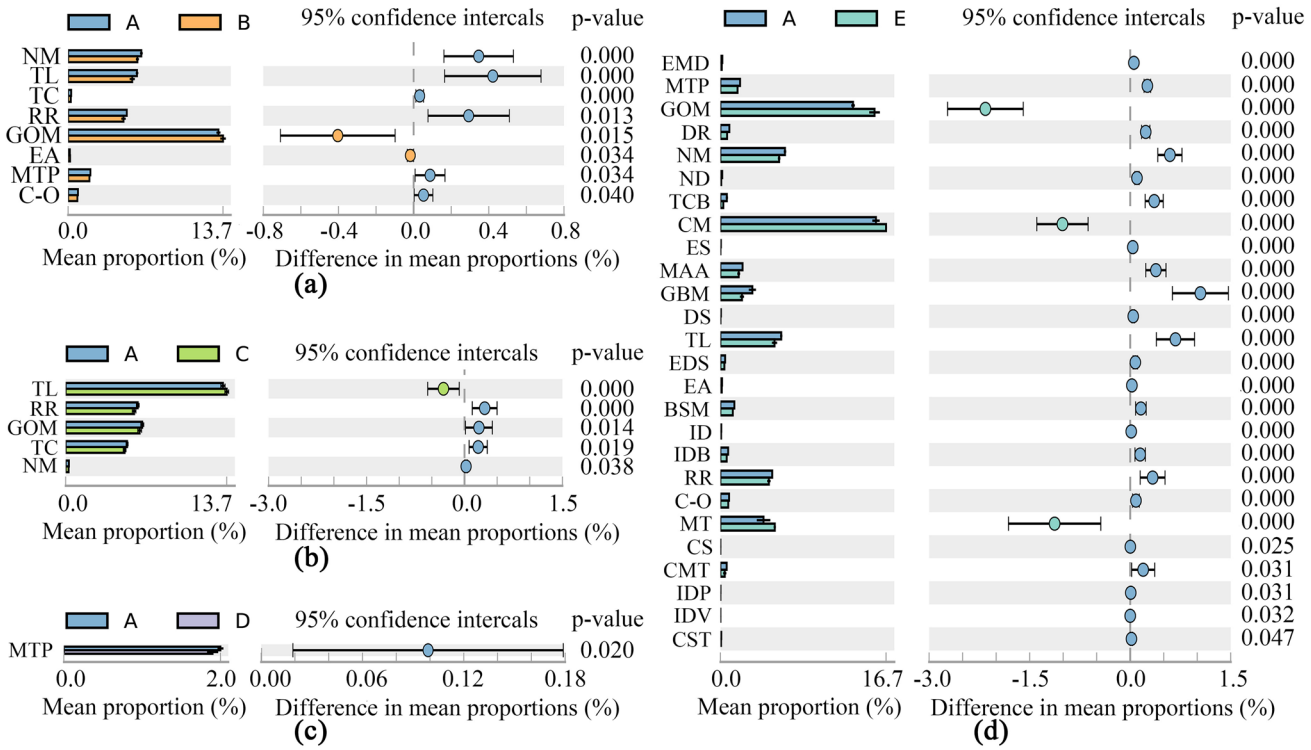


Fig. 6 Functional prediction differences between the control group and the other groups based on the KEGG database. *NM* nucleotide metabolism, *TL* translation, *TC* transcription, *RR* replication and repair, *GOM* global and overview maps, *EA* environmental adaptation, *MTP* metabolism of terpenoids and polyketides, *C-O* cancers: overview, *EMD* endocrine and metabolic diseases, *DR* drug resistance, *ND* neurodegenerative, *TCB* transport and catabolism, *CM* carbohydrate metabolism, *ES* excretory system, *MAA* metabolism of other amino acids, *GBM* glycan biosynthesis and metabolism, *DS* digestive system, *EDS* endocrine system, *BSM* biosynthesis of other secondary metabolites, *ID* immune diseases, *IDB* infectious diseases: bacterial, *MT* membrane transport, *CS* circulatory system, *CMT* cell motility, *IDP* infectious diseases: parasitic, *IDV* infectious diseases: viral, *CST* cancers: specific types. **a** Control group; **b** low-dose group; **c** moderate-dose group; **d** high-dose group; **e** cefixime capsule group

S. scandens has some antibiotic properties (Li et al. 2018), we did not observe any bacterial drug resistance, which suggested that the ethanol extract of *S. scandens* does not result in bacterial drug resistance, unlike other antibiotics in vivo.

In conclusion, our study suggested that the ethanol extract of *S. scandens* is able to maintain gut microbiota diversity in mice. The ethanol extract could increase sIgA and MUC2 concentrations. The high dose of the ethanol extract significantly upregulated some species of *Bifidobacterium* and *Lactobacillus* in the gut. In addition, the ethanol extract did not result in bacterial drug resistance.

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Compliance with ethical standards

Conflict of interest The authors declare no conflicts of interest.

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