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RESEARCH ARTICLE

Exploring the Therapeutic Potential of *Prunus mume* Ethanol Extract Against Multidrug-Resistant *Escherichia coli* in Mice

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ABSTRACT

The emergence of multidrug-resistant *Escherichia coli (E. coli)* necessitates the development of antibiotic alternatives. The screening of natural antibiotics with a higher therapeutic window and a low risk of antimicrobial resistance (AMR) is the aim of this study. In the current study, *E. coli* was isolated and purified from fresh diarrheal yak calves' fecal samples, collected from the Linzhou area of Tibet, by the plate streaking method, biochemical testing, and PCR. Drug sensitivity assay was performed following the "Kirby-Bauer disk diffusion" protocol. Through PCR detection of various drug resistance genes and mouse pathogenicity tests, isolation of highly resistant pathogenic *E. coli* (LZ-2) was achieved. The water and ethanol extracts of seventeen herbs, including *Caesalpinia sappan L.* and *Prunus mume,* were prepared, and their minimum inhibitory concentrations (MICs) against resistant *E. coli* were estimated. The results showed that the ethanol extract of *Prunus mume* has significant ($P<0.05$) anti-*E. coli* effects, with a MIC of 6.25 mg/ml. *In vivo* results indicated that when *Prunus mume* (ethanol extract) was administered intragastrical, the mortality proportion and damage to the intestine, spleen, and liver due to *E. coli* were reduced. It also significantly ($P<0.05$) improved the villus length, crypt depth, villus-to-crypt ratio (V:C), the serum antioxidant enzymes levels (SOD, MDA, GSH-Px and T-AOC) and significantly ($P<0.05$) reduced the level of pro-inflammatory mediators (IL-1β, IL-6, IL-10 and TNF-α), concluding the significant inhibitory action of *Prunus mume* against multidrug-resistant *E. coli*, providing a potential herbal alternative to antibiotics.

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INTRODUCTION

The World Health Organization (WHO) has notified AMR as among the top ten global health challenges, found to be directly accountable for 1.27 million mortalities in 2019 and an additional 4.95 million mortalities worldwide that year (Marston *et al*., 2016; Walsh *et al*., 2023). In this context, multidrug-resistant *E. coli* is a leading global health problem in humans and animals, with 45-52 cases per 100,000 people between 2010-2014, leading to an estimated annual cost of £14.3 million (Hiruy *et al*., 2024). *E. coli* leads to bloody diarrhea, fatigue, and nausea, and in severe cases, complications like hemolytic uremic syndrome (HUS) can result in impaired renal function and prove lethal (Nasrollahian *et al*., 2024). Calves are the main vectors for the pathogenic and zoonotic transmission of *E. coli*, with clinical symptoms including profuse loose stools and dehydration (Kolenda *et al*., 2015). Additionally, *E.*

coli disrupts the intestinal microflora, reducing beneficial bacteria, hence increasing the risk of secondary infections (Acres, 1985).

The recent shift from antibiotics to natural products is attracting scientists worldwide due to AMR (Matsakidou *et al*., 2019; Meng *et al*., 2024; Hu *et al*., 2025; Zhu *et al*., 2025). Previous studies showed the inhibitory effect of herbs and their components on *E. coli*. For instance, *Caesalpinia sappan* L. has shown potential anti-*E. coli* activity at a concentration of 200µg/mL (Puttipan *et al*., 2018; Pattananandecha *et al*., 2022). In the same way, *Forsythia suspensa*, due to the presence of triterpenoids and phenylethanoid glycosides, also proved effective against a variety of pathogens, including *E. coli* (Kuo *et al*., 2014; Zhou *et al*., 2022). Similarly, herbs like *Coptis chinensis* and *Punica granatum* have proven significant as broad-spectrum antimicrobials (Kuo *et al*., 2014).

Recent studies have shown that extracts from *Prunus mume* (Japanese apricot) have demonstrated significant inhibitory potential against different foodborne pathogens (Matsakidou *et al*., 2019; Mitani *et al*., 2018). The use of such herbal therapeutics helps to cope with the side effects of antibiotic usage (Gong *et al*., 2021). To the best of our knowledge, this is the first study evaluating the effectiveness of *Prunus mume* against resistant *E. coli*, providing a reference for the use of herbal products against *E. coli*-induced diarrhea.

MATERIALS AND METHODS

Experimental animals: A total of 18 fresh diarrheal fecal samples were collected randomly from free-range yak calves in the Linzhou region of Tibet, China. The collected samples were put into a 50mL sterile centrifuge tube in dry ice and transported to Nanjing Agricultural University for further analysis. No intervention was made on the calves other than fecal collection. Mice were purchased from the Comparative Medical Center of Yangzhou University, Yangzhou, China, for the second part of the experiment.

Ethical approval: All the experiments were conducted after approval from the Animal Welfare Committee on the Ethics of Animal Care and Use, Nanjing Agricultural University (NJAU.No.20230413054).

Herbs used in the given experiment: *Caesalpinia sappan* L, *Rhus chinensis* Mill, *Forsythia suspensa, Atractylodes macrocephala* Koidz, *Prunus mume, Isatis tinctoria, Adina rubella* Hance, *Viola yedoensis* Makino, *Punica granatum* L, *Macleaya cordata, Gardenia jasminoides* J. Ellis, Galla Turcica, Bupleuri Radix, *Lonicera japonica* Thunb, *Portulaca oleracea* L, *Rheum palmatum L* and *Coptis chinensis* Franchare were purchased from the Shenao Pharmacy, Bozhou, China (Institute of Cancer Research). All were cultivated in China under GAP standards.

Isolation and staining microscopy of pathogenic bacteria: Fecal samples of diarrhea yaks were inoculated into a nutrient broth medium and cultured at 37°C, 180 rpm for 12 h. This culture was inoculated into an LB solid medium and incubated at 37°C for 12h. Single colonies were selected, streaked on EMB agar, and cultured overnight at 37°C. Distinct colonies with green-metallic sheen were selected and further inoculated on MacConkey agar for pure culture. After five rounds of culture, single colonies were selected for Gram staining, followed by microscopy. The pure bacterial cultures were stored at -20°C in glycerol for further use.

Molecular identification of *E. coli* species: *E. coli* isolates were identified using PCR and 16S rRNA sequencing. Distinct single colonies from MacConkey agar were picked and inoculated into the nutrient broth. Cultures were incubated at 37°C with shaking at 180 rpm for 16 h, then centrifuged at 12000 rpm for 5 minutes. The supernatant was discarded, and the precipitate was collected. Genomic DNA was extracted according to the instructions on the respective DNA extraction kit (Solarbio Biotechnology Co. Ltd., Beijing, China), and the extracted DNA was stored at -4°C for further analysis.

For PCR amplification of the 16S rRNA gene, universal primers 27F (AGAGTTTGATCCTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) were used. The PCR mix consisted of 12.5µL 2 ×Taq PCR MasterMix, 1µL (10µM) of each forward and reverse primer, 9.5µL ddH_2O, and 1µL template DNA (thoroughly mixed and centrifuged), making a total volume of 25µL. The thermal cycling parameters were as follows: the reaction consisted of 35 cycles with initiation at 95°C for 3 min (required only once at the start of the response), followed by denaturation at 95°C for 15s, primer annealing at 53°C for 15s, elongation at 72°C for 30s, and a final extension at 72°C for 5 min. The PCR products were analyzed by electrophoresis on a 1% agarose gel. The parallel PCR products were sent to Shanghai Shenggong Bioengineering Co., Ltd., Shanghai, China, for phylogenetic sequencing. Sequences were analyzed by the Basic Local Alignment Search Tool (BLAST) through the National Center for Biotechnology Information (NCBI). Finally, a phylogenetic tree was constructed using the neighbor-joining method in Molecular Evolutionary Genetics Analysis (MEGA) version 11 software.

Antibiotic susceptibility testing (AST): According to the disk diffusion method specified by the Clinical and Laboratory Standards Institute (CLSI), 17 types of antibiotics were selected for AST. The isolated *E. coli* strains were evenly spread on nutrient agar plates. Then the antibiotic disks were evenly dispensed on the surface of the medium and incubated at 37°C for 16 h. The size of the inhibition zones for each antibiotic was observed and measured (Table 1).

Detection of antibiotic resistance genes: The drug-resistance genes of *E. coli* isolates were identified using PCR, with standard primer sequences for *E. coli-resistant* genes. The resistant genes detected included; β-lactam resistant genes (*SHV, blaCTX-M-1, blaTEM*), macrolide-resistant genes (*ermA, ermB, ermC*), tetracycline resistant genes (*Tet(A), Tet(B), Tet(C), Tet(D), Tet(M), Tet(K)*), aminoglycoside resistant genes (*ant (3'') -Ia, aac (6 ') -Ib, rmtB)*, and quinolone-resistant genes (*qnrS, qepA, oqxA*), using primer sequences for these antibiotic-resistance genes (Table 2).

Table 1: Type of antibiotic used and related zone of inhibition to estimate antibiotic sensitivity or resistance to *E. coli.*

Categories of antibiotics	Antibiotic	Zone of Inhibition (mm/diameter)		
		Resistant (R)	Intermediate (I)	Susceptible (S)
Sulfonamide	Sulfamethoxazole	≤10	11-15	≥16
Chloramphenicol	Chloramphenicol	≤25	26-28	≥29
Nitrofuran	Nitrofurantoin	≤14	15-16	≥17
Quinolone	Levofloxacin	≤13	14-16	≥17
	Ciprofloxacin	≤15	16-20	≥21
	Norfloxacin	≤12	13-16	≥17
Aminoglycoside	Kanamycin	≤13	14-17	≥18
	Fosfomycin	≤12	13-18	≥19
	Streptomycin	≤11	12-14	≥15
	Gentamicin	≤12	13-14	≥15
	Spectinomycin	≤14	15-17	≥18
Tetracycline	Tetracycline	≤14	15-18	≥19
	Minocycline	≤13	15-18	≥19
Beta-lactam	Ampicillin	≤11	12-14	≥15
	Amoxicillin	≤13	14-17	≥18
	Ceftriaxone	≤13	14-22	≥23
Polypeptide	Polymyxin B	≤8	9-11	≥12

Table 2: Primers targeting *E. coli* antibiotic resistance genes.

Gene	Sequences (5'-3')	Length (bp)	Annealing temperature (°C)
SHV	F:ATGCGTATATTCGCCTGTG R:CCTCATTCAGTTCCGTTTCC	502	53
blaCTX-M-1	F:CGCGCTACAGTACAGCGATA R:CCTTAGGTTGAGGCTGGGTG	434	58
blaTEM	F:TCGCCGCATACACTATTCTCAGAATGA R:ACGCTCACCGGCTCCAGATTTAT	445	60
ermA	F:GTTCAAGAACAATCAATACAGAG R:GGATCAGGAAAAGGACATTTTAC	557	51
ermB	F:CGAGTGAAAAAGTACTCAACC R:GCCGTGTTTCATTGCTTGATG		60
ermC	F:GCTAATATTGTTTAAATCGTCAATTCC R:GGCTCAGGAAAAGGGCATTTTAC		56
TetA	F:GCTACATCCTGCTTGCCTTC R:CATAGATCGCCGTGAAGAGG	210	56
TetB	F:TTGGTTAGGGGCAAGTTTTG R:GTAATGGGCCAATAACACCG	659	53
TetC	F:CTTGAGAGCCTTCAACCCAG R:ATGGTCGTCATCTACCTGCC	418	56
TetD	F:AAACCATTACGGCATTCTGC R:GACCGGATACACCATCCATC	787	54
Tet(M)	F:GAGGTCCGTCTGAACTTTGCG R:AGAAAGGATTTGGCGGCACT	594	58
TetK	F:ATAAATTGTTTCGGGTCGGTAAT R:AACCAGCCAACTAATGACAATGAT	1159	53
ant(3")-Ia	F:ATCTGGCTATCTTGCTGAC R:TATGACGGGCTGATACTGG	284	53
aac(6')-Ib	F:ATGACCTTGCGATGCTCTAGA R:CGAATGCCTGGCGTGTTT	717	56
rmtB	F:ATGAACATCAACGATGCCCTC R:TTATCCATTCTTTTTTATCAAGTATAT	756	53
qnrS	F:GCAAGTTCATTGAACAGGGT R:TCTAAACCGTCGAGTTCGGCCG	428	56
qepA	F:CTTGCACTTAGTTAAGCGCC R:GAGGTTTTGATAGTGGAGGTAGG	866	54
oqxA	F:GACAGCGTCGCACAGAATG R:GGAGACGAGGTTGGTATGGA	339	56

Mouse lethality assay: To evaluate the potential pathogenicity of the isolates, mice were challenged with three highly resistant *E. coli* strains (LZ-2, LZ-6, and LZ-11). To this end, twenty 6-week-old ICR mice with an average weight of 33 g were randomly divided into a control group and three test groups (LZ-2, LZ-6, and LZ-11) after three days of acclimatization, with five mice in each group. The colonies of *E. coli* were picked with a sterile inoculation loop and cultured at 37°C, 180 rpm until the OD_{600} (optical density at a wavelength of 600 nm using a spectrophotometer) of the *E. coli* suspension was 1.0 (~1 × 10^9 *E. coli* cfu/mL). Mice in the three test groups were injected with 0.2 mL *E. coli* suspension subcutaneously (S/C), however, mice in the control group received the same dose of sterile saline solution S/C. Clinical manifestations and mortality proportions were recorded at 6, 12, 24, 36, 48, and 72 h post-challenge. Deceased mice were dissected to examine gross and histopathological lesions in their organs.

Preparation of aqueous and alcohol extracts of herbs: For an aqueous extract after the herbs were crushed, 100g of each herb was weighed, soaked in 500 mL distilled water for 12h, decocted over high heat until boiling, then simmered over low heat for 1 hour, then filtered with gauze, and the residue was then decocted again using the same method as described above. The filtrate was combined in two batches and concentrated to 100 mL using a rotary evaporator. For the alcohol extract after crushing, 100g of each herb was weighed and added with 500 mL of 95% ethanol, sealed with plastic wrap, left for 24 h, and processed using an ultrasonic disperser. The ultrasonic dispersion parameters were set as follows: temperature 50 ~ 70°C, frequency 40 ~ 60 kHz, and duration 35 ~ 45 minutes. The ultrasonic dispersion was performed 3 times, with a 30s interval between each dispersion. The solution was filtered and concentrated to 100 mL using a rotary evaporator, with a drug extract ratio of 5:1. A freeze-dryer was used to freeze-dry the filtrate to obtain the herbal water extract, which was then stored at 4°C for future use.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of herbs against *E. coli:* A highly pathogenic and highly resistant *E. coli* (LZ-2) was selected as the test strain to test the antibacterial activity of herbs. The MIC and MBC were determined using the broth microdilution method per CLSI guidelines (Humphries *et al*., 2021). Herbal extracts at twice the concentrations (2x) were added into sterilized 96-well polypropylene microtiter plates, diluted in Luria-Bertani (LB) broth to a volume of 100μL, and mixed with an equal volume of bacterial suspensions (1×10^6 cfu/mL). The MIC was recorded as the lowest concentration at which there was no visible bacterial growth after incubation for 18-20 h at 37°C, then 10μL of liquid culture was taken from each well and incubated at 37°C for 48 hours to check the bacterial growth. The MBC was recorded as having the lowest concentration with no visible bacterial colonies after 48 hours of incubation at 37°C.

Effect of herbal extracts on the growth curve of *E. coli:* According to the experimental protocol designed by Wei *et al*. (2023), 200μL LB medium was added to a sterile 96-well plate, and then 1 MIC concentration of herbal extract was added and thoroughly mixed. In each well, 20μL bacterial suspension (1×10^6 cfu/mL) was added and cultured at 180 rpm/min in a gas bath shaker at 37°C. The OD_{600} was measured every 2 hours using a spectrophotometer, and the growth curve was drawn.

Estimation of anti-*E. coli* activity of ethanol extract of *Prunus mume* against *E. coli* infection in mice: After three days of acclimatization, ten ICR mice aged four

weeks with an average weight of 29g were randomly divided into the model group (n=5) and the treatment group (n=5). Each group was challenged intraperitoneally (IP) with 0.2 mL of *E. coli* suspension (OD_{600}=1.0). After the *E. coli* challenge, the mice in the treatment groups were administered the ethanol extract of *Prunus mume* (1000mg/kg). In contrast, the model group was administered an equal volume of sterile saline. Clinical manifestations and the mortality proportions were recorded to estimate mice survival rates.

Protective effect of *Prunus mume* ethanol extract against *E. coli* infection in mice: Forty-four-week-old ICR mice, with an average weight of 29g each, were acclimatized for three days and then randomly divided into the model group (n = 20) and the prevention group (n = 20). In addition to the basic feed, each mouse in the model group was orally gavaged with 0.2 mL sterile normal saline daily, while each mouse in the prevention group received ethanol extracts of *Prunus mume* (1000mg/kg) daily through oral gavage and was weighed daily. From the 8th day, the mice in both groups were IP administered with 0.2 mL of *E. coli* suspension (OD_{600} = 1.0). After 24 h of the *E. coli* challenge, blood samples were collected from the eyeballs of each mouse in both groups. The mice were then dissected and observed for visceral lesions. The heart, liver, spleen, lung, and kidney were collected precisely after removing the fascia and adipose tissue. The organ-to-body weight index was calculated (organ index = organ weight (g)/body weight of mice (g)).

For further analysis, three mice were randomly selected from each group. After blood collection from the eyeballs, about 0.1g of heart, liver, spleen, lung, kidney, duodenum, jejunum, ileum, cecum, colon, and rectal tissues were weighed and placed in a sterilized 2 mL centrifuge tube, 1 mL of normal saline and 2 grinding beads were added. After grinding, the supernatant was taken and diluted to 10 times its original volume. The diluted 20μL bacterial solution was inoculated into the MacConkey agar containing azithromycin and cultured in the incubator at 37°C for 18 h. The colony number was recorded, and the bacterial load in each organ and intestine was calculated. Additionally, three random samples were chosen for each of the duodenum, jejunum, and ileum and subjected to hematoxylin and eosin (H&E) staining to evaluate the histopathological lesions.

After standing at 4°C for 4 h, the collected blood was centrifuged at 4°C and 4000 rpm for 10 minutes. Serum was collected and the level of antioxidant enzymes; glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), malondialdehyde (MDA), and total antioxidant capacity (T-AOC) were checked using kits purchased from Jiancheng Institute of BioEngineering, Nanjing, China. Levels of inflammatory mediators: Interleukin-6 (IL-6), IL-10, IL-1β, and tumor necrosis factor-α (TNF-α), were quantified using commercial detection kits (purchased from Shanghai Jianglai Biotechnology Co., LTD., Shanghai, China) according to the manufacturer's recommendations.

Statistical analysis: Analysis of variance (ANOVA) to compare groups, IBM SPSS (version 26.0) for detailed statistical computations, and GraphPad Prism (version 8.0) for graphical representation, considering P-values <0.05 as statistically significant.

RESULTS

Isolation, purification, and identification of *E. coli:* Eleven strains of bacteria were isolated and purified from eighteen yak calves' fecal samples. After culturing on MacConkey agar, smooth colonies with pink, round, convex, moist, and well-defined edges were formed (Fig. 1a). On EMB agar, the colonies appeared round and smooth with a green metallic sheen (Fig. 1b). Gram staining revealed the colonies as pink, round bacilli (Fig. 1c). The eleven strains were labeled subsequently from LZ-1 to LZ-11.

Molecular biological identification: The 16S rRNA PCR product displayed a distinct bright band at 1500 bp on a 1% agarose gel. The sequencing results were analyzed for homology using the BLAST tool on the NCBI website. All strains exhibited 99% homology with *E. coli*. The sequences were compared with 16S rRNA sequences of the other bacteria using BLAST, and a phylogenetic tree was subsequently constructed. The selected reference sequences included *E. coli* (MN153456.1, MN208206.1, OQ891222.1, ON849073.1, OK253988.1, OQ405419.1) and *Staphylococcus aureus* (NZ KV839669.1) (Fig. 2a, b).

Antibiotic sensitivity testing (AST): The results of AST conducted on eleven isolated *E. coli* strains are presented in Fig. 3. All isolates exhibited 100% resistance to chloramphenicol. Additionally, some isolated strains demonstrated resistance to kanamycin, fosfomycin, spectinomycin, ampicillin, amoxicillin, and ceftriaxone, indicating multidrug resistance. However, they were relatively sensitive to the other antibiotics.

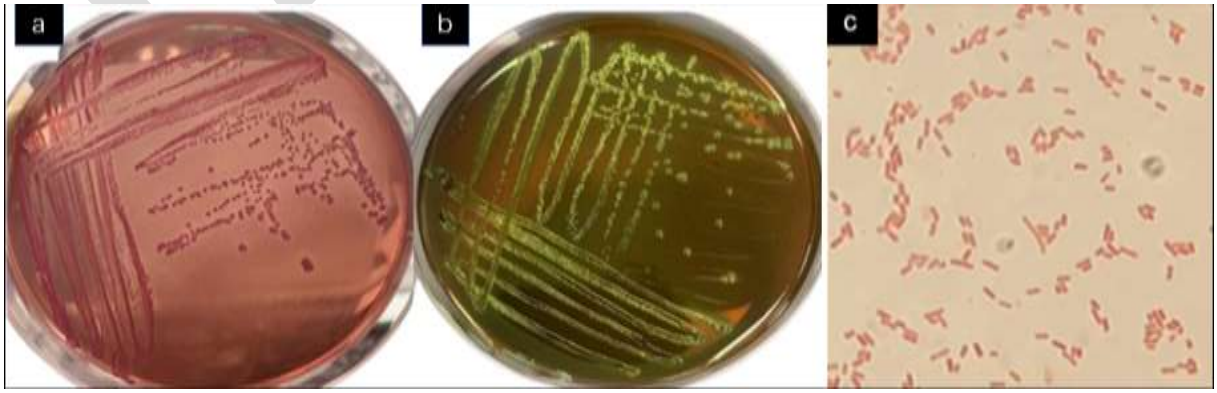

Fig. 1: (a) Colony characteristics of *E. coli* (LZ-2) on MacConkey agar; (**b)** Colony characteristics of *E. coli* on EMB agar; (**c)** Gram staining of the *E. coli* (×1000).

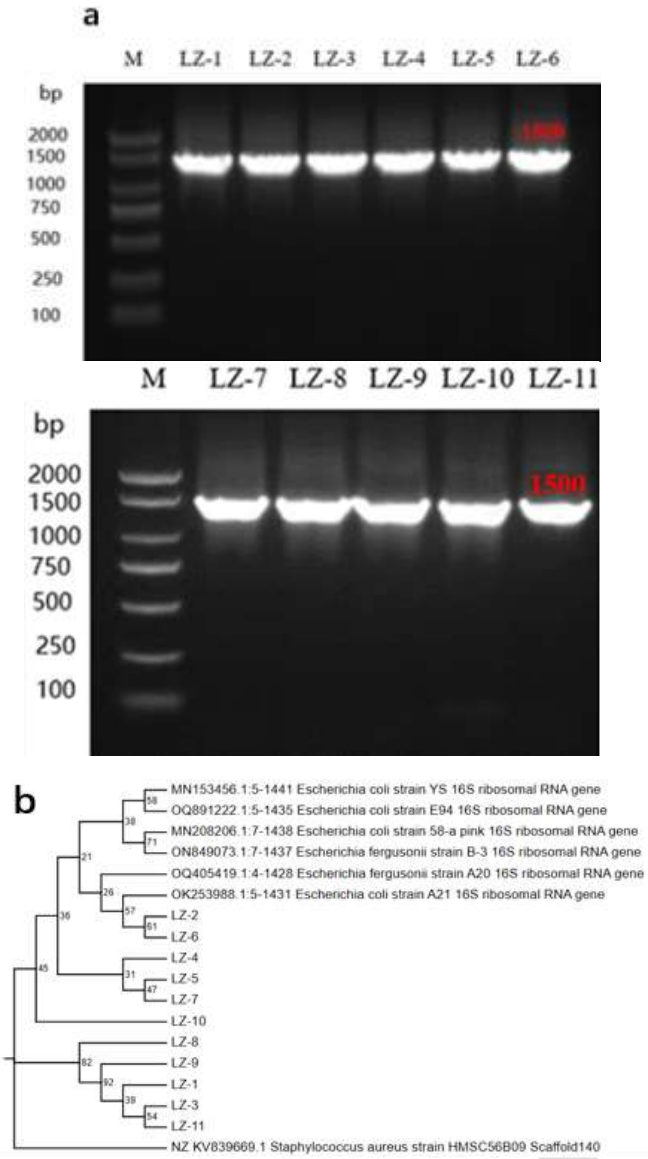

Fig. 2: Detection results of 16S rRNA products from 11 isolated strains, with M representing the 2000bp DNA marker (a). Phylogenetic tree analysis based on 16S rRNA gene sequences of isolated bacteria (b).

Detection of antibiotic resistance genes: PCR amplification results revealed that isolates carried multiple antibiotic resistance genes, including β-lactam resistant genes (*blaSHV, blaCTX-M-1*, and *blaTEM*), macrolides-resistant genes (*ermA, ermB, ermC*), tetracycline resistant genes (*TetK, TetB, TetC, TetD, TetA,* and *TetM*), aminoglycoside resistant genes (*ant (3 ") -Ia, aac(6')-Ib,* and *rmtB*), and quinolone-resistant genes (*qnrS, qepA, oqxA*). No other drug-resistant genes were detected (Fig. 4). As shown in Fig. 5, the prevalence of major drug-resistant genes ranged from 9.1% to 100%.

Pathogenicity of *E. coli* strains in mice: Necropsy revealed hemorrhage foci in the liver and duodenum, along with intestinal bleeding, swelling, rupture, and leakage of intestinal contents in different groups challenged with *E. coli* (Fig. 6). Based on the results of AST and mouse pathogenicity experiment, LZ-2 exhibited strong antibiotic resistance and high pathogenicity, making it the strain selected for further experiment.

Inhibitory activity of herbal extracts against *E. coli:* The MIC and MBC of each herbal extract against *E. coli* (LZ-2) are shown in Table 3. The water extracts of *Prunus mume* and Galla Turcica exhibited the strongest MIC of 3.125 mg/mL and 12.5mg/mL, respectively, along with MBC values of 12.5mg/mL and 25mg/mL. Among the ethanol extracts, *Caesalpinia sappan* L and *Prunus mume* demonstrated the strongest MIC values of 1.563mg/mL and 6.25mg/mL, respectively, along with MBC values of 1.563mg/mL and 12.5mg/mL, respectively. At a concentration of 3.125mg/mL, both water and ethanol extracts of *Prunus mume* demonstrated significant inhibition of *E. coli*. Similarly, the ethanol extract of *Caesalpinia sappan* L at a concentration of 1.563mg/mL also exhibited a significant inhibitory effect on *E. coli* growth. However, the ethanol extract of *Caesalpinia sappan* L had a very low extraction yield of only 1%. Hence, *Prunus mume* was selected for use in further experiments (Fig. 7A, B).

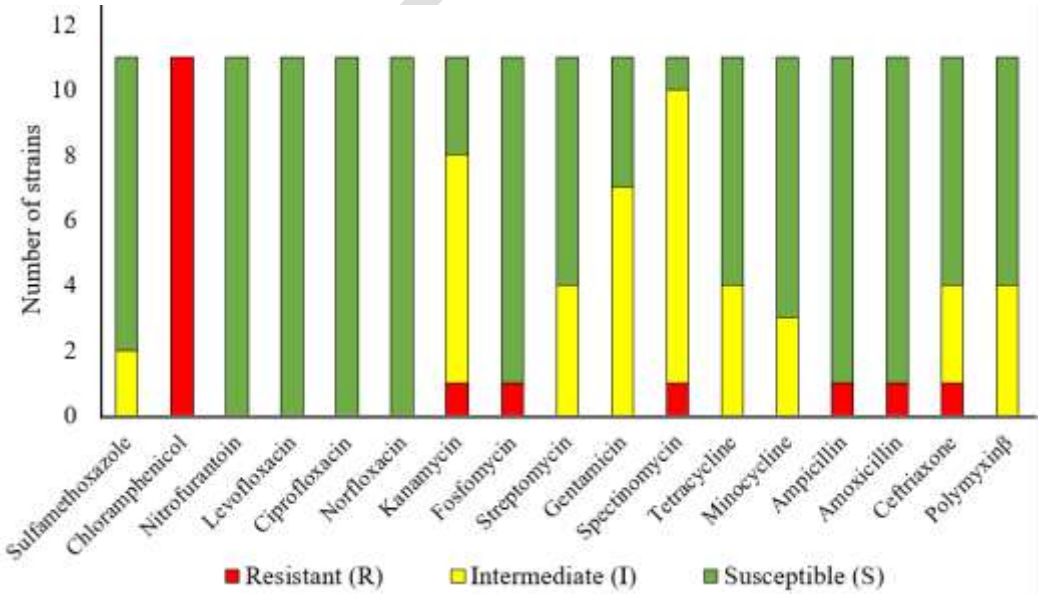

Fig. 3: Drug sensitivity results of 11 *E. coli* strains. The bar chart represents the *E. coli* strains classified as resistant (R), intermediate (I), or susceptible (S) to 11 antibiotic treatments with 100% resistance to chloramphenicol, intermediate resistance to sulphamethoxazole, kanamycin, streptomycin, gentamicin, spectinomycin, tetracycline, minocycline, and polymyxin, and susceptibility to rest of the antibiotics.

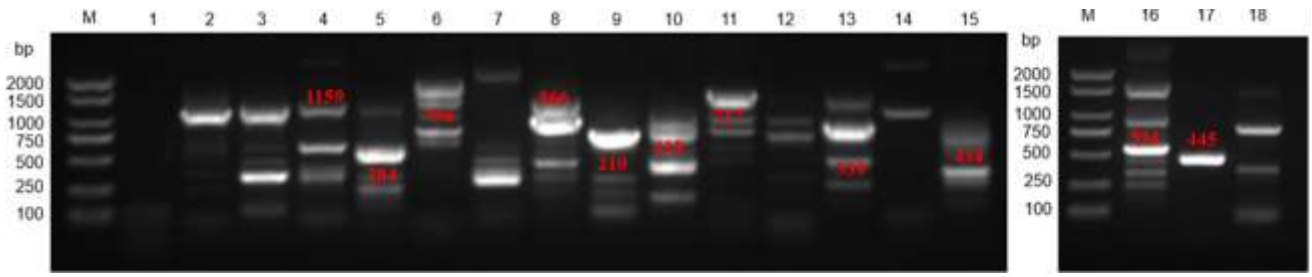

Fig. 4: M.DNA Marker DL2000;1. ermA;2. SHV;3. TetB;4. TetK;5. ant(3")-Ia;6. rmtB;7. TetD;8. qepA;9. TetA;10. TetC;11. aac(6')-Ib;12.qnrS;13. oqxA;14. ermC;15. blaCTX-M-1;16. Tet(M);17. blaTEM;18. ermB.

Table 3: MIC and MBC of 17 water- and ethanol-based herbal extracts (mg/mL) on *E. coli*.

Herbs	MIC (water extraction)	MBC (water extraction)	MIC (ethanol extraction)	MBC (ethanol extraction)
Caesalpinia sappan L	50	50	1.563	1.563
Rhus chinensis Mill	50	50	12.5	25
Forsythia suspensa	50	50	50	100
Atractylodes macrocephala Koidz	200	＞200	200	200
Prunus mume	3.125	12.5	6.25	12.5
Isatis tinctoria	100	＞200	200	＞200
Adina rubella Hance	100	＞200	25	25
Viola yedoensis Makino	50	50	25	100
Punica granatum L	25	50	12.5	50
Macleaya cordata	25	100	50	200
Gardenia jasminoides J.Ellis	100	＞200	200	200
Galla Turcica	12.5	25	100	100
Bupleuri Radix	＞200	＞200	＞200	＞200
Lonicera japonica Thunb	50	50	50	100
Portulaca oleracea L	＞200	＞200	100	200
Rheum palmatum L	＞200	＞200	50	100
Coptis chinensis Franch	50	100	50	50

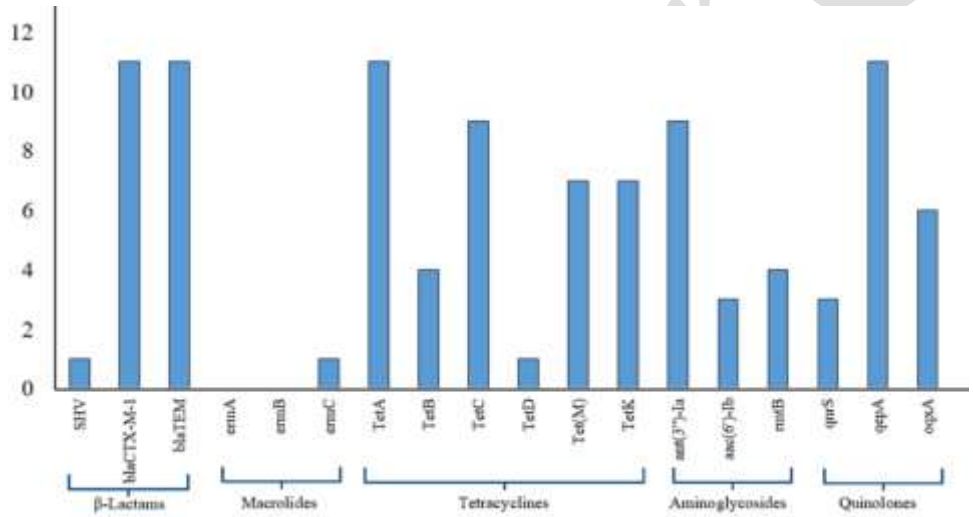

Fig. 5: Distribution of antibiotic resistance genes in *E. coli* isolates. The bar chart demonstrates the prevalence of antibiotic-resistance genes detected in *E. coli* isolates. It represents the variability in gene distribution across different *E. coli* isolates.

Comparative survival rate of *E. coli*-challenged and treated mice: Following the *E. coli* challenge, both the model and treatment groups exhibited clinical symptoms including general malaise, loss of appetite, ruffled fur, and chills. These symptoms were less severe in the treatment group compared to the control group, indicating the strong pathogenicity of *E. coli* in mice. After 36 hours post-challenge, all mice in the model group succumbed, showing 0% survivability. In contrast, the treatment group showed a survival rate of 60%, with some mice dying at 36 and 48 h (Fig. 8A, B).

Protective effect of *Prunus mume* (ethanol extract) against *E. coli*-induced damage: Daily weighing revealed that the intragastric administration of ethanol extract of *Prunus mume* did not affect the growth performance of mice. After the challenge with *E. coli*, mice in the model group exhibited ruffled fur, severe lethargy, anorexia, and a significant purulent red discharge at the corner of their eyes. The prevention group exhibited the same symptoms but significantly ($P<0.05$) less severe. Upon dissection, mice in the model group had severe liver and spleen congestion, enteritis, and a shortened small intestine compared to the prevention group (Fig. 9A, B).

Microscopically, mice in the prevention group demonstrated an increased villus height, enlarged intestinal epithelial surface area, shallow crypts, and enhanced maturation rate of the intestinal epithelial cells. Consequently, the ratio of villus height to crypt depth, an

Fig. 6: Gross pathological lesions in experimental groups after challenge with different *E. coli* strains in mice.

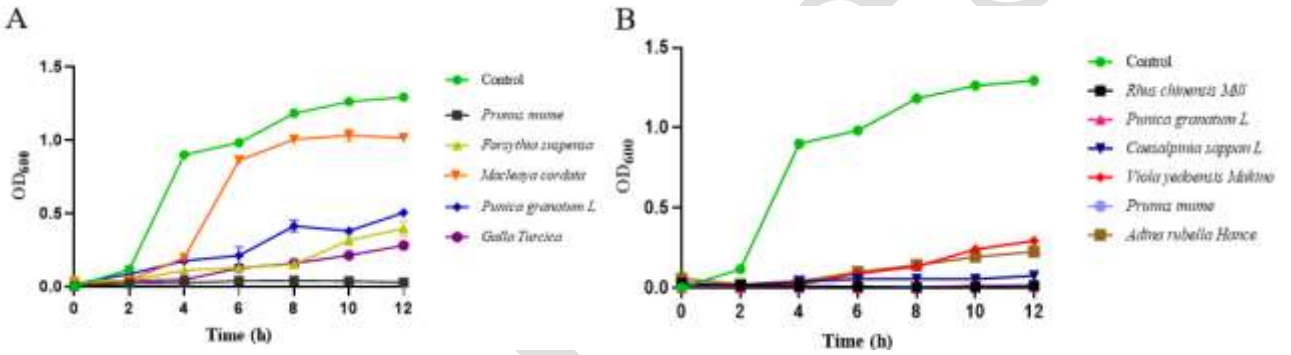

Fig. 7: Anti-*E. coli* activity of the water and ethanol extracts of herbs over 12 hours. It can be seen that both water and ethanol extracts of *Prunus mume* exhibited higher anti-*E. coli* activity, significantly suppressing *E. coli* growth. Error bars showed standard deviations (SD) from threefold replicas. A: Water extract, B: Ethanol extracts.

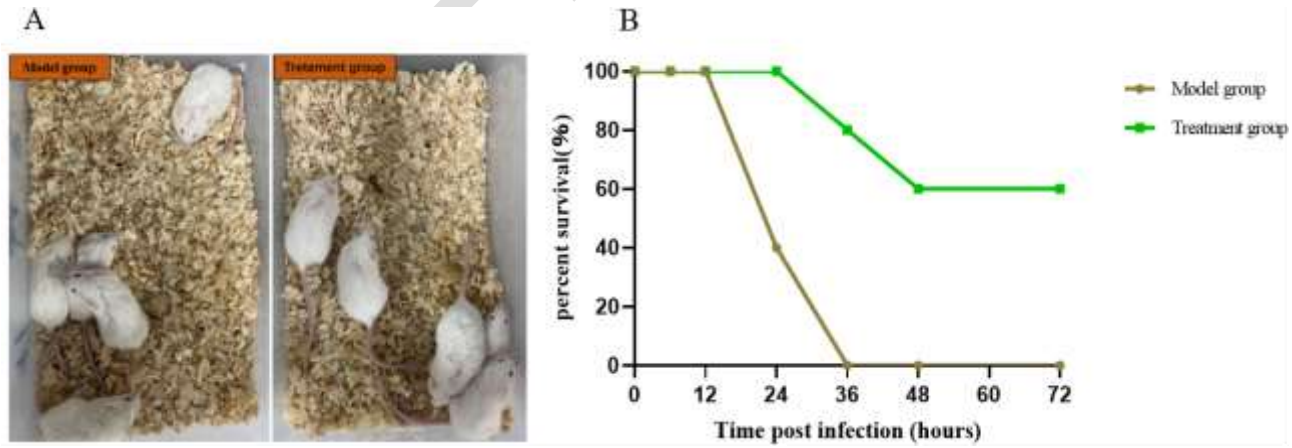

Fig. 8: (A) Clinical symptoms of mice infected with *E. coli*. (B) Mouse survival rate curve.

An indicator of intestinal absorption function showed significant improvement. Thus, the intestinal morphology indicated that the ethanol extract of *Prunus mume* could significantly ($P<0.05$) mitigate damage to the intestinal tissue structure caused by *E. coli* invasion (Fig. 9C).

Estimation of organ indices and bacterial load in organs of mice: As shown in Fig. 10A, the spleen index of the model group was significantly ($P<0.05$) higher than that of the prevention group, indicating that the intragastric administration of *Prunus mume* ethanol extract

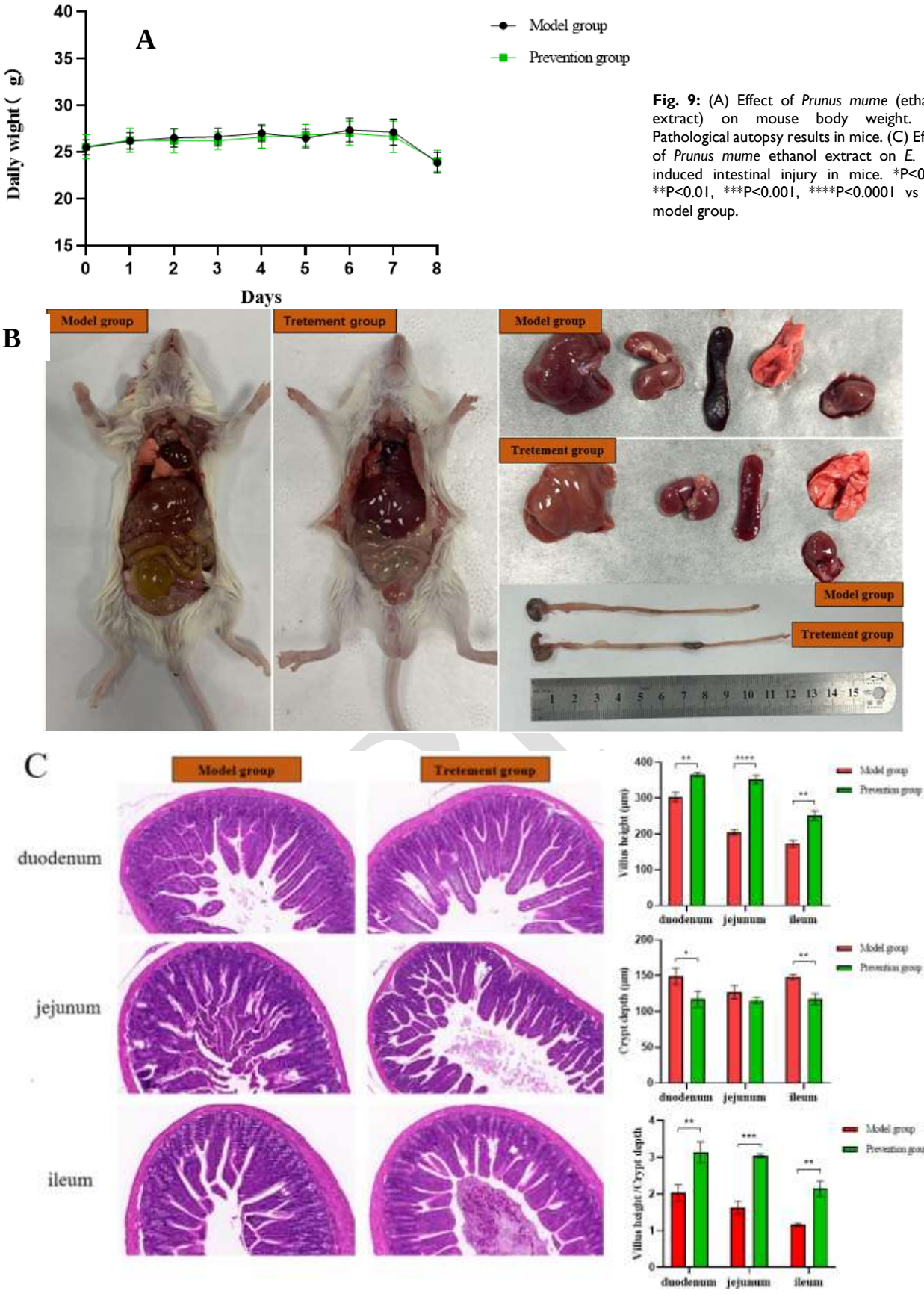

Fig. 9: (A) Effect of *Prunus mume* (ethanol extract) on mouse body weight. (B) Pathological autopsy results in mice. (C) Effect of *Prunus mume* ethanol extract on *E. coli*-induced intestinal injury in mice. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs the model group.

reduced spleen congestion and edema caused by *E. coli* infection, offering a protective effect on the spleen without significantly affecting other organs. Twenty-four hours after the challenge, the concentration of *E. coli* in various organs and intestinal segments was considerably higher in the model group. Daily gavage with *Prunus mume* ethanol extract significantly (P<0.05) reduced *E. coli* colonization in multiple organs and intestinal segments of the mice, exerting a protective role (Fig. 10 B).

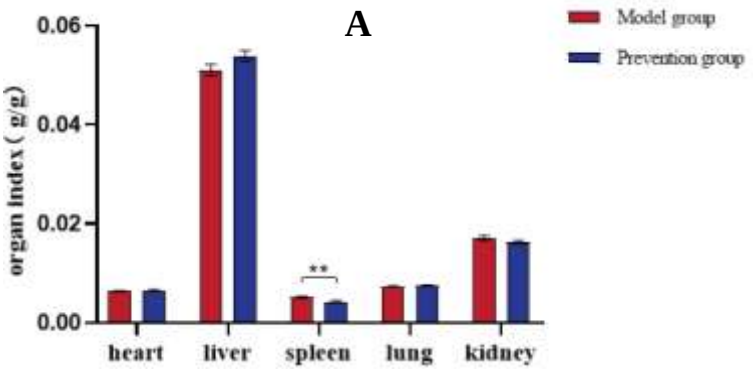

Fig. 10: (A) Effect of *Prunus mume* ethanol extract on organ index in mice. (B) The effect of ethanol extract of *Prunus mume* on bacterial count in organs and intestines of mice. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 vs the model group.

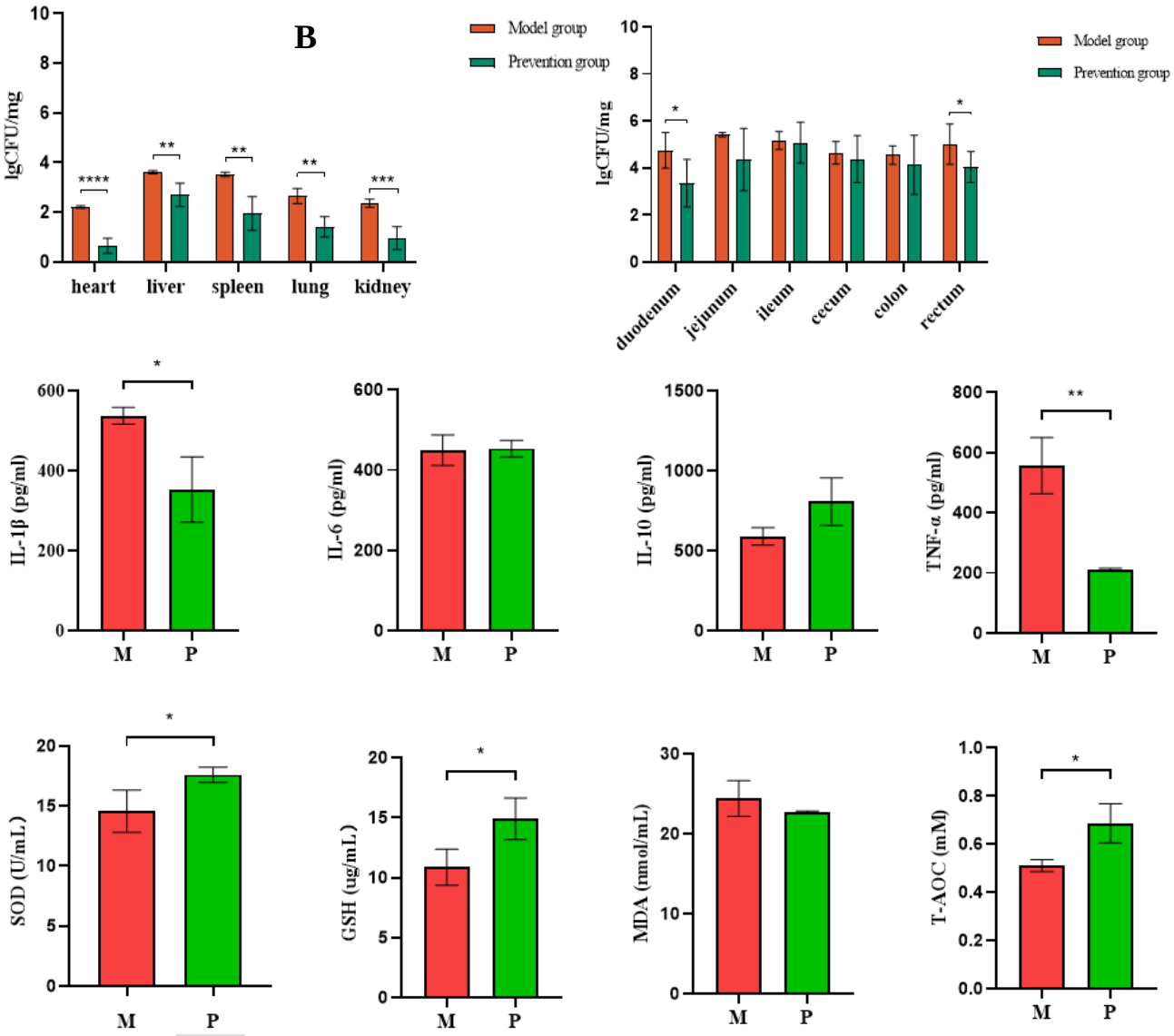

Fig. 11: Effect of ethanol extract of *Prunus mume* on antioxidant enzyme levels and inflammatory mediators in the serum of mice in the prevention and model groups.

Ethanol extract of *Prunus mume* alleviates inflammation and oxidative stress: As shown in Fig. 11, intragastric administration of the ethanol extract of *Prunus mume* significantly (P<0.05) enhanced T-AOC in the serum of mice and significantly (P<0.05) reduced the level of TNF-α in the prevention group compared to the model group. It indicated that the ethanol extract of *Prunus mume* can alleviate the inflammatory response in mice induced by the *E. coli* infection.

DISCUSSION

Diarrheagenic *E. coli* strains demonstrate alarming antimicrobial resistance (in ~ 80% of cases) against commonly used antibiotics like cotrimoxazole, complexing the therapeutic avenues (Abbasi *et al*., 2020; Yandag *et al*., 2023). In the given experiment, all strains demonstrated 99% genetic concordance to *E. coli* in BLAST analysis,

consistent with observations from comparable studies that highlight the accuracy of 16S rRNA sequencing for bacterial characterization (Patel, 2001; FR *et al*., 2019). Expanding phylogenetic studies to encompass a wider range of bacterial strains may provide valuable understanding between various enteric pathogens and *E. coli* (Saraceno *et al*., 2024).

The AST results are per the previous studies regarding *E. coli*, highlighting its resistance to different antimicrobials, e.g., aminoglycosides and β-lactams (Budiarso *et al*., 2021). The multidrug-resistant strains (resistant to ceftriaxone, fosfomycin, and kanamycin) are a significant threat to both public health and the livestock sectors, as these strains are increasingly linked to therapeutic inefficiency and critical infections (Verma, 2024). Additionally, the PCR results confirming the presence of genes linked to antibiotic resistance, e.g., qnrS, ermA, blaCTX-M-1, and blaSHV, highlighted the genetic foundation underlying the detected phenotypic resistance (Hsiao *et al*., 2024). The variations in gene distribution among the isolates suggest complex evolutionary dynamics and potential horizontal gene transfer mechanisms that facilitate the spread of resistance traits (Cookson *et al*., 2022).

Necropsy findings of hemorrhagic foci and enteric damage highlight the severe tissue damage of *E. coli*, aligning with its known role in intestinal pathology (Bernabeu *et al*., 2024). Despite advancements, the role of antibiotic resistance in enhancing the pathogenicity of the *E. coli* strain LZ-2 is still unclear. Examining host factors and microbiota variations could clarify *E. coli* pathogenesis and treatment (Herzog *et al*., 2023). The literature highlighted the antimicrobial properties of *Prunus mume* and different herbal extracts against various bacterial strains (Barnes, 2003; Bessalah *et al*., 2023). *Prunus mume*, has shown notable protective effects on intestinal health, particularly in preserving the morphology of intestinal villi. The literature highlighted that *Prunus mume* ethanol extracts improved the villus height and villus-to-crypt depth ratios, key indicators of enteric health and absorption, in mice infected with *E. coli* (Kim *et al*., 2020). The results of this study are also in alignment with the existing literature, indicating the mitigation of inflammatory reactions and oxidative stress levels, which otherwise can lead to gut dysfunction (Xia *et al*., 2010; Shin *et al*., 2013).

Research indicated that *Prunus mume* exhibits significant anti-inflammatory effects, as evidenced by the downregulation of pro-inflammatory cytokines, e.g., TNF-α and IL-6 (Kim *et al*., 2005). The daily gavage of *Prunus mume* reduced *E. coli* colonization in different organs, underscoring its antibacterial efficacy (Gong *et al*., 2021). Regarding oxidative stress, in the current study, a significant increase in T-AOC in *Prunus mume*-treated mice was observed, which is consistent with previous findings where *Prunus mume* extracts significantly lowered MDA through upregulating the levels of SOD and GSH-Px activities (Lee *et al*., 2016; Zapata *et al*., 2021). These enzymes are crucial in alleviating the oxidative stress due to infections, hence, preserving the integrity and functioning of different organs (Jo *et al*., 2006). However, the identification of particular biologically active ingredients with their associated modes of action, and interrelations between the composition of herbal products and varied host factors, needs to be focused on in the future.

Conclusions: The current study results indicated that *Prunus mume* ethanol extract inhibited resistant *E. coli*, exhibiting its anti-inflammatory, antioxidant, and antibacterial potential. The extract also decreased systemic and enteric damage by reducing *E. coli* proliferation in vital organs, upregulating the levels of antioxidant enzymes, and downregulating the pro-inflammatory mediators in the murine model. These findings demonstrated that *Prunus mume* could prove an effective natural alternative to antibiotics to cure *E. coli*-induced damage. However, still, research is needed to purify and understand the active ingredients and their effectiveness in diverse promises.

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