In Vitro Antibacterial Activities of the Tibetan Herbal Medicines Against the Staphylococcus Isolated From Mastitis of Guanzhong Dairy Goat

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Research Article

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Abstract

Background: Dairy goat mastitis is one of the most significant disease with effect on Guanzhong dairy goat breeding. The purpose of this study was to screen the pathogenic bacteria from milk samples of the mastitis of Guanzhong dairy goat and evaluate the antibacterial activities of six Tibetan herbal medicines, including *Swertia bimaculata*, *Gentiana urnula*, *Uncaria rhynchophylla*, *Aconitum flavum*, *Dracocephalum tanguticum* and *Lagotis brachystachy* on Staphylococcus isolated.

Results: Fifty-two bacteria species, and a total of 149 different isolates were identified from 55 milk samples, and Staphylococcus was the predominant species as it accounted for 36.91% (55/149) of the identified isolates, and *S. aureus* (26/55) was the majority of the Staphylococcus isolates. Histopathological examination showed that obvious pathological changes were observed in the liver, kidney and lung tissues of the mice infected with *S. aureu*s. Most of the Staphylococcus isolates expressed the multidrug resistance except for *S. aureus* and *S. hemolyticus*. The water extracts of several Tibetan medicinal plants exhibited distinguished *in vitro* antibacterial activities for the Staphylococcus, especially the *S. aureus* as well as the MDR isolates. *Lagotis brachystachy* has the most significant antibacterial activity, and followed by the *Aconitum flavum*, *Uncaria rhynchophylla*, *Swertia bimaculata*, and *Dracocephalum tanguticum*, while the *Gentiana urnula* showed the weaker inhibitory effects to the tested Staphylococcus isolates.

Conclusion: This study indicated that Tibetan herbal medicines could be a potential candidate for the treatment the mastitis of dairy goat in clinical application.

Background

Mastitis is the most serious disease in dairy goats due to financial losses attributed to its negative impact on milk quantity and components [1]. Mastitis is caused by multiple etiological agents. While, the Staphylococcus, especially the *Staphylococcus aureus* (*S. aureus*) is considered to be one of the major etiological agents causing of mastitis in livestock [2, 3]. Although antimicrobial therapy remains the mainstay of treatment for goat mastitis, the antimicrobial agents were gradually restricted due to the increasing incidence of antimicrobial resistance and residue problems in milk products, which is harmful to human health [4, 5]. Therefore, it is extremely urgent to screen the safe, novel, cost effective and alternative therapeutic drugs, such as the bioactive plant-derived products. Lots of literatures showed that herbs are interesting alternatives to synthetic antimicrobial drugs or antiseptics as they can be very safe under the suitable dosage [6]. Tibetan medicine has been widely used for more than 1000 years as one of the China's traditional medical systems, and it is receiving greater attention from the public, scholars, and the media. Tibetan herbal medicines usually grow on the Qinghai-Tibet Plateau with special living environment of high elevation and strong sunlight irradiation, and they have particular biological activity, such as anti-inflammatory, antioxidant and immunotropic activities [7]. Multiple Tibetan herbal medicines have well-documented antimicrobial activity [7–9]. Moreover, they are safe to use, and there has been no report of resistance among the pathogens. However, we have a lack of understanding about the efficacy of Tibetan herbal medicines on the pathogenic bacteria isolated from the mastitis of dairy goat.

The current study aimed to screen the pathogenic bacteria, and further evaluate the *in vitro* antibacterial activities of six Tibetan herbal medicines, including *Swertia bimaculata*, *Gentiana urnula*, *Uncaria rhynchophylla*, *Aconitum flavum*, *Dracocephalum tanguticum* and *Lagotis brachystachy* on Staphylococcus isolated from the mastitis of Guanzhong dairy goat and to develop as an alternative to antibiotic treatment in therapy mastitis of Guanzhong dairy goat.

Results

Bacteriological examinations

In this study, the preliminary clinical examination of the mammary glands of 3240 dairy goats indicated that 55 (1.7%) goats with clinical mastitis, of which 8/55 (14.5%) were primiparous and 47/55 (85.5%) were multiparous. Of the 55 goats, 43 (78.18%) goats had only one udder half affected.

We analyzed 55 milk samples collected from 55 dairy goats with clinical mastitis. Ultimately, one hundred and forty-nine bacteria isolates were identified and divided into fifty-two different bacterial species, and Staphylococcus appeared in each milk sample, and it was the predominant species as it accounted for 36.91% (55/149) of the identified isolates, followed by Enterobacter 15.44% (23/149), Proteus 12.08% (18/149), Klebsiella 10.07% (15/149), and others 25.5% (38/149). Among the 55 Staphylococcus isolates, *S. aureus* (n = 26) was the predominant as it isolated in 26 samples, followed by *S. haemolyticus* (n = 10), *S. epidermidis* (n = 6), *S. saprophyticus* (n = 5), *S. gallinarum* (n = 4), *S. galilis* (n = 1), *S. coni* (n = 1), *S. simulans* (n = 1), and *S. equorum* (n = 1). While the Streptococcus was not isolated in this study.

Antimicrobial susceptibility profile

The antimicrobial resistant phenotype of fifty-five Staphylococcus isolates from dairy goat mastitis to 9 antimicrobial agents were showed in Table 1. The tested Staphylococcus isolates were resistance to all tested β-lactam antibiotics (penicillin, ampicillin, and ceftriaxone). *S. aureus* and *S. haemolyticus* were sensitive to amikacin, clindanlycin, ciprofloxacin, acetylisovale-ryltylosin and doxycycline.More than 60% of *S. epidermidis*, *S. saprophyticus* and *S. gallinarum* were resistance to the tested antimicrobial agents, and most of them expressed the MDR phenotype. *S. galilis*, *S. coni*, *S. simulans* and *S. equorum* were resistance to all tested antimicrobial agents.

Pathogenicity of the isolated bacteria

The *S. aureus* isolates were used in the pathogenicity experiment as it was the most prevalent bacteria based on the bacteriological examinations. We observed the general performance of the tested mice in order to know the effects of *S. aureus* on clinical symptoms of mice. We found that the majority of mice infected with *S. aureus* exhibited clinical signs, including mental depression, slow response, lethargy, reduced spontaneous activity and anorexia,. The mice of the tested group began to die after two days. After 7 days, the survival rate of mice in the control group was 100%, whereas the fatality rate in the tested group was 80%.

Histopathological examinations of the main organs of mice in tested group and negative control group were performed. In the control group, the liver, heart, lung and kidney tissues of mice exhibited normal structure (The Histopathological images were not shown). Compared to the control group, obvious pathological changes were observed in the liver, kidney and lung tissues of the mice in the tested group. Necrotic foci were observed in liver, some hepatocytes occurred the cytoplasmic vacuoles and karyopyknosis. Moreover, some hepatocytes were reduced in size, with hyperchromatic nuclei (Fig. 1A). For kidney tissues, renal tubular epithelial cells fused, the nucleolus disappeared, and separated from the basement membrane of the renal tubule. The cellular necrosis and neutrophil infiltration were seen in the glomerular capillary network (Fig. 1B). Alveolar walls fused, and the alveolar space was filled with numerous inflammatory exudates, neutrophils and lymphocytes (Fig. 1C). However, no significant pathological changes were observed in the heart tissue of infected mice, except the nucleolus of the some myocardial cells disappeared, and the striated muscle disappeared (Fig. 1D).

The antimicrobial activities of the Tibetan herbal medicines

The *in vitro* antibacterial activities of the water extracts of six Tibetan herbal medicines against the Staphylococcus isolates were showed in Fig. 2, Table 1 and Table 2 based on the Kirby-Bauer disc diffusion and microdilution methods. The results indicated that the six Tibetan herbal medicines exhibited various degrees of antimicrobial activity, and the inhibition zone diameter ranged from 7.0 to 25.5 mm. Among the tested Staphylococcus isolates, the larger inhibition zone diameter (Ø 25.5mm) was against *S. aureus* and the smallest (Ø 7.0 mm) was against *S. epidermidis* and *S. coni*. In the comparative study, the water extracts of *Lagotis brachystachy* exhibited the best antibacterial effect to all tested Staphylococcus isolates, followed by *Aconitum flavum*, 89.1% (49/55) of Staphylococcus isolates were highly sensitive to *Aconitum flavum*. Others, 98.2% (54/55), 89.1% (49/55) and 83.6% (46/55) of Staphylococcus isolates were moderately sensitive to *Uncaria rhynchophylla*, *Swertia bimaculata*, and *Dracocephalum tanguticum*, respectively. While the water extracts of *Gentiana urnula* showed the weakest antibacterial activity to Staphylococcus isolates.

Table 1

The susceptibility of 55 Staphylococcus isolates from dairy goat mastitis to 9 antimicrobial agents

Isolates	Minimal inhibitory concentration (MIC, μg/mL)																			
	Penicillin			Amoxicillin			Ceftriaxone			Ceftiofur			Amikacin			Clindanlycin			Ciprofloxac	
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I
	≤0.5	1	≥2.0	≤0.5	1	≥2.0	≤2	4	≥8	≤2	4	≥8	≤16	32	≥64	≤0.5	1-2	≥4	≤0.5	1-2
S. aureus(n=26)	8-32			4-32			8-32			1-8			0.125-16			8-32			0.125-8	
S. haemolyticus (n=10)	8-32			8-32			8-64			1-16			0.125-8			1-16			2-8	
S. epidermidis(n=6)	16-64			8-64			16-128			2-32			16-128			2-8			16-64	
S. saprophyticus (n=5)	4-32			16-64			16-256			2-32			1-256			4-32			2-64	
S. gallinarum(n=4)	4-32			8-32			16-128			4-128			1-64			8-32			1-32	
S. galilis(n=1)	16			16			64			16			0.5			4			128	
S. coni(n=1)	32			32			64			32			128			32			64	
S. simulans(n=1)	256			>256			128			128			>256			>256			64	
S. equorum(n=1)	16			16			16			32			4			8			8	
ATCC 25923 (n=1)	0.5			0.125			0.5			0.25			0.125			0.125			0.5	
Note: "S" means sensitive; "I" means intermediate; "R" means resistant.																				

Table 2
The results of inhibition zone of six Tibetan medicinal plants against Staphylococcus isolates

Isolates	Inhibition zone diameter(mm)						
	Lagotis brachystachy	*Aconitum flavum*	*Uncaria rhynchophylla*	*Swertia bimaculataaa*	*Dracocephalum tanguticum*	*Gentiana urnula*	*Lonicera japonica*
S. aureus (n = 26)	19.3–25.5	15.1–20.3	13.3–18.3	12.2–15.3	12.5–15.7	9.1–13.9	14.4–18.0
S. haemolyticus(n = 10)	18.2–24.2	16.2–19.8	13.5–16.4	10.0-15.8	9.3–14.7	8.3–12.5	13.2–17.8
S. epidermidis(n = 6)	19.2–25.3	14.6–19.4	11.1–17.3	10.2–16.9	11.3–13.5	7.3–10.0	12.0-16.5
S. saprophyticus(n = 5)	16.2–24.3	14.2–18.8	11.3–16.0	10.2–14.3	10.2–12.6	9.4–11.5	12.3–17.7
S. gallinarum(n = 4)	18.1–24.0	14.4–17.6	10.4–15.6	9.4–14.6	10.4–13.6	8.2–10.8	11.4–15.3
S. galilis(n = 1)	21.5	18.4	14.2	13.4	10.8	9.9	14.4
S. coni(n = 1)	17.4	15.2	14.9	10.8	10.5	9.5	15.7
S. simulans(n = 1)	20.7	17.2	14.4	12.5	11.6	11.4	14.5
S. equorum(n = 1)	19.3	17.7	15.2	12.1	10.7	10.2	17.6
ATCC 25923(n = 1)	24.4	19.4	16.7	14.8	12.7	10.7	18.3

Note: Inhibition zone > 16 mm indicate highly sensitivity; 11–15 mm indicate moderate sensitivity; 10 mm indicate light sensitivity; <10 mm indicate the resistance or ineffective.

The MIC and MBC of six Tibetan herbal medicines were determined in comparison with *Lonicera japonica* against ten *S. aureus* isolates (Table 3). According to the results, five of six Tibetan herbal medicines showed certain antibacterial activity. Among them, the *Lagotis brachystachy* extract has the strongest antibacterial activity on *S. aureus*. The MIC and MBC values of *Lagotis brachystachy* were smaller than other Tibetan herbal medicines. The MIC values of *Lagotis brachystachy*, *Aconitum flavum*, *Uncariarhync hophylla*, *Swertia bimaculata* and *Dracocephalu mtanguticu*m extracts were 1.95–7.8 mg/ml, 3.9–15.6 mg/ml, 7.8-31.25 mg/ml, 15.6–62.5 mg/ml and 15.6–62.5 mg/ml, respectively, and the corresponding MBC values were 7.8-31.25 mg/ml, 7.8–62.5 mg/ml, 31.25–125 mg/ml, 62.5–250 mg/ml and 62.5–250 mg/ml, respectively (Table 3).

Table 3
The MIC and MBC of six Tibetan medicinal plants against *S. aureus*

Isolates	MIC (MBC) (mg/mL)						
	Lagotis brachystachy	*Aconitum flavum*	*Uncaria rhynchophylla*	*Swertia bimaculata*	*Dracocephalum tanguticum*	*Gentiana urnula*	*Lonicera japonica*
S. aureus 1	1.95 (15.6)	3.9 (15.6)	15.6 (62.5)	31.25 (125)	31.25 (125)	125 (500)	7.8 (31.25)
S. aureus 2	7.8 (31.25)	15.6 (62.5)	31.25 (125)	62.5 (250)	62.5 (125)	250 (500)	31.25 (125)
S. aureus 3	3.9 (15.6)	3.9 (15.6)	7.8 (31.25)	15.6 (62.5)	31.25 (62.5)	62.5 (125)	7.8 (31.25)
S. aureus 4	3.9 (15.6)	7.8 (31.25)	15.6 (62.5)	31.25 (125)	31.25 (125)	62.5 (250)	7.8 (31.25)
S. aureus 5	3.9 (62.5)	7.8 (15.6)	15.6 (62.5)	62.5 (250)	62.5 (250)	125 (500)	15.6 (62.5)
S. aureus 6	3.9 (15.6)	3.9 (15.6)	7.8 (31.25)	31.25 (125)	31.25 (62.5)	125 (500)	31.25 (125)
S. aureus 7	7.8 (15.6)	7.8 (15.6)	7.8 (31.25)	31.25 (125)	31.25 (125)	62.5 (250)	7.8 (31.25)
S. aureus 8	3.9 (15.6)	3.9 (15.6))	15.6 (62.5)	31.25 (62.5)	31.25 (62.5)	250 (500)	15.6 (62.5)
S. aureus 9	3.9 (15.6)	7.8 (31.25)	15.6 (62.5)	62.5 (250)	62.5 (250)	250 (500)	7.8 (31.25)
ATCC 25923	1.95 (7.8)	3.9 (7.8)	7.8 (31.25)	15.6 (125)	15.6 (62.5)	62.5 (250)	7.8 (31.25)

Effect of water extracts of Tibetan herbal medicines on growth curve of S. aure**us isolates**

In the Kirby-Bauer disc diffusion and broth microdilution tests, the water extracts showed the varying degrees of effect against Staphylococcus isolates, indicating that the decoctions were activated as antimicrobial against the Staphylococcus isolates. The isolates in the negative control group showed a normal adaptation, exponential and stationary growth phases. Whereas the presence of water extracts of different Tibetan herbal medicines interfered with the culture growth of *S. aureus* isolates, delayed the beginning of the multiplication step, and prolonging the lag phase compared with the *S. aureus* isolate in negative control, except for the *Gentiana urnul* (Fig. 3A-F). The *S. aureus* isolates treated by water extracts of different Tibetan herbal medicines, in varying degrees, showed a reduction in cell density in the stationary phase according to the $OD_{660\ nm}$ values, especially for the *Lagotis brachystachy* and *Aconitum flavum*, which had more than 80% reduction, while it has about 30% reduction for *Gentiana urnul*.

Discussion

Mastitis is a common disease among dairy goats that is responsible for the milk production losses and decreased dairy goat product quality [10]. Bacteriological examinations revealed the characteristics of the etiology of clinical mastitis, and Staphylococcus, especially *S. aureu*s was the most commonly isolated bacteria as it was identified in 47.3% (26/55) samples, it is significantly higher than that in a previous report that showed the prevalence of *S. aureus* in raw goat milk of healthy goats in Shaanxi province was 17.6% [11]. However, Streptococci were the least isolated. It is consistent with the previous studies that, unlike bovine mastitis, Streptococci were very rarely the cause of mammary infections in goats [2, 12, 13]. Staphylococci mastitis in goats is due to the presence of the commensal germs of the mammary integument and *S. aureu*s can cause both clinical mastitis and subclinical mastitis in goats [14]. Therefore, contamination usually occurs during milking operations. Moreover, the higher prevalence of mammary infections with *S. aureus* can cause a public health hazard if the *S. aureus* isolates cross the species barrier into humans [15]. Pathogenicity test indicated that most of infected mice appeared mental depression, slow response, lethargy, reduced spontaneous activity and anorexia and other symptoms. Further histopathological examination indicated that multiple organs were damaged by *S. aureus* isolates, such as necrotic foci in livers, some liver hepatocytes were reduced in size, nuclear condensation of liver cells; renal tubular epithelial cells fused, the nucleolus disappeared, and filled with exudation; alveolar walls fused, and the alveolar space was filled with numerous inflammatory exudates, and neutrophils and lymphocytes.

The occurrence of goats mastitis resulted in the widespread use of antimicrobials, which can lead to the appearance and spread of the antibiotic resistance and the antibiotic residues in the human food chain [16]. Based on the antimicrobial susceptibility test, most of the Staphylococcus in this study expressed the MDR phenotype, which is one of the health and socioeconomic challenge all over the world, and it is even faster than the speed of progress in new technology and drug developments. These problem highlight the need to search for alternative sources of antibacterial agents, such as the plant extracts or essential oils with antimicrobial activities [17, 18]. Meanwhile, many attempts have been made to explore the potential role of plant extracts to overcome the antibiotic resistance [19].

Traditional Chinese medicine is commonly used as disease treatment or the supplement to the health care. Among of them, the Tibetan medicinal plants are unique sources capable of inhibiting drug resistance growth. Tibetan medicine has a long history as one of the world's oldest known medical systems, and it plays an important role in the health care system in Qinghai-Tibet Plateau of China. Moreover, Tibetan medicine has gradually developed into a unique medical system by incorporating the theories of early traditional Chinese medicine, India medicine, and Arabia medicine. Our preliminary experiments indicated that there was no significant difference in the antimicrobial activities between the water extracts and ethanol extracts, while, the water extracts were more economical than ethanol extracts. Hence, water extracts were used in the current study. According to the results of the *in vitro* antibacterial activities, the water extracts of several Tibetan herbal medicines had stronger activity against the Staphylococcus isolates, even the MDR phenotype isolates. The water extracts of *Lagotis brachystachy* exhibited the best antibacterial effect to all tested Staphylococcus isolates, followed by *Aconitum flavum* and *Uncariarhync hophylla*. The growth curve can test the growth and death of bacteria and was used to evaluate the effect of antibacterial agents [20]. The growth curve analysis in this study demonstrated that the extracts of *Lagotis brachystachy* decreased the growth of *S. aureus* up to 80% compared to the negative control. Therefore, *Lagotis brachystachy*, *Aconitum flavum* and *Uncariarhync hophylla* can be considered as strong candidates against drug-resistant Gram-positive bacteria. Although no previous study has reported the activity of Tibetan herbal medicines against the bacteria isolated from mastitis in dairy goats, a few studies have reported the antibacterial effects of several Tibetan herbal medicines included in this study [7, 21].

The limitation of this study was that the experiments were based on crude extracts of Tibetan herbal medicines, while the extracts were not chemically characterized, and the chemical constituents to the antimicrobial activities were not identified. Hence, we might have overestimated their importance of antibacterial effective in clinical trials. However, this study highlights that some Tibetan herbal medicines can be the new medicinal resources for antimicrobial agents that can be used in the Staphylococcal mastitis, including the drug resistant pathogenic bacteria. Therefore, more studies, including the extraction, separation, purification, and *in vivo* experiments need to further exploited in the future.

Conclusion

In conclusion, our findings showed a high prevalence of Staphylococcus, especially *S. aureus* in mastitis of Guanzhong dairy goats, and *S. aureus* isolates can cause significant damage of livers, kidneys and lungs of mice. We exemplified the potent antibacterial activities of several Tibetan herbal medicines against the Staphylococcus, especially the *S. aureus* as well as the MDR isolates from mastitis of dairy goats. Tibetan herbal medicines could be a potential candidate for the treatment the mastitis of dairy goat. Moreover, further phytochemical and pharmacological studies are required for proper scientific validation of the tested Tibetan herbal medicines.

Methods

Ethical approval

The study was carried out in compliance with the ARRIVE guidelines. The animal studies of the present study was approved by the Animal Ethical and Welfare Committee of Northwest A&F University. Sampling was carried out in accordance with the standard protocols and with the consent of farm owners or administration.

Sample Collection

Between March and October 2020, fifty-five milk samples of mastitis goats were collected from 6 different commercial dairy goat farms (200-2,000 goats per farm) located in Fuping County, the important dairy goat base of Shaanxi Province, also known as the famous hometown of the national dairy goat in China. The milk samples were collected from the goats with clinical mastitis before once-daily milking. Approximately 10 mL individual milk sample was collected in a 50 ml sterile centrifuge tube after disinfection of teat surface with ethyl alcohol, washing with clean warm water, and discarding the first streams of milk. The samples were labeled and placed into an ice box, and then transported to the laboratory of the College of Veterinary Medicine within 4 h for bacteriological examination.

Bacteriological examination and Antimicrobial resistant phenotypes

All collected raw milk samples were subjected to the routine isolation and identification of bacteria using the methods described in previous studies [2, 22]. Briefly, 10 μl of each sample was spread on 5% sheep blood agar medium. After incubation for 24–48 h at 37°C, the agars were examined for the presence and appearance of bacterial colonies. Bacteria identification was performed through conventional methods (differential medium, Gram stain, biochemical tests) and molecular methods based on 16S rRNA gene sequencing. Additionally, a 2 mL milk aliquot was stored at -80°C until further DNA extraction.

All Staphylococcus isolates were processed to evaluate the antimicrobial resistant phenotype using twelve antimicrobials representing six antimicrobial classes, β-lactams, including penicillins (penicillin and ampicillin), the third-generation cephalosporins (ceftriaxone and ceftiofur), aminoglycosides (amikacin), lincosamides (clindanlycin), quinolones (ciprofloxacin), macrolides (acetylisovale-ryltylosin) and tetracyclines (doxiciclina). All minimum inhibitory concentrations (MICs) were performed in triplicate, with ATCC 25923 serving as quality control. The results were interpreted according to the guidelines of Clinical Laboratories Standards Institute (CLSI) [23]. The isolates resistant to three or more antimicrobial classes were defined as multiple drug resistance (MDR).

Pathogenicity of the isolated bacteria in mice

Female specific-pathogen-free BALB/c mice (8 weeks, 18–22 g) were purchased from the Laboratory Animal Centre of Xi'an Jiaotong University (Xi'an, China), and were used to determine the pathogenic role of the most prevalent bacteria species based on bacteriological examination. All mice were adaptively bred for 7 d before the experiment was conducted, and they were allowed free access to a normal diet and water.

The mice were randomly divided into tested group and negative control group, and 10 mice in each group. 0.4 ml bacterial suspension contained approximately 10^8 CFU/ml or the same volume of sterile physiological saline solution (0.9%) were intraperitoneally injected into the mice of two groups, respectively. Infected mice were monitored for mortality and possible signs every 12 h for 7 d. At the end of experiment, the mice were anesthetized with 2% isoflurane and euthanized via cervical dislocation. Subsequently, the main organs, including the heart, liver, spleen, lung, and kidney were collected to conduct the histopathological examination. Briefly, the tissue samples were fixed in 4% paraformaldehyde for 24 h and embedded in paraffin wax. Subsequently, paraffin-embedded specimens were consecutively sectioned into the slides of 5 μm thickness and stained with hematoxylin and eosin. The signal acquisition and analysis system was used for histopathological analysis.

Preparation of the water extracts of Tibetan herbal medicines

Six Tibetan herbal medicines (*Swertia bimaculata*, *Gentiana urnula*, *Uncaria rhynchophylla*, *Aconitum flavum*, *Dracocephalum tanguticum* and *Lagotis brachystachy*) were purchased from a pharmacy located in Lhasa City of Tibet, they were naturally collected at an elevation of 3700 m, in July 2019 from Shannan City, in the southern part of Tibet, and these plants have been authenticated by the pharmacy and Dr. Jin of Tibet Vocational Technical College. All herb extracts were made in an Electrical Herb Pot (Cangnan Dongqi electric apparatus Co. Ltd., Zhejiang, China). Firstly, the oven-dried Tibetan medicinal plants were smashed into coarse powder, respectively. Secondly, fifty grams of each ground herb was soaked in 500 ml of the distilled water for 24 hours, the powdered herb was boiled for 1 h, and filtered through the sterile four-layer gauze, the decoction and filtration were performed in triplicate, and the filtrates were combined. Finally, the combined filtrates were further concentrated till the volume reduced to 100 ml. It was centrifuged at 4000 rpm for five minutes and filtered through a 0.22 μm membrane. The filtrates were further concentrated till the volume reduced to 50 ml, and the concentration of water extracts were 1 g/ml. The prepared decoctions were stored in the fridge.

In vitro **antibacterial activities of the Tibetan herbal medicines against the Staphylococcus**

The Kirby-Bauer disc diffusion method was applied to evaluate the *in vitro* antibacterial activities of six Tibetan herbal medicines against Staphylococcus of dairy goat mastitis according to CLSI protocols. All tests were performed on Mueller-Hinton (MH) agar. Firstly, a sterile cotton swab was soaked in a bacterial suspension with a turbidity of 0.5 McFarland, and squeezing the extra suspension, and then lightly and uniformly inoculated on the surface of MH agar. *Lonicera japonica* was used as a positive control. Secondly, six-millimeter-diameter sterile filter paper disks were immersed in prepared decoctions, and dried at room temperature. The impregnated with prepared decoctions were placed at equal distances on the MH agar. Finally, the plates were incubated at 37°C for 18–24 h to measure the diameter of inhibition zones.

Moreover, the MICs of the Tibetan herbal medicines to nine *S. aureus* isolates were performed with microdilution method. *Lonicera japonica* was used as a positive control, and MH broth was used as a vehicle control. MIC was recorded as the plant extracts with the lowest concentration and has shown absolute inhibition of observable growth [24]. Minimum bactericidal concentration (MBC) was determined following the MIC assay [25]. Wells that exhibited no evident growth had 5 mL of a sample taken and streaked on to MH plates, and this was followed by incubation at 37°C for 18–24 h. The MBC was then recorded as the concentration at which there was minimum growth/colony of bacteria.

Effect of water extracts of Tibetan herbal medicines on growth curve of S. aureus **isolates**

The effects of crude water extracts from Tibetan herbal medicines on the growth curve of *S. aureus* were performed as described previous study with modifications [26, 27]. *S. aureus* was selected to test as it is the major identified pathogen. Briefly, 0.5 mL bacterial suspension were added to 50 mL LB broth containing sub-MIC concentration of crude water extracts, respectively, and then mixed on a vortex mixer for one min. The mixes without the water extracts served as a negative control, and ciprofloxacin was used as a positive control. The mixes were cultured under shaking conditions (150 rpm) at 37°C, and the growth curves of the *S. aureus* isolates were determined by measuring the absorbance at 660 nm every hour using a spectrophotometer.

Abbreviations

MDR: multiple drug resistance; MIC: minimum inhibitory concentrations; MBC: minimum bactericidal concentration; CLSI: Clinical Laboratories Standards Institute.

Declarations

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Authors' contributions

Fang Qiu, Siemg Hou and Lianjie Liu performed sampling and experiments. Jingjing Guo revised and edited the manuscript. Xiaoqiang Liu designed the study, supervised research, performed the statistical analyses and drafted and edited the manuscript. All the authors have read and approved the manuscript, and agreed to be accountable for their own contributions.

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Availability of data and materials

All the data used to support the findings of this study is available in the manuscript or supplementary materials. Raw datasets may also be requested from the corresponding author provided that all ethical requirements have been met.

Ethics approval and consent to participate

The animal studies of this report was approved by the Animal Ethical and Welfare Committee of Northwest A&F University (Permit Number: 2021052).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

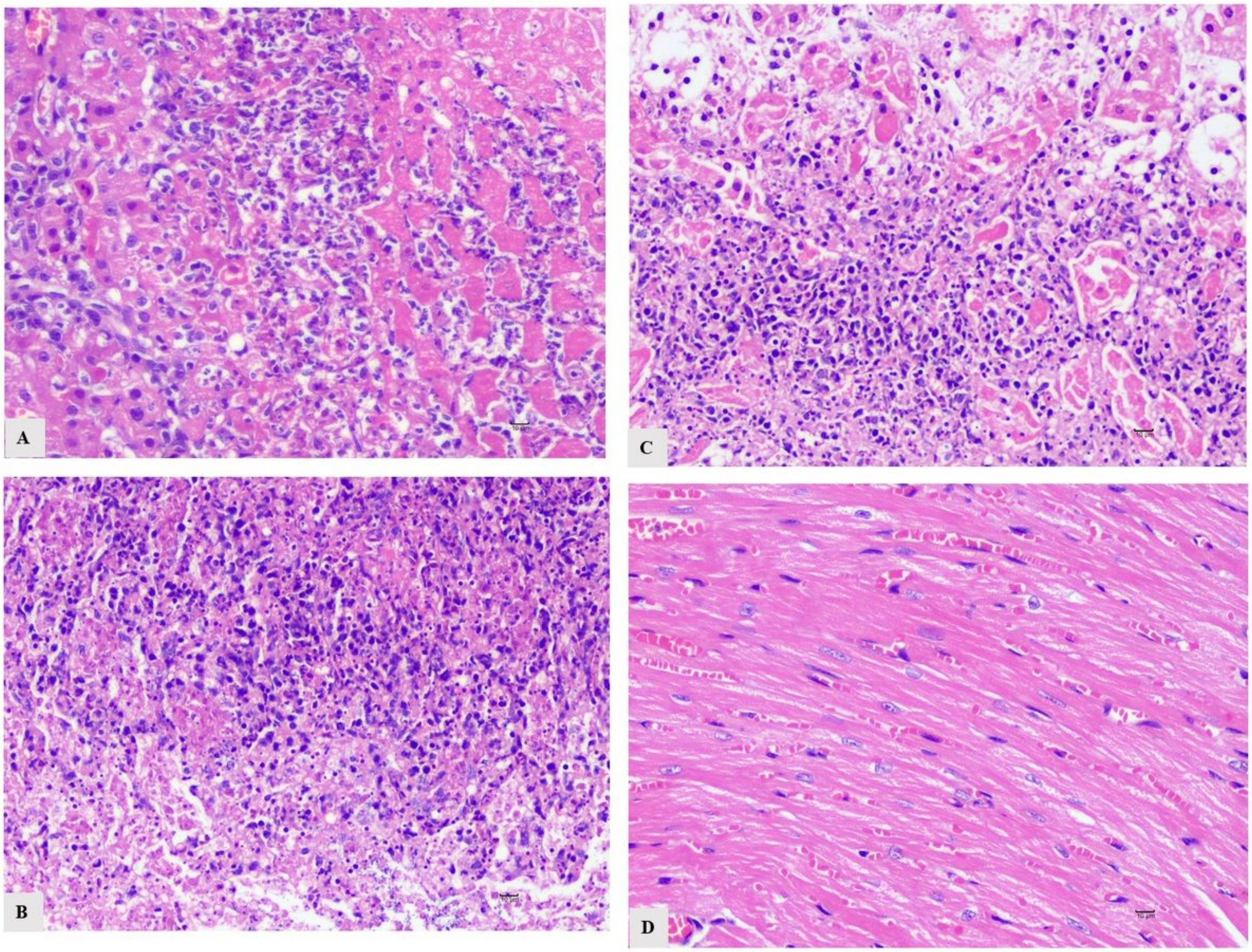

Figure 1

Histopathogological observation analysis of infected mice by S. aureus （HE stain ×40） A. liver B. kidney C. lungs D. heart

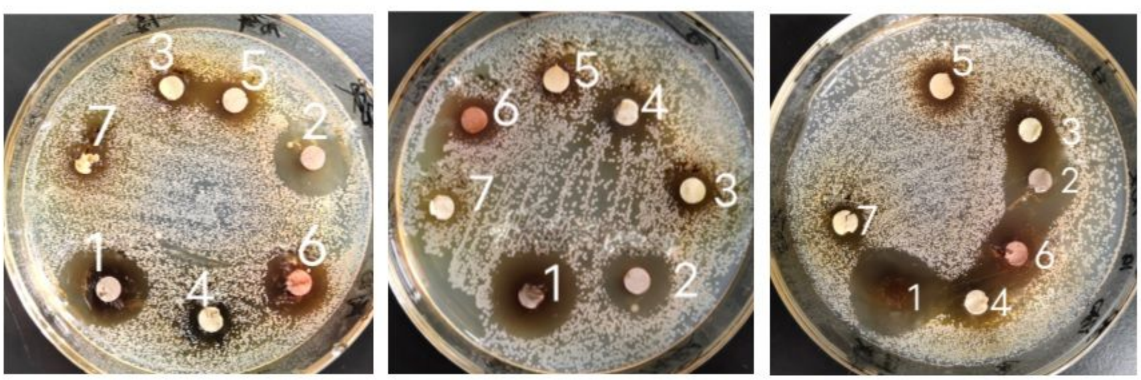

Figure 2

The results of in vitro antimicrobial test of Tibetan herbal medicines against the Staphylococcus isolates Note: 1. Lagotis brachystachyum, 2. Aconitum flavum, 3. Dracocephalum tanguticum, 4. Lonicera japonica, 5. Swertia bimaculata, 6. Uncaria rhynchophylla, 7. Gentiana urnula

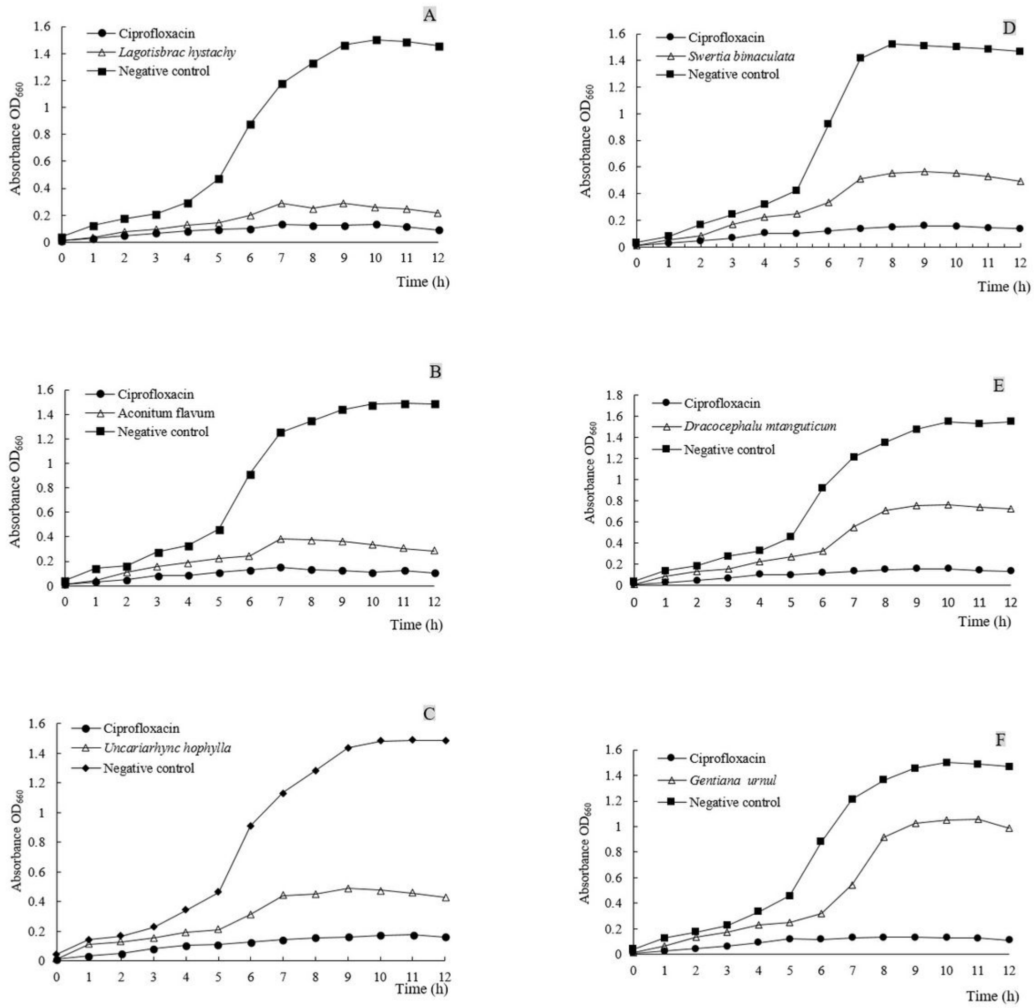

Figure 3

The effect of different Tibetan herbal medicines (sub-MIC) on the growth curve of S. aureus (A) Lagotisbrac hystachy; (B) Aconitum flavum; (C) Uncariarhync hophylla; (D) Swertia bimaculata; (E) Dracocephalu mtanguticum; (F) Gentiana urnul. The mixes without the water extract served as a negative control. Ciprofloxacin was used as positive control.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementary1.docx
- Supplementary2Growthcurves.xlsx