

Antibacterial Activity of Aqueous and Ethanolic Extracts of *Portulaca Oleracea* L and *Taraxacum Mongolicum* Against Pathogenic Bacteria of Cow Mastitis

Shuai Peng¹

Weichang Dai¹

Hansong Yu¹

Yuhua Wang¹

Xuelin Wang³

Shumin Sun^{2,3*}

¹ College of Food Science and Engineering Jilin Agricultural University, Changchun, 130118, Jilin, China

² College of Animal Science and Technology, Inner Mongolia University for Nationalities, Tongliao, Inner Mongolia 028000, China.

³ Key Laboratory of Zoonosis Research, Ministry of Education, Institute of Zoonosis, Jilin University, Changchun 130062, China.

*Corresponding author: shums1975@126.com

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ABSTRACT

As the problems of bacterial resistance and safety of dairy products have become more prominent, alternative medicine for antibiotics in treating cow mastitis is needed. In the present study, antibacterial activity of aqueous and ethanolic extracts of *Portulaca oleracea* L and *Taraxacum mongolicum* against the main pathogenic bacteria (*Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus dysgalactiae*) of cow mastitis was evaluated using disc diffusion method. The results showed that aqueous and ethanolic extracts of the two herbs both could inhibit the four pathogenic bacteria of cow mastitis at different level. All extracts of the two herbs displayed the highest antibacterial activities

against *Escherichia coli* than other bacteria. For *Portulaca oleracea* L, ethanolic extracts had higher antibacterial activities than aqueous extracts except for against *Escherichia coli*. However, for *Taraxacum mongolicum*, ethanolic extracts had lower antibacterial activities than aqueous extracts. These results indicate that extracts of *Portulaca oleracea* L and *Taraxacum mongolicum* have the potential to be used in treating cow mastitis.

INTRODUCTION

Mastitis causes huge economic losses in dairy industry including reduced milk production, discarded milk, treatment costs, and higher risk of abortion.¹ It has been estimated that the economic losses associated with clinical mastitis was approximately \$179 per case of mastitis in the United States.² As clinical mastitis frequently occurs in dairy cows, reasonable treatment of the disease is necessary. Traditionally, antibiotics have

Table 1. Antibacterial activities of aqueous and ethanolic extracts of *Portulaca oleracea* L

Name of the bacteria	Diameter of inhibition zone at different concentration levels (mm)					
	Aqueous extract			Ethanolic extracts		
	0.5 g/ml	0.25g/ml	0.125g/ml	0.5 g/ml	0.25 g/ml	0.125 g/ml
<i>Escherichia coli</i>	22.7±0.24	19.2±0.19	14.5±0.17	22.5±0.18	18.4±0.27	16.6±0.16
<i>staphylococcus aureus</i>	12.2±0.18	8.4±0.17	0	18.3±0.23	15.6±0.19	12.3±0.15
<i>Streptococcus agalactiae</i>	17.5±0.28	15.4±0.23	13.7±0.15	19.8±0.22	17.3±0.18	15.4±0.13
<i>Streptococcus dysgalactiae</i>	18.2±0.25	15.6±0.21	13.8±0.17	19.6±0.22	17.4±0.19	14.8±0.18

been widely used in treating cow mastitis. However, as the problems of bacterial resistance and safety of dairy products have become more prominent,³ searching for alternative medicine to antibiotics in treating cow mastitis has been a hot spot.

Plant extracts have been proposed as an important direction of searching for alternative medicine for antibacterial agent, and intensive studies have been conducted in recent years.⁴ Some plant extracts have been used in treating clinical mastitis on organic dairy farm and demonstrated with good therapeutic effect.⁵ *Portulaca oleracea* L, a medicinal herb, had been widely used in treating diarrhea of human and animals in China and demonstrated with anti-inflammatory and anti-oxidation effects by recent studies.^{6,7} Previous studies had shown that *Taraxacum mongolicum*, a traditional Chinese herbal medicine, had anti-inflammatory effects.⁸ In the present study, inhibitory effects of aqueous and ethanolic extracts of *Portulaca oleracea* L and *Taraxacum mongolicum* on pathogenic bacteria of cow mastitis were evaluated.

MATERIALS AND METHODS

Bacterial Strains

Escherichia coli, *staphylococcus aureus*, *Streptococcus agalactiae*, and *Streptococcus dysgalactiae* were used in this study. All these bacterial were isolated from pathologic specimens of cow mastitis, and had been

confirmed with pathogenicity in our lab.

Medicinal Herbs

Portulaca oleracea L and *Taraxacum mongolicum* (dry spices) were purchased from a local drugstore in Hohhot.

Preparation of the Aqueous Extracts of Medicinal Herbs

Portulaca oleracea L (100 g) and *Taraxacum mongolicum* (100 g) were crushed and sieved through mesh cloth to get the fine powder, and then the powders were put into beakers containing distilled water to be soaked for 30 min, respectively. Finally, the powders were boiled for 30 min and sieved through double layer of sterile fine mesh cloth. The filtrates were heated at 50-60°C and concentrated to 100 ml. These extracts (1 g/ml) were stored at 4°C in refrigerator. Extracts of the two herbs were further diluted to make different concentrations (0.5 g/ml, 0.25 g/ml and 0.125 g/ml) by mixing with appropriate volumes of distilled water.

Preparation of the Ethanolic Extracts of Medicinal Herbs

Soxhlet extraction method was used to obtain the ethanolic extracts of medicinal herbs. Briefly, powders of the two herbs were extracted with 95% ethanol at 70°C for 6 h, then the ethanol was retrieved from the filtrates by reducing pressure. Finally, 1 g/ml of ethanolic extracts of medicinal herbs were obtained and stored at 4°C in refrigerator. The extracts were further diluted to make

Table 2. Antibacterial activities of aqueous and ethanolic extracts of *Taraxacum mongolicum*

Name of the bacteria	Diameter of inhibition zone at different concentration levels (mm)					
	Aqueous extract			Ethanolic extracts		
	21.3±0.16	18.2±0.17	15.6±0.12	18.5±0.21	15.3±0.15	13.6±0.14
<i>Escherichia coli</i>	18.5±0.18	15.7±0.21	13.3±0.14	15.1±0.19	12.3±0.17	10.2±0.13
<i>staphylococcus aureus</i>	19.9±0.18	16.8±0.17	13.7±0.15	17.8±0.22	14.1±0.18	9.7±0.16
<i>Streptococcus agalactiae</i>	19.7±0.25	17.5±0.21	13.9±0.18	18.3±0.24	15.5±0.21	11.1±0.14
<i>Streptococcus dysgalactiae</i>	18.2±0.25	15.6±0.21	13.8±0.17	19.6±0.22	17.4±0.19	14.8±0.18

different concentrations (0.5 g/ml, 0.25 g/ml and 0.125 g/ml) by mixing with appropriate volumes of distilled water.

Antimicrobial Activity of the Extracts Using Disc Diffusion Method

Escherichia coli and *staphylococcus aureus* were inoculated into broth medium, while *Streptococcus agalactiae* and *Streptococcus dysgalactiae* were inoculated into broth medium supplemented with 2% fetal bovine serum. All the bacteria were incubated at 37°C for 24 h. The test microorganisms were diluted to a count of 10⁸ CFU/ml and then transferred from nutrient broth to sterile Muller Hinton agar plates. Subsequently, filter paper discs (5 mm in diameter) saturated with different concentration of ethanolic extract and aqueous extract (20 µL) were placed on surface of each inoculated plate. The plates were incubated at 37°C for 24 h. Diameters of the inhibition zone were measured in mm. For each treatment, 3 duplicates were performed. Diameters of inhibition zone of <10 mm zone was considered as low sensitivity; 10-14 mm as medium sensitivity; while 15-19 mm as high sensitivity and ≥20mm as extreme sensitivity, according to Standard for pharmacology of traditional Chinese medicine.⁹ The results were expressed as mean ± SD.

RESULTS

Antibacterial activities of aqueous and ethanolic extracts of *Portulaca oleracea* L

and *Taraxacum mongolicum* are shown in Table 1 and Table 2. The results showed that aqueous and ethanolic extracts of the two herbs both could inhibit the four pathogenic bacteria of cow mastitis at different level. For *Portulaca oleracea* L, ethanolic extracts had higher antibacterial activities than aqueous extracts except for against *Escherichia coli* (Table 1). However, for *Taraxacum mongolicum*, ethanolic extracts had lower antibacterial activities than aqueous extracts (Table 2). Extracts of the two herbs displayed higher antibacterial activities against *Escherichia coli* than other bacteria, in which the maximum zone of 22.7 mm appeared in *Portulaca oleracea* L extracts (Table 1). It was demonstrated that concentration of extracts of the two herbs significantly affect the antibacterial activities, in which the higher the concentration was, the higher antibacterial activity was.

DISCUSSION

As using of antibiotics has caused serious problems such as multidrug-resistant bacteria, antibiotic overuse and antibiotic residues in food, etc,³ new materials to replace antibiotics to treat bacterial infections is needed. Previous studies have shown that plants extracts can be used to treat a variety of disorders including inflammatory conditions, bacterial infections, cancer and other diseases.¹⁰ In the present study, antibacterial activities of aqueous and ethanolic extracts

of *Portulaca oleracea* L and *Taraxacum mongolicum* against pathogenic bacteria of cow mastitis were evaluated. The results showed that extracts of *Portulaca oleracea* L and *Taraxacum mongolicum* have the potential to be used in treating cow mastitis.

It has been reported that *Escherichia coli*, *staphylococcus aureus*, *Streptococcus agalactiae* and *Streptococcus dysgalactiae* are the main pathogenic bacteria of cow mastitis; therefore, these strains were used in this study.¹¹ The results showed that all the extracts could inhibit these bacteria at different level in which ethanolic extracts of *Portulaca oleracea* L generally had higher antibacterial activities than aqueous extracts.

It is widely accepted that the antimicrobial component may be different between ethanolic extracts and aqueous extracts of the plants. Our results indicate that the antimicrobial component of the two herbs may more soluble in ethanol as compared to water. Previous studies supported this result. A recent study has shown that the flavonoid apigenin isolated from ethanolic extracts of *Portulaca oleracea* L has antibacterial property and could be used to develop antibacterial drugs.¹² Our results also showed that ethanolic extracts of *Taraxacum mongolicum* generally had higher antibacterial activities than aqueous extracts. In china, aqueous extract of *Taraxacum mongolicum* has commonly been used in treating inflammatory disorders during lactation of women. A recent study has shown that *Taraxacum mongolicum* significantly inhibited production of NO and pro-inflammatory cytokines in LPS stimulated bovine mammary epithelial cell line, proving its role on anti-inflammation.⁸

In conclusion, our results indicate that extracts of *Portulaca oleracea* L and *Taraxacum mongolicum* have the potential to be used in treating cow mastitis. Further research is required to investigate the bioactive molecules of the two herbs and their clinical outcome in treating cow mastitis.

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