

BIOAG PROJECT PROGRESS OR FINAL REPORT

TITLE: Searching for Organic Antimicrobials

PRINCIPAL INVESTIGATOR(S) AND COOPERATOR(S): Drs.Meijun Zhu, Lawrence Fox, Karen Killinger, Min Du, Shuming Zhang.

KEY WORDS: Bovine mastitis, *Staphylococcus* spp. Dandelion, Cinnamon oil

ABSTRACT

Organic food production in Washington is expanding rapidly. However, this industry is hampered by the lack of effective antimicrobial agents which can be used in organic food production. Plants rely on innate immunity to defend microbial invasion, depending on the antimicrobial effects of phytochemicals. Plants with high antimicrobial contents were used to treat infections since ancient times, though their use in recent decades has been largely abandoned due to the discovery of antibiotics. However, these phytochemicals are excellent candidates for developing organic antimicrobials to treat infection in organic livestock, in particular cow mastitis. Dandelion has long been used as an antimicrobial herb to treat mastitis, and cinnamon oil is a natural plant-derived essential oil that has been traditionally used to preserve foods. In this study, we test their efficacy in inhibiting the growth of pathogens causing cow mastitis, focusing on *Staphylococcus aureus* and *E. coli*, and found that these natural phytochemicals are highly effective in inhibiting/killing these pathogens. The development of effective phytochemical extracts against microbial infection, so called "organic antimicrobials", facilitates the development and profitability of organic farming, further boosting the prosperity of organic agricultural industry at Washington State and the US.

PROJECT DESCRIPTION

The plant relies on its innate immunity for defending microbial invasion. During evolution, plants have developed an array of compounds which have strong antimicrobial activities. Herbs enriched with certain antimicrobial compounds have been used for treating infections from the ancient times. With the discovery of antibiotics in the last century, however, studies on these antimicrobial phytochemicals were halted, and their uses as antimicrobials have been largely abandoned. Recently, consumers have been regaining interests on these natural antimicrobials, owing to the increasing awareness of harmful effects associated with the excessive use of antibiotics and the surge in organic food production where antibiotic use is banned. The overall goal of this project was to explore natural antimicrobial agent(s) to treat infection in organic livestock. We have been pursuing two specific objectives: 1) To optimize procedures for preparing an antimicrobial extract from dandelion with antimicrobial activities against *Staphylococcus* spp. and *E. coli* strains isolated from bovine mastitis; and 2) To search other alternative natural antimicrobial extracts against pathogenic bacteria causing bovine mastitis.

OUTPUTS

We have developed an antimicrobial extract from dandelion (Fig. 1). Based on HPLC analysis, the major component of dandelion stem, flower, root and leaf was chicoric acid, and a total of 12 major chemicals were identified.

In addition, we found that cinnamon oil is another natural extract highly effective against pathogenic *E. coli*. At a concentration as low as 0.01875% (v/v), it completely inhibited the growth of pathogenic *E. coli*. A concentration of 0.15% (v/v) resulted in a 5 Log reduction of pathogenic *E. coli* within 30 min (Sheng and Zhu, 2014).

Furthermore, it is very effective in inhibiting or even eliminating *Escherichia coli* and several tested strains of *Staphylococcus aureus* isolated from mastitic cows. As little as 0.4 μ l cinnamon oil per disc resulted in a ~20 mm and ~27 mm inhibition zone in *S. epidermidis* and *S. aureus*, respectively (Figure 2). Their minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against pathogenic isolates from mastitic cow are listed in Table 1.

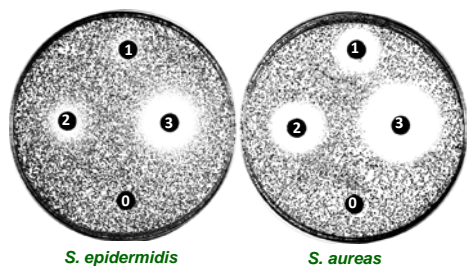


Figure 2. Antimicrobial activity of Cinnamon oil against *S. aureus* and *S. epidermidis* by disc diffusion method. A. 0, vehicle control; 1, 0.1 μ L; 2, 0.2 μ L; 3, 0.4 μ L.

Table 1. MIC and MBC of *C. cassia* oil against bovine mastitis isolates

	MIC (% , v/v)	MBC (% , v/v)
<i>S. aureus</i>	0.0125	0.05
<i>S. epidermidis</i>	0.0125	0.05
<i>S. hyicus</i>	0.00625	0.025
<i>S. xylosus</i>	0.0125	0.05
<i>E. coli</i> 29	0.025	0.10

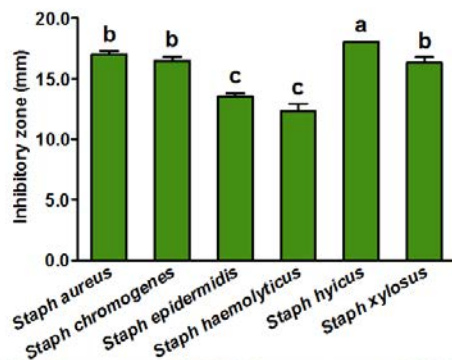


Figure 1. Antimicrobial activity of dandelion leaf crude ethanolic extract against *Staphylococcus spp* by disc diffusion method. A. The average diameter inhibitory zones (mm) of dandelion leaf extract at 4 mg per disc against six *S. spp*. B. Maps of plates for the real antimicrobial activity of dandelion leaf extract against six *S. spp* at 10^5 CFU/mL sterile phosphate buffered saline (PBS, pH7.4): 0, vehicle control; 1, 10 μ L 100 mg/mL of leaf extract; 2, 20 μ L 100 mg/mL of leaf extract; 3, 40 μ L 100 mg/mL of leaf extract.

We also tested their kill kinetic against two major mastitic pathogens, *S. aureus* and *E. coli*. The survival curves (Figure 3A and B) showed that 0.1% and

0.2% of cinnamon oil in culture media respectively, had bactericidal effects on *S. aureus* and *E. coli* 29 within 30 min. *C. cassia* oil at 0.2% and 0.4% for *S. aureus* and *E. coli* 29, respectively, caused 6 Log reduction of *S. aureus* and *E. coli* 29 within 30 min (Figure 3). We further tested the miscibility and antimicrobial activity of *C. cassia* oil in milk, which provides essential information for its application in bovine mastitis. 0.2% and 0.4% solution of *C. cassia* caused a reduction of ~6.0 Log of *S. aureus* and *E. coli* 29, respectively, and to an undetectable level within 8 h (Figure 3C & D).

We further found that cinnamon oil treatment at MIC impaired membrane integrity of *E. coli* and *S. aureus*, which was followed by a decrease in ATP synthesis. Collectively, our data show that Cinnamon oil provides an exciting potential to be used as an alternative antimicrobial for bovine mastitis in organic dairy farms.

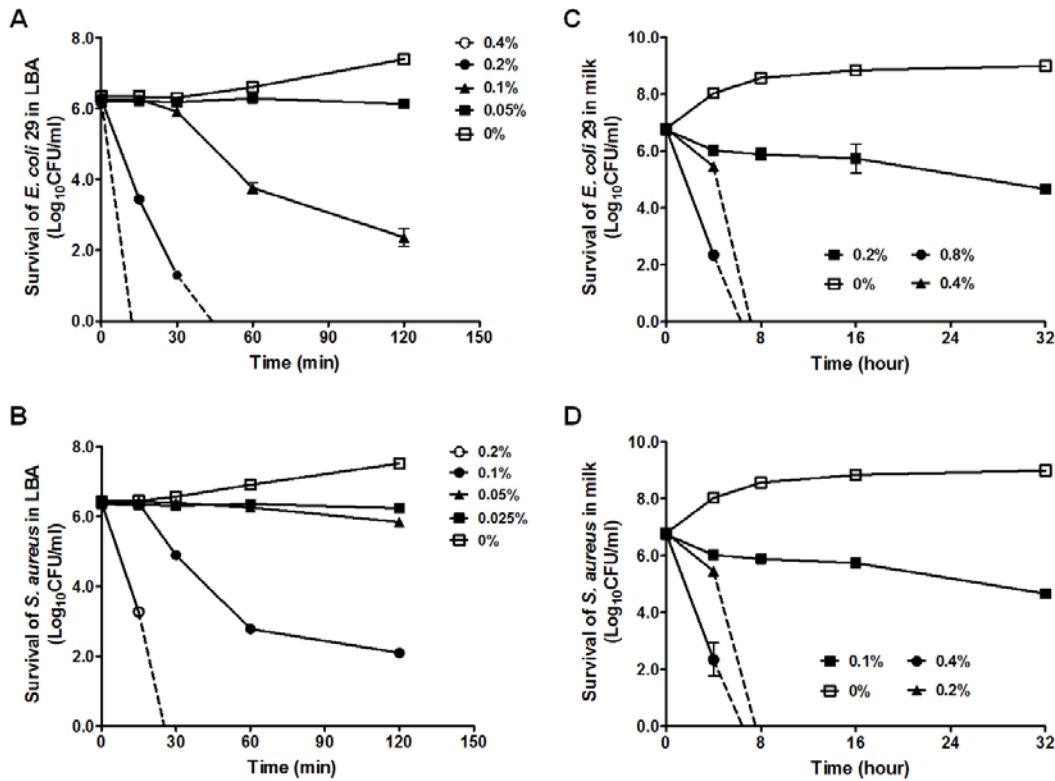


Figure 3. Survival curves of bovine mastitis isolates in culture media or milk containing different concentrations of *C. cassia* oil. A. *E. coli* 29 in LBA broth; B. *S. aureus* in LBA broth; C. *E. coli* 29 in milk; D. *S. aureus* in milk. Mean \pm SEM, n = 3.

Publications, Handouts, Other Text & Web Products:

1. Sheng, L., M. J. Zhu. 2014. Inhibitory effect of *Cinnamomum cassia* oil on non-O157 Shiga toxin-producing *Escherichia coli*. Food Control, 46: 374-381.
2. Zhu H., M. Du, L. Fox, M. J. Zhu. 2015. Bactericidal effects of Cinnamomum cassia oil against bacterial pathogens of bovine mastitis. Submitted to Food Control.
3. Zhang S., L. Sheng, M. Du, L. Fox, M. J. Zhu. 2015. Antioxidants and antimicrobial activity of dandelion extracts. Submitted to International Journal of Pharmacology and Phytochemistry.

IMPACTS

Short-term: We have formed an effective multidisciplinary team that covers food microbiology, animal science and pathology, and extension in developing new natural antimicrobials. Through proposed studies, we have demonstrated the effectiveness of natural antimicrobial extracts in the inhibition of pathogens associated with mastitis.

Long-term: Our long-term objective is to develop an effective organic antimicrobial cocktail which can be used as an antibiotic alternative for organic food production. Successful use of organic antimicrobials will reduce pathogen contamination and prevalence in organic foods, improving the wholesomeness of organic food products, as well as the profitability of organic farm operation.

ADDITIONAL FUNDING APPLIED FOR / SECURED

Using the data generated we are planning to submit proposals to USDA Food Safety, USDA Organic Transitions (USDA-ORG) and/or USDAOREI program in 2016.

GRADUATE STUDENTS FUNDED

This project has supported doctoral students and postdoctoral fellows: (1) Lina Sheng in School of Food Science; (2) Hongmei Zhu in School of Food Science; (3) Shuming Zhang in School of Food Science.

RECOMMENDATIONS FOR FUTURE RESEARCH

Future researches will need to be directed towards their application in organic farms. Furthermore, because pathogen infection is directly associated with farm animal's immune responses, it will be intriguing to investigate their immune boosting functions on farm animals in addition to their antimicrobial effects.