J. Dairy Sci. 97:5587–5591
http://dx.doi.org/10.3168/jds.2013-7806

Short communication: An in vitro assessment of the antibacterial activity of plant-derived oils

K. A. E. Mullen,* A. R. Lee,† R. L. Lyman,† S. E. Mason,‡ S. P. Washburn,* and K. L. Anderson†[1]
*Department of Animal Science, North Carolina State University, Raleigh 27695
†Department of Population Health and Pathobiology, North Carolina State University, Raleigh 27607
‡Department of Biological Sciences, Campbell University, Buies Creek, NC 27506

ABSTRACT

Nonantibiotic treatments for mastitis are needed in organic dairy herds. Plant-derived oils may be useful but efficacy and potential mechanisms of action of such oils in mastitis therapy have not been well documented. The objective of the current study was to evaluate the antibacterial activity of the plant-derived oil components of Phyto-Mast (Bovinity Health LLC, Narvon, PA), an herbal intramammary product, against 3 mastitis-causing pathogens: *Staphylococcus aureus*, *Staphylococcus chromogenes*, and *Streptococcus uberis*. Plant-derived oils evaluated were *Thymus vulgaris* (thyme), *Gaultheria procumbens* (wintergreen), *Glycyrrhiza uralensis* (Chinese licorice), *Angelica sinensis*, and *Angelica dahurica*. Broth dilution testing according to standard protocol was performed using ultrapasteurized whole milk instead of broth. Controls included milk only (negative control), milk + bacteria (positive control), and milk + bacteria + penicillin-streptomycin (antibiotic control, at 1 and 5% concentrations). Essential oil of thyme was tested by itself and not in combination with other oils because of its known antibacterial activity. The other plant-derived oils were tested alone and in combination for a total of 15 treatments, each replicated 3 times and tested at 0.5, 1, 2, and 4% to simulate concentrations potentially achievable in the milk within the pre-dry-off udder quarter. Thyme oil at concentrations ≥2% completely inhibited bacterial growth in all replications. Other plant-derived oils tested alone or in various combinations were not consistently antibacterial and did not show typical dose-response effects. Only thyme essential oil had consistent antibacterial activity against the 3 mastitis-causing organisms tested in vitro. Further evaluation of physiological effects of thyme oil in various preparations on mammary tissue is recommended to determine potential suitability for mastitis therapy.

Key words: mastitis, plant-derived oils, alternative to antibiotics, organic

Short Communication

Organic dairy products represented almost 6% of the entire US dairy market in 2010 (Organic Trade Association, 2011). Because organic dairies are prohibited from using synthetic antibiotics in cattle (Electronic Code of Federal Regulations, 2013), a need exists for effective alternatives to synthetic antibiotics to treat mastitis. Dairies with a 6-mo average bulk tank SCC over 250,000 cells/mL had significantly higher prevalence of *Staphylococcus aureus*, *Streptococcus* spp., CNS, and *Streptococcus agalactiae* in organic herds compared with conventional herds (Pol and Ruegg, 2007a). In contrast, organic and conventional herds in North Carolina had similar prevalence of *Staph. aureus*, *Streptococcus* spp., CNS, and *Corynebacterium* spp. (Mullen et al., 2013).

Organic dairies have been reported to use a wide variety of nonantibiotic treatments for mastitis, from *Aloe vera* to vitamin supplements (Pol and Ruegg, 2007b). Peer-reviewed clinical efficacy studies evaluating those alternatives are scarce (Ruegg, 2009), but anecdotal reports exist of the efficacy of plant essential oils for improvement of milk quality in dairy cattle (Karreman, 2007). Plant-derived oils are the main ingredients in one intramammary product, Phyto-Mast (Bovinity Health LLC, Narvon, PA), labeled for improvement of milk quality and approved for use in organic production by the Ohio Ecological Food and Farm Association (Columbus). Phyto-Mast has some effectiveness in curing infections during the dry period when used as dry cow therapy (Mullen et al., 2014). Presence of the essential oil of *Thymus vulgaris* (thyme) in the formula may account for antibacterial action. Essential oil of thyme has strong antibacterial activity (Cowan, 1999; Kalemba and Kunicka, 2003) and contains a phenolic molecule called thymol that has strong activity against gram-negative bacteria (Helander et al., 1998) and common mastitis-causing pathogens (Ananda Baskaran et al., 2009).

Received December 5, 2013.
Accepted June 5, 2014.
[1]Corresponding author: kevin_anderson@ncsu.edu

The study objective was to investigate antimicrobial activity of oils from each herbal component of PhytoMast in milk against common mastitis-causing pathogens cultured in vitro.

Bacterial Cultures

Single isolates of *Staph. aureus*, *Staphylococcus chromogenes*, and *Streptococcus uberis* were obtained from clinical mastitis cases on North Carolina dairy farms by the Milk Quality and Mastitis Laboratory at the College of Veterinary Medicine at North Carolina State University (Raleigh). Isolates were plated on Trypticase soy agar with 5% sheep blood (**TSA**; Becton, Dickinson and Co., Franklin Lakes, NJ). Purity of each culture was confirmed by Gram stain, morphology on mannitol salt agar (*Staph. aureus* and *Staph. chromogenes*), and morphology on blood agar (*Strep. uberis*). Bacteria were grown on TSA plates for 18 h in preparation for testing, and then kept refrigerated for the duration of the experiment. For each replication of each treatment tested, 1 colony was removed from the refrigerated plate and placed into 3 mL of Mueller-Hinton broth. Bacteria were grown to the midpoint of the log phase [6 h for *Staph. aureus* and *Staph. chromogenes* (Fujikawa and Morozumi, 2006), and 3 h for *Strep. uberis* (Almeida and Oliver, 1993)] before beginning the experiment.

Herbal Oils

Canola oil extractions of 4 different herbs and essential oil of thyme were obtained from Herbal Vitality Inc. (Sedona, AZ). The 4 herbs included *Angelica dahurica*, *Angelica sinensis*, *Gaultheria procumbens* (wintergreen), and *Glycyrrhiza uralensis* (Chinese licorice). Plant oils were kept refrigerated in brown glass bottles to prevent light degradation or volatilization of the oils.

Experimental Design

Herbal oils were tested at concentrations of 0.5, 1, 2, and 4% (vol/vol). Those concentrations would theoretically be achievable in the pre-dry-off udder quarter, provided that milk production was approximately 2.45 kg/quarter per day (5.4 lb/quarter per day). Because of the known antibacterial activity of thyme essential oil, it was tested by itself at concentrations of 1, 2, and 3% (vol/vol). The other 4 herbal oils were tested alone and in 15 combinations at each test concentration (0.5, 1, 2, and 4%), for a total of 60 combinations. Combination treatments were prepared by mixing equal (vol/vol) amounts of each of the oils to a total volume of 5 mL in a 10-mL vial: 6 mixes of 2 oils each at 2.5 mL, 4 mixes of 3 oils each at 1.67 mL, and 1 mix of 4 oils each at 1.25 mL. Vials containing oil preparations were kept sealed and refrigerated when not in use. All oil-containing vials were vortexed for 15 s before beginning each trial.

Several controls were included in every testing of each treatment. Milk was cultured alone as a negative control to ensure that pasteurization was successful. An antibiotic control included milk + bacteria + penicillin-streptomycin at 1 and 5% concentrations, using the same antibiotic control as in a dry cow study (Mullen et al., 2014). The positive control was milk + bacteria, to document growth of bacteria in the milk. PhytoMast was also tested at 1, 2, 3, and 4% concentrations. Canola oil was tested at 1 and 70% concentrations to determine if it had an antibacterial effect without the herbal extract. For each bacterial species, 3 replicates of every treatment were tested at every concentration and control. Replicates were randomized by date, bacterium, and treatment to minimize experimental bias.

Antibacterial Activity Testing

Herbal oils were tested using a modified protocol for broth dilution testing (CLSI, 2008). Whole UHT pasteurized and homogenized organic milk was purchased from a grocery store and used instead of Mueller-Hinton broth as the growth medium. Vials were prepared for testing each control and treatment by first adding a calculated volume of milk, and then adding the volume of treatment solution to reach the percentage by volume (0.5, 1, 2, or 4%) tested. Following addition of treatments to milk, vials were vortexed for 90 s. Ten microliters of the inoculated Mueller-Hinton broth was added, and then the test vials were vortexed for another 15 s and placed in an incubator at 37°C for 24 h. Test vials contained a total of 1 mL of liquid.

Following incubation, vials were vortexed for 15 s. Serial dilution was used to determine bacterial counts, using a 0.1-mL aliquot from the vortexed vial and sterile 0.85% saline solution to create eight 10-fold dilutions. Dilutions were plated on eighths of a TSA plate and incubated for 24 h at 37°C. Colony-forming units of all dilutions were recorded. Colony counts from the lowest readable dilution were used in the analysis (Miles and Misra, 1938).

Analyses

Results are reported as growth of bacteria in the treatment sample relative to the growth of the milk + bacteria control, as a percentage of the growth of the control. Treatments were considered successful at reducing bacterial growth if all 3 replications resulted in reduction of bacterial growth compared with the