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DIETARY PLANT-BASED BIOACTIVE COMPOUNDS FOR SUSTAINABLE MASTITIS CONTROL IN LIVESTOCK

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Abstract: The increasing antimicrobial resistance among mastitis pathogens necessitates alternative therapeutic approaches in dairy cattle. This study evaluated the antimicrobial efficacy of selected plant extracts against both reference and production strains of mastitis-associated microorganisms in vitro, and assessed the *in vivo* effects of an extruded feed additive (the wormwood–poplar extract additive) containing *Artemisia absinthium* and *Populus balsamifera* extracts, on udder health and milk quality in cows with subclinical mastitis. In vitro assays demonstrated significant antimicrobial activity of citrus peel (*Citrus* spp.) and spruce (*Picea abies*) extracts against *Candida* spp., *Escherichia coli*, and *Staphylococcus aureus*, including resistant field isolates. The 15-day in vivo trial involving 60 Holstein cows revealed a 68% reduction in somatic cell counts ($p = 0.017$) following supplementation, achieving compliance with top-grade milk standards without compromising milk composition or sensory attributes. These findings underscore the potential of phytogenic compounds as effective, natural alternatives for mastitis control, promoting sustainable dairy production and reduced antibiotic reliance.

Key words: Mastitis; *Artemisia absinthium*, antimicrobial activity, somatic cell count, milk quality, phytogenic feed additive

Introduction

Mastitis is one of the most common and economically detrimental diseases in dairy and meat-producing livestock worldwide, inflicting substantial losses due to decreased productivity, animal culling, and veterinary treatment costs (Chen et al., 2023). The disease is primarily caused by bacterial infection, with pathogens such as Staphylococcus aureus, Streptococcus agalactiae, and Escherichia coli frequently implicated (Kerro Dego and Vidlund, 2024). Conventional mastitis control relies heavily on antibiotics; however, this practice has contributed to a worrying increase in antimicrobial resistance (AMR), particularly among pathogens commonly associated with mastitis. Systematic studies have shown resistance rates exceeding 45 % for penicillin in S. aureus, with trends of increasing resistance noted across multiple antimicrobial classes over recent decades (Leon et al., 2025). In China, a study reported that 58.7% of S. aureus isolates from bovine mastitis were resistant to penicillin. Multidrug resistance was also observed, with 11.6% of isolates showing resistance to multiple antimicrobial classes (Yang et al., 2023). In Poland found penicillin resistance rates of 62.3% among S. aureus isolates from bovine mastitis, and studies in Turkey reported rates of 62.1% (Szweda et al., 2014). Given the global crisis of AMR and its potential impact on both animal and human health, there is a critical need to identify and implement viable alternatives to antibiotics. Dietary interventions using plant-derived bioactive compounds have emerged as a sustainable and promising strategy in this regard. These include phytochemicals such as essential oils, and polyphenols, which demonstrate antibacterial, anti-inflammatory, antioxidant, and immunomodulatory actions. Many studies have demonstrated inhibitory effects of compounds derived from Eucalyptus globulus, Juglans regia, Schinus molle, and Taraxacum mongolicum against mastitis pathogens, along with suppression of inflammatory pathways like NF-κB and MAPK (Da Silva et al., 2023; Kaseke et al., 2023; Mezzasalma et al., 2025).

Moreover, local strategies such as intramammary administration of phytochemicals, probiotics, and bacteriocins are gaining traction, supported by growing evidence from biomimetic and plant-based therapeutic formulations. For example, intramammary flavonoid fractions have shown clinical efficacy against coagulase-negative Staphylococci in dairy cows (Gutiérrez-Reinoso et al., 2023). Such interventions not only target pathogens directly but also enhance host immune defenses and reduce inflammation, aligning with the One Health framework.

Plant-based bioactive compounds are naturally occurring compounds derived from herbs, spices, fruits, seeds, and other plant parts, known for their functional properties beyond basic nutrition. These compounds, often secondary metabolites, play crucial roles in plant defense and offer diverse pharmacological

benefits such as antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory effects (Wang et al., 2024). Their broad spectrum of biological activities has attracted increasing attention in veterinary science, particularly as sustainable alternatives to antibiotics in the management of livestock diseases, including mastitis.

Based on their biosynthetic origin and structural characteristics, plant bioactive compounds are commonly grouped into three main classes: polyphenols, terpenes and terpenoids, and alkaloids. Polyphenols, including flavonoids, phenolic acids, and tannins, exhibit strong antioxidant, anti-inflammatory, and antimicrobial activities, making them valuable in mitigating both pathogen load and host tissue damage in mastitis. For example, catechins and quercetin derivatives have demonstrated inhibitory effects on S. aureus and E. coli, alongside modulation of inflammatory pathways such as NF-κB (Li et al., 2019; Wu and Brown, 2021).

Terpenes and terpenoids, particularly those found in essential oils, such as thymol, carvacrol, eugenol, and menthol, are volatile lipophilic compounds that disrupt microbial membranes, impair quorum sensing, and interfere with biofilm formation. Their rapid action and broad-spectrum efficacy make them especially promising in both dietary and topical applications.

Alkaloids, a diverse group of nitrogen-containing compounds like berberine and sanguinarine, show potent antimicrobial and immunomodulatory effects and are increasingly explored as feed additives or components in phytogenic formulations.

While other compounds such as saponins or coumarins may also contribute to antimicrobial activity, they are generally considered subclasses or functional extensions of the main groups above. The multifunctionality of these bioactives, particularly their combined antibacterial, anti-inflammatory, and antioxidant effects, makes them attractive candidates for integrated mastitis prevention strategies, especially in light of rising antimicrobial resistance and consumer demand for antibiotic-free production systems.

This study aims to assess current knowledge and identify effective dietary plant-based bioactives, especially essential oils, and polyphenols, for mastitis prevention and treatment in livestock. We focus on elucidating mechanisms, evaluating antimicrobial and immunomodulatory effects, and discussing pathways for integrating these compounds into sustainable livestock health management.

Material and Methods

In vitro study

Testing of traditional and alternative antimicrobial agents

To address the challenge of antimicrobial resistance among mastitis pathogens, the efficacy of commonly used udder treatment products, including chlorhexidine bigluconate, povidone-iodine, and other commercially available preparations sourced from two dairy farms, was evaluated against production (field) strains isolated from mastitic cows. Standard disk diffusion assays revealed that these traditional agents were largely ineffective against the production strains. While some products exhibited antimicrobial activity against reference laboratory strains (such as *Candida* spp., *Escherichia coli*, *Staphylococcus aureus*, and *Serratia marcescens*), they failed to inhibit the growth of the field isolates. This finding underscores a marked decline in the effectiveness of conventional antimicrobials when used against contemporary, farm-derived pathogens.

Preparation and testing of plant extracts

In light of the limited efficacy of traditional antimicrobials, the study proceeded to investigate plant-based alternatives. Extracts were prepared from burdock leaves (*Arctium lappa folium*), citrus peel (*Citrus* spp. pericarpium), spruce (*Picea abies*), aloe vera (*Aloe vera folium*), and celandine (*Chelidonium majus*) using hot Soxhlet extraction with 70% ethanol as the solvent. The antimicrobial activity of these phytogenic extracts was assessed using the standardized disk diffusion method in accordance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Both reference laboratory strains and production strains isolated from clinical samples were tested. The diameters of inhibition zones were measured to quantify the antimicrobial efficacy of each extract. This comparative approach allowed for a direct evaluation of plant extracts versus traditional agents in combating both standard and field-derived mastitis pathogens.

***In vivo* study**

Production of the feed additive

The extruded multi-functional feed additive was manufactured at the feed facility of “NFT-KATU” LLP, located within the Faculty of Veterinary and Livestock Technologies at the NCJSC “S.Seifullin Kazakh Agrotechnical Research University”. The additive was based on barley and oats as carrier materials, enriched with two phytogenic components: bitter wormwood (*Artemisia absinthium*) extract and balsam poplar (*Populus balsamifera*) bud extract. The wormwood extract, standardized to contain ≥1.2% absintin (HPLC), was incorporated at 0.01% w/w, while the balsam poplar bud extract was included at 0.015% w/w. The production process of the feed additive involved barothermal extrusion, which combined high pressure and temperature to treat grain forages,

specifically barley and oats. This advanced processing method enhanced the digestibility and bioavailability of the nutrients in the grains. Following barothermal treatment, the mixture was formed into pellets, resulting in a uniform extrudate. The pelleted form facilitated convenient handling and feeding while ensuring consistent distribution of the active components throughout the animal feed. The extrusion line in the production and testing workshop utilized equipment produced in the Kostanay region of Kazakhstan, provided by LLP "Agrotekhservice-12". The machinery included a PD-2000 pneumatic hammer crusher, an SG-800 kg mixer equipped with load cells and a cyclone, a frequency-controlled feed hopper, a PE-350 extruder, and a PG-600 flat matrix pelletizer.

For the purposes of this manuscript, the feed additive composed of barley and oats enriched with bitter wormwood (Artemisia absinthium) extract (0.01% w/w) and balsam poplar (Populus balsamifera) bud extract (0.015% w/w) is hereafter referred to as the wormwood–poplar extract additive.

Experimental design

The study was conducted at LLP "Agrofirm Rodina" in the Akmola region to evaluate the effectiveness of the extruded feed additive in the diets of dairy cows. The study involved 60 high-yielding Holstein cows of both black and brown varieties. A total of 120 milk samples were collected from animals diagnosed with mastitis. The affected cows received the wormwood–poplar extract additive for a 15-day treatment period. The details were previously described (Abakanova et al., 2025).

Isolation and identification of mastitis pathogens

To assess the current effectiveness of traditional mastitis control methods and to illustrate the severity of antimicrobial resistance, preliminary studies were conducted using commercial strains of microorganisms isolated from cows with subclinical and clinical mastitis. Pathogens identified included *Candida spp.*, *Escherichia coli*, as well as representatives of the general microflora, including staphylococcal and streptococcal bacteria and yeasts.

Assessment of dairy production and milk quality

Milk yield was monitored through controlled milking over the 15-day period. Milk samples were collected in sterile disposable bottles both prior to the introduction of the feed additive and throughout the 15-day experimental phase. The qualitative characteristics of the milk were assessed using the "Expert Super Plem Kombo" analyzer, which measured fat, protein, skimmed milk powder, lactose content, acidity, density, temperature, freezing point, and conductivity. The somatic cell count was determined with the "Ekomilk Scan" analyzer. Sensory

properties of the milk were evaluated according to the Republic of Kazakhstan standard 1732-2007, which outlines the organoleptic method for determining quality indicators. These assessments were conducted by university employees.

Diagnosis of mastitis

Latent mastitis in the cows was initially identified using the California test at LLP "Agrofirm Rodina" in the Akmola region.

Ethical approval

All animal procedures were reviewed and approved by the local ethical committee of the Faculty of Veterinary Science and Technology of Animal Husbandry at NCJSC «S.Seifullin Kazakh Agrotechnical Research University» (protocol №6, dated 28.03.2022).

Statistical analysis

Statistical analyses were conducted using SAS software, version 9.4 (SAS Institute, Cary, NC). The Kolmogorov–Smirnov test was used to assess the normality of the data distribution. Due to significant deviations from normality, all variables except for freezing point underwent logarithmic transformation. The differences between measurements taken before and after treatment were analyzed using the General Linear Model (GLM), with treatment as a fixed factor and individual animals as a random factor to account for variability among cows.

Results

***In vitro* antimicrobial activity of plant extracts**

The preliminary investigation revealed a high level of antimicrobial resistance among mastitis pathogens isolated from dairy cows. Traditional udder treatment agents, including chlorhexidine bigluconate, povidone-iodine, and other commercially available products, demonstrated low efficacy against production strains of microorganisms such as *Candida spp., Escherichia coli*, and various staphylococcal and streptococcal bacteria. Notably, some products that were effective against reference (laboratory) strains failed to inhibit the growth of field isolates, indicating a significant decline in the effectiveness of conventional antimicrobials. In contrast, several plant extracts exhibited promising antimicrobial activity. The disk diffusion assay showed that citrus peel extract (*Citrus spp. pericarpium*) was active against both reference and production strains of *Candida spp., E. coli, and Staphylococcus aureus*, and also reduced the overall microbial

load. Spruce extract (*Picea abies*) demonstrated broad-spectrum activity, particularly against Staphylococcus aureus and in reducing total contamination by production strains. Burdock leaf extract (*Arctium lappa folium*) was effective against *E. coli* and *Staphylococcus aureus* (reference strains), as well as against commercial strains of *E. coli* and in lowering total microbial counts. Aloe vera and celandine extracts showed some degree of antimicrobial activity, but their effects were less pronounced or more specific to certain strains. The comparative antimicrobial activity of the tested plant extracts is summarized in Table 1. The detailed results of the antimicrobial activity of each plant extract, including inhibition zones against both reference and production strains, are presented in Table 2. These results indicate that certain plant extracts, especially those from citrus peel and spruce, possess significant antimicrobial activity against both reference and field strains of mastitis pathogens, including those resistant to conventional treatments.

Table 1. Summary of antimicrobial activity of plant extracts against reference and production mastitis pathogens

Plant extract	Activity against reference strains	Activity against production strains	Notes on efficacy
Citrus peel (*Citrus spp*.)	Active against *Candida* spp., *E. coli*, *S. aureus*	Active against *Candida* spp., *E. coli*, *S. aureus*; reduced total microbial load	Most broadly effective, potential universal agent
Spruce (*Picea abies*)	Active against *S. aureus* and others	Active against *S. aureus*; significantly reduced total contamination	Broad-spectrum activity, especially strong vs. *S. aureus*
Burdock leaf (*Arctium lappa*)	Active against *E. coli*, *S. aureus*	Active against *E. coli* and reduced total microbial load	Effective against key bacterial strains
Aloe vera (*Aloe vera folium*)	Limited or no activity	Limited or no activity	Less pronounced antimicrobial effect
Celandine (*Chelidonium majus*)	Some activity against *E. coli*, *S. aureus*	Data limited	Moderate, strain-specific activity

Table 2. Inhibition zone diameters (mm) of plant extracts against reference and production strains of mastitis pathogens

Extract	Reference strains			Commercial strains		
	Candida spp.	*E. coli*	*Staph. aureus*	*Candida* spp.	*E. coli*	Total contamination
Burdock leaf extract (*Arctium lappa folium*)	–	6.0 ± 0.0	8.3 ± 2.3	–	8.7 ± 0.9	5.2 ± 0.4
Citrus peel extract (*Citrus spp. Pericarpium*)	12.3 ± 1.1	8.3 ± 0.9	11.5 ± 6.8	12.1 ± 1.8	8.8 ± 2.8	4.8 ± 1.4
Spruce extract (*Picea abies*)	6.9 ± 0.1	7.9 ± 0.1	17.3 ± 3.0	11.9 ± 1.5	11.9 ± 2.5	11.5 ± 1.8
Aloe vera extract (*Aloe vera folium*)	–	–	–	–	–	9.9 ± 0.0
Celandine extract (*Chelidonium majus*)	–	8.8 ± 0.2	10.5 ± 0.3	–	–	–

Note: "–" indicates absence of inhibition zones.

Case study: Efficacy of the wormwood–poplar extract additive in mastitis management

A 15-day trial involving 60 Holstein cows with subclinical mastitis showed that supplementation with the wormwood–poplar extract additive led to a significant improvement in udder health, as evidenced by a 68% reduction in somatic cell count (SCC) from 402,111 ± 106,680 cells/mL at baseline to 127,000 ± 6,360 cells/mL post-treatment ($p = 0.02$), thereby meeting the standard for top-grade milk (≤200,000 cells/mL) (Figure 1). No statistically significant changes were observed in key milk quality parameters, including fat, protein, lactose, and density, all of which remained within regulatory norms. The only significant change observed in milk physico-chemical parameters was an increase in temperature by 1.9°C (from 21.0°C to 22.9°C, $p < 0.001$).

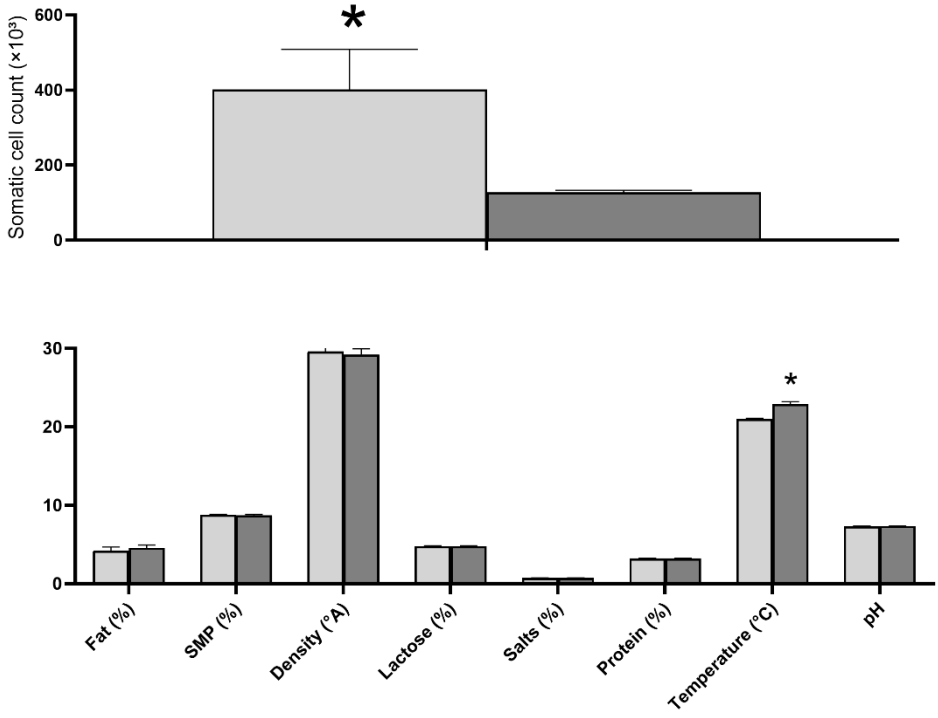

Figure 1. Physico-chemical parameters of milk from mastitic cows before and after treatment with the extruded additive. Data are presented as mean ± standard deviation (n=15). A significant reduction in somatic cell count was observed post-treatment (p=0.02)

Organoleptic properties (color, odor, consistency) also complied with established standards, indicating that the intervention effectively reduced inflammation without adversely affecting milk quality.

Discussion

The findings of our study highlight a critical issue in contemporary dairy practice: the increasing AMR among mastitis pathogens and the declining efficacy of conventional udder treatment agents. The high resistance observed in production strains of *Candida spp.*, *Escherichia coli*, and staphylococcal and streptococcal

bacteria aligns with global reports documenting the limited effectiveness of traditional antiseptics and antibiotics in mastitis management (Yang et al., 2023; Pires et al., 2024). The failure of agents like chlorhexidine bigluconate and povidone-iodine to inhibit field isolates, despite their activity against laboratory strains, underscores the urgent need for alternative therapeutic strategies (Okello et al., 2023; Rana et al., 2022). In our study, citrus peel extract exhibited broad-spectrum efficacy against both reference and commercial strains of *Candida spp.*, *E. coli*, and *Staphylococcus aureus*, as well as a reduction in overall microbial load. These findings are in line with recent studies that have shown citrus-derived extracts and eco-enzymes possess significant antibacterial properties against mastitis-associated pathogens, attributed to their content of flavonoids, essential oils, and polyphenols (Rizqan et al., 2023).

The present study demonstrated that dietary supplementation with the wormwood–poplar extract additive significantly reduced SCC in the milk of cows with subclinical mastitis, while maintaining the physico-chemical and organoleptic quality of milk. The observed 68% reduction in SCC is clinically meaningful, as SCC is a widely recognized biomarker of udder inflammation and milk quality (Ruegg, 2017). Achieving SCC levels below 200,000 cells/mL is particularly important, as it aligns with international standards for premium-grade milk and is associated with improved udder health and reduced economic losses (Ruegg, 2017; Sharma et al., 2011).

The anti-inflammatory and antimicrobial properties of wormwood and related phytogenic compounds have been previously documented. Wormwood extracts are rich in flavonoids, sesquiterpene lactones, and phenolic acids, which have demonstrated both in vitro and in vivo efficacy against mastitis-associated pathogens and in modulating immune responses (Juteau et al., 2003; Kordali et al., 2005). The efficacy of wormwood and other medicinal plants in mastitis management has been increasingly supported by recent research. Several studies have shown that plant extracts, including those from wormwood, possess significant antimicrobial activity against mastitis-associated pathogens such as *Staphylococcus aureus*, a major etiological agent in bovine mastitis (Coutinho et al., 2010). Moreover, a recent study evaluating the antimicrobial effect of plant-derived products found that these natural formulations were effective in reducing bacterial load and somatic cell counts in mastitic cows, in some cases outperforming conventional antibiotic treatments (Pașca et al., 2017; 2020). The antimicrobial efficacy of wormwood has also been demonstrated in a rat model, where topical application of its extract significantly reduced Staphylococcus aureus counts in infected surgical wounds compared to untreated controls (Moslemi et al., 2012).

The maintenance of milk quality parameters, such as fat, protein, lactose, and sensory characteristics, following supplementation with the wormwood–poplar extract additive, is in line with previous reports indicating that phytogenic additives do not adversely affect the physico-chemical or organoleptic properties of milk (Pașca et al., 2020). This is an important consideration for the dairy industry, as it ensures that interventions aimed at improving udder health do not compromise product quality or marketability.

The only significant change observed in milk physico-chemical parameters was an increase in temperature. This rise is likely attributable to improved udder blood circulation following the resolution of inflammation, as increased local temperature is a recognized physiological response during recovery from mastitis and is associated with vascular dilation and enhanced tissue perfusion in the mammary gland (Gayathri et al., 2024). Additionally, daily fluctuations in udder temperature due to circadian rhythms and environmental factors, such as the timing of milking, may have contributed to this variation, as previous studies have shown that udder temperature can vary predictably throughout the day and in response to external conditions. Thus, the observed temperature increase likely reflects both post-inflammatory physiological changes and the influence of circadian or procedural variables.

The use of plant-based therapies for mastitis is gaining momentum as a strategy to address the growing problem of antimicrobial resistance associated with conventional antibiotic use in livestock (Coutinho et al., 2010). Plant extracts offer a multifaceted approach, combining antimicrobial, anti-inflammatory, and immunomodulatory effects, and are less likely to contribute to resistance development. The results of this study support the integration of the wormwood–poplar extract additive into mastitis control programs, potentially reducing reliance on antibiotics and contributing to sustainable dairy production. Future research should focus on elucidating the mechanisms of action of wormwood and poplar *in vivo*, optimizing dosing regimens, and evaluating long-term effects on udder health and milk production in larger cohorts.

Conclusion

This study demonstrated that traditional antimicrobial agents currently used for mastitis control are largely ineffective against field strains of mastitis-associated pathogens, underscoring the urgency for alternative strategies. *In vitro* experiments showed that plant extracts, particularly those derived from citrus peel (*Citrus spp.*) and spruce (*Picea abies*), exhibited notable antimicrobial activity against both reference and commercial strains of *Candida spp., Escherichia coli*, and *Staphylococcus aureus*.

The *in vivo* trial further confirmed the practical benefits of phytogenic supplementation. Administration of the wormwood–poplar extract additive to cows with subclinical mastitis resulted in a significant reduction in SCC, indicating improved udder health. Importantly, this intervention did not adversely affect key milk quality parameters or organoleptic properties, maintaining compliance with regulatory standards.

Thus, these results support the integration of plant-based antimicrobials and feed additives into mastitis management protocols as effective, natural alternatives to conventional antibiotics. Further research should focus on elucidating the mechanisms of action, optimizing formulations, and evaluating long-term impacts on animal health and milk safety in commercial dairy herds.

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