

## ANTIBACTERIAL ACTIVITY OF PLANT ESSENTIAL OILS AGAINST *STAPHYLOCOCCUS AUREUS* ISOLATED FROM BOVINE MASTITIS

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### ABSTRACT

Bovine mastitis is a disease of cows and buffaloes, mainly caused by *Staphylococcus aureus*, which not only affects milk production but also causes enormous economic losses. Various antibiotics have been used for their treatment, but due to long-term and inappropriate use, resistance has developed in *S. aureus* to these drugs. That's why mostly plant essential oils have been used as an alternative treatment against *S. aureus* for the treatment of this disease. Moreover, essential oils from various plants have been shown to exhibit different antibacterial activities against *S. aureus* isolated from mastitic cows and buffaloes (bovine mastitis) at different concentrations. Furthermore, these plant-derived essential oils contain various compounds, and their combined effect makes it difficult for bacteria to develop resistance to these oils. These essential oils also exert their antibacterial activity against *S. aureus* by disrupting cell membrane integrity, decreasing metabolic activity, and diminishing energy production. This results in the leakage of cellular components and insufficient ATP production, which leads to bacterial cell death. This review discusses the importance of plant essential oils relative to antibiotics for treating *S. aureus*. It also explains the antibacterial activity of different plant-derived essential oils at various concentrations and under different conditions against *Staphylococcus aureus* isolated from mastitic cows and buffaloes. Limitations of these essential oils are also briefly described here.

**Keywords:** Antibacterial activity, Bovine mastitis, Cows and buffaloes, Limitations, Mechanism of action, Plant essential oils.

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### 1. INTRODUCTION

Mastitis is classified into two types, clinical and subclinical (Gonçalves et al. 2020; Sharun et al. 2021; Tomanic et al. 2023). In clinical mastitis, signs such as udder inflammation, swelling, redness and warmth are visible. Along with these udder signs, changes in milk, such as discoloration and clots, are also seen (Sharun et al. 2021). Subclinical mastitis lacks visible signs (Gonçalves et al. 2018; Gonçalves et al. 2020; Susanty et al. 2025). Mastitis increases culling rates, huge financial and economic losses, and decreased milk production and quality (Gonçalves et al. 2018; Sharun et al. 2021; Du et al. 2022; Wani et al. 2022; Arikan et al. 2024). According to one study, a farmer typically earns 6000-8000 Rs per month from one milking cow. From multiple cows (all dairy farming activities), a farmer earns 9000–10000 Rs per month. And milk yield loss due to mastitis in a cow is 6–7kg of milk/day, while a healthy cow produces 9–10kg of milk/day, which causes a huge drop in milk production (milk production–4kg/day). Due to a decrease in milk production from the cow, daily earnings drop from 413–458 Rs to almost 306–335 Rs (Wani et al. 2022). In cows and buffaloes this disease is called bovine mastitis (Ali et al. 2021). Bovine mastitis is a common and economically important disease of dairy cows and buffaloes that is mainly caused by *Staphylococcus aureus* (Zaatout et al. 2020; Abdeen et al. 2021; Mishra et al. 2024; Abd El-Razik et al. 2025). *Staphylococcus aureus* (*S. aureus*) is a coccus, a facultative anaerobe, gram-positive, and catalase-positive. It has a capacity for growth in various temperatures and pH levels, and is a major cause of foodborne outbreaks (Bhunia

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2008; da Silva et al. 2020). Antibiotics such as, cefixime, cephalothin, neomycin, penicillin, streptomycin, tetracycline, ampicillin, and ceftaxime have been mostly used for the treatment of bovine mastitis. But due to long-term and inappropriate usage of antibiotics, resistance have been developed in *S. aureus* (Abdeen et al. 2021; Cășaru et al. 2024; Salman et al. 2024). Although antibiotics cause problems, but some of them are still effective against *S. aureus* in bovine mastitis. That's why, there is need to find some alternatives, so that in future if they cause problems, there should be some alternatives that can be used for the treatment of this disease (Ogbuadike et al. 2023; Liu et al. 2023). Therefore, many alternatives have been discovered for the treatment of this disease and control of antibiotic resistance (Cheng and Han 2020; Tomanić et al. 2023; Cășaru et al. 2024). One of them is the use of plant essential oils (Cășaru et al. 2024).

Plant essential oils are natural, volatile, and have promising antimicrobial potential (Cerioli et al. 2018; Abers et al. 2021; Salman and Imran 2022). Essential oils can be extracted from every part of plant like, leaves, flowers, buds, bark, roots, rhizomes and stem (Batoool et al. 2023; Bülbül et al. 2023; Sharma et al. 2023). They are extracted from plants by using different extraction techniques, but steam distillation and hydrodistillation are commonly used techniques globally. Commonly found phytochemicals in these essential oils are terpenoids, hydrocarbons (of low molecular weight), and their oxygenated derived compounds (Sharma et al. 2023). These oils have different kinds of biological potential, like antimicrobial, insecticidal, anti-inflammatory, antioxidant, insect repellent, pain relievers, anticancer, and cytotoxic (Bangulzai et al. 2022; Salman and Imran 2022; Sharma et al. 2023; Özüüçli and Baykalir 2024). That's why, they have been used in different industries and fields like, cosmetics, aromatherapy, food preservation, biopesticides, insecticides industries, incense, perfume, and fragrance (Sharma et al. 2023).

In the medical field, essential oils have been used for the control and treatment of different disease-causing pathogens and diseases (Imran and Alsayeqh 2022; Al-Hoshani et al. 2023; Issa 2024; Ratajac et al. 2024). One of them is bovine mastitis in which essential oils have been shown their best antibacterial effects for the treatment of this disease (Gonçalves et al. 2024; Munive Nuñez et al. 2024). These essential oils are safe for host at specific concentration. Like, essential oils of *Minthostachys verticillata* stem and leaves are also safe for the cows' mammary glands upto 250 µg/ml concentration (Moliva et al. 2023). These plant-derived essential oils have also been used instead of antibiotics because bacteria cannot easily develop resistance against these oils due to the combined effect of different compounds in them, as compared to antibiotics, which contain a single molecule (Bassolé and Juliani 2012; Ramsay et al. 2018). Moreover, these are promising substitutes for conventional drugs because they can block several cellular mechanisms of bacteria (Budri et al. 2015). That's why these essential oils have been mostly used in the treatment of this disease, alone or their combination with antibiotics due to their best antibacterial activities against *S. aureus* (Buldain et al. 2020). These oils also have biofilm-inhibiting activity produced by *S. aureus* (Moliva et al. 2023), which is not explained here. This review discusses the importance of plant essential oils as compared to antibiotics against *S. aureus*. It also explains antibacterial activity of different plant-derived essential oils at different concentrations and conditions, against *Staphylococcus aureus* that is isolated from mastitic cows and buffaloes. Limitations of these essential oils are also briefly described here.

### 1.1. Importance of Essential Oils as Compared to Antibiotics against *S. aureus*

Essential oils have been giving best results as compared to antibiotics against *S. aureus*. Such as, in one study, *S. aureus* showed resistance against several ampicillin, chloramphenicol, cefixime, kanamycin, cephalothin, novobiocin, penicillin, and streptomycin, while, essential oils of different plants (*Thymus serpyllum*, *Cinnamomum zeylanicum*, *Syzygium aromaticum*, *Mentha piperita*) showed their best antibacterial activities against this multidrug-resistant bacteria (Cășaru et al. 2024). In another study, *S. aureus* also showed high resistance against amoxicillin-clavulanic, ampicillin, cefoxitin, and ceftaxime. While, EOs like, cactus oil showed inhibitory effects against *S. aureus* at 100, 50 and 25% concentrations. Apart from tea, geranium, and thyme oils, which also exhibited antibacterial activity at different concentrations (Salman et al. 2024), Abd El-Hamid et al. (2023) concluded that *S. aureus* showed complete resistance to cefoxitin, while highly resistant to ampicillin, gentamicin, and erythromycin. As compared to these antibiotics, essential oils of eugenol, carvacrol, and linalool showed excellent antibacterial activities by forming ZOI of 20-37mm and giving MIC values of 0.5-8µg/mL range, against this bacterium (Abd El-Hamid et al. 2023). These results underscore the importance of plant essential oils and conclude that they can be used as an alternative treatment for *S. aureus*, compared with antibiotics.

### 1.2. Antibacterial Activity of Plant Essential Oils against *Staphylococcus aureus*

Different plant essential oils exhibit antibacterial activity against *Staphylococcus aureus*, isolated from mastitic cows and buffaloes (Cășaru et al. 2024; Munive Nuñez et al. 2024), at different concentrations, as shown in Table 1. Like, Aouadhi et al. (2024) investigated antibacterial activity of eight plant essential oils and concluded that *Thymus capitatus* and *Trachyspermum ammi* exhibit best antibacterial activities against *S. aureus*, by forming ZOI of 26-45

and 25-40 mm (range from small to significant value), respectively as compared to the other plant oils. While the range (from small to significant value) of ZOIs obtained from other plant essential oils e.g., *Eucalyptus globulus*, *Eucalyptus camaldulensis*, *Artemisia absinthium*, *Myrtus communis*, *Mentha pulegium*, and *Cymbopogon citratus* were 14-25, 16-19, 14-17, 16-21, 14-15, and 18-44mm, respectively (Aouadhi et al. 2024). Schlemper et al. (2023) also concluded that 625 and 2500µg/mL concentrations of thyme and geranium essential oils inhibited 80 and 60% *S. aureus*,

**Table 1:** Antibacterial activity of plant essential oils against *Staphylococcus aureus* that is isolated from bovine mastitis (2023-2024)

Plant	Plant parts used for oil extraction	Conditions	Concentrations	<i>Staphylococcus aureus</i> isolated/obtained from/samples obtained from	Results	References
<i>Artemisia absinthium</i>	N. A	37°C, 24h	0.07-50%	Clinical bovine mastitis	MIC=3.125-12.5%	Aouadhi et al. 2024
<i>Azadirachta indica</i>	N. A	N. A	N. A	Mastitic cows	Effective	Kouaho et al. 2024
<i>Citrus limon</i>	N. A	N. A	N. A	Mastitic cows	Strong inhibition	Kouaho et al. 2024
<i>Cinnamomum zeylanicum</i>	N. A	N. A	N. A	N. A	MIC= 0.098%	Caneschi et al. 2024
<i>Cinnamomum zeylanicum</i>	N. A	24h	Integral EO	Mastitic cows	ZOI= 26 mm	Caşaru et al. 2024
<i>Cinnamomum zeylanicum</i>	N. A	24h	1/2 dilution	Mastitic cows	ZOI=22 mm	Caşaru et al. 2024
<i>Cinnamomum zeylanicum</i>	N. A	24h	1/4 dilution	Mastitic cows	ZOI=20 mm	Caşaru et al. 2024
<i>Citrus aurantium bergamia</i>	N. A	37°C, 24 h	N. A	Cows with mastitis	Subclinical MIC=0.277–4.435	Munive Nuñez et al. 2024
<i>Coriandrum sativum</i>	N. A	N. A	1-3%	Mastitic cows	Inhibit bacterial growth	Albuquerque et al. 2023
<i>Copaifera reticulata</i>	N. A	37°C, 24 h	N. A	Cows with mastitis	Subclinical MIC=1.11–17.76	Munive Nuñez et al. 2024
<i>Cymbopogon citratus</i>	Leaves	37±1°C, 24h (incubation)	0.39-50mg/mL	Clinical mastitis cases	MIC range (small to large value) =0.39-1.56mg/mL	Lopes et al. 2023
<i>Cymbopogon citratus</i>	N. A	37°C, 24h	0.07- 50%	Clinical bovine mastitis	MIC=3.125%	Aouadhi et al. 2024
<i>Cymbopogon citratus</i>	N. A	N. A	30 µl/mL	Mastitic cows	Effective	Gonçalves et al. 2024
<i>Eucalyptus camaldulensis</i>	N. A	37°C, 24h	0.07-50%	Clinical bovine mastitis	MIC=3.125-12.5%	Aouadhi et al. 2024
<i>Eucalyptus globulus</i>	N. A	37°C, 24h (incubation)	0.07-50%	Clinical bovine mastitis	MIC=6.25%	Aouadhi et al. 2024
<i>Eugenia brejoensis</i>	Leaves	37°C, 24 h	1,024 to 8µg/mL	Bovine mastitis	MIC range=64-256µg/mL	da Silva et al. 2024
<i>Eugenia gracillima</i>	Leaves	37°C, 24 h	1,024 to 8µg/mL	Bovine mastitis	MIC range=64-1,024µg/mL	da Silva et al. 2024
<i>Eugenia pohliana</i>	Leaves	37°C, 24 h	1,024 to 8µg/mL	Bovine mastitis	MIC and MBC range=64-256µg/mL	da Silva et al. 2024
<i>Eugenia stictopetala</i>	Leaves	37°C, 24 h	1,024 to 8µg/mL	Bovine mastitis	MIC range= 64-512µg/mL	da Silva et al. 2024
<i>Lippia graveolens</i>	Leaves	24 and 48 h	25 µL	Bovine mastitis	Largest ZOI	Vargas-monter et al. 2023
<i>Lippia origanoides</i>	N. A	N. A	60 µl/mL	Mastitic cows	Effective	Gonçalves et al. 2024
<i>Melaleuca alternifolia</i>	Leaves	N. A	10%	Bovine mastitis	100% growth inhibition	Carrasco et al. 2024
<i>Mentha piperita</i>	N. A	N. A	N. A	N. A	Showed antibacterial effects	Rahchamani et al. 2023
<i>Mentha piperita</i>	N. A	24h	Integral EO	Mastitic cows	ZOI= 12 mm	Caşaru et al. 2024
<i>Mentha piperita</i>	N. A	24h	1/2 dilution	Mastitic cows	ZOI=10 mm	Caşaru et al. 2024
<i>Mentha piperita</i>	N. A	24h	1/4 dilution	Mastitic cows	ZOI= 6 mm	Caşaru et al. 2024
<i>Mentha pulegium</i>	N. A	37°C, 24h	0.07-50%	Clinical bovine mastitis	MIC=6.25-12.5%	Aouadhi et al. 2024
<i>Mentha pulegium</i>	N. A	N. A	N. A	N. A	Showed antibacterial effects	Rahchamani et al. 2023
<i>Minthostachys verticillata</i>	Leaves and stem		4 and 6mg/mL	Clinical cases of bovine mastitis	Decrease bacterial load	Moliva et al. 2023
<i>Myrtus communis</i>	N. A	37°C, 24h	0.07-50%	Clinical bovine mastitis	MIC=3.125%	Aouadhi et al. 2024
<i>Ocimum basilicum</i>	N. A	37°C, 24 h (incubation)	N. A	Cows with mastitis	Subclinical MIC=0.149–2.378	Munive Nuñez et al. 2024
<i>Origanum vulgare</i>	N. A	N. A	N. A	Bovine mastitis	Significant antibacterial activity	Hartfiel et al. 2024
<i>Origanum vulgare</i>	N. A	N. A	1-3%	Mastitic cows	Inhibit bacterial growth	Albuquerque et al. 2023
<i>Pelargonium graveolens</i>	N.A	36.5°C, 24 h	N. A	Bovine mastitis	MIC <sub>60</sub> =2500µg/mL	Schlemper et al. 2023
<i>Syzygium aromaticum</i>	N. A	N. A	1-3%	Mastitic cows	Inhibit bacterial growth	Albuquerque et al. 2023
<i>Salvia Rosmarinus</i>	N. A	N. A	N. A	Bovine mastitis	Significant antibacterial activity	Hartfiel et al. 2024

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<i>Syzygium aromaticum</i>	N. A	24h	Integral EO	Mastitic cows	ZOI=13 mm	Căşaru et al. 2024
<i>Syzygium aromaticum</i>	N. A	24h	1/2 dilution	Mastitic cows	ZOI=10 mm	Căşaru et al. 2024
<i>Syzygium aromaticum</i>	N. A	24h	1/4 dilution	Mastitic cows	ZOI=6 mm	Căşaru et al. 2024
<i>Trachyspermum ammi</i>	N. A	37°C, 24h	0.07-50%	Clinical bovine mastitis	MIC=0.048-0.195%	Aouadhi et al. 2024
<i>Thymus capitatus</i>	N. A	37°C, 24h	0.07- 50%	Clinical bovine mastitis	MIC=0.048-0.195%	Aouadhi et al. 2024
<i>Thymus serpyllum</i>	N. A	24h	Integral EO	Mastitic cows	ZOI=20 mm	Căşaru et al. 2024
<i>Thymus serpyllum</i>	N. A	24h	1/2 dilution	Mastitic cows	ZOI=18 mm	Căşaru et al. 2024
<i>Thymus serpyllum</i>	N. A	24h	1/4 dilution	Mastitic cows	ZOI=12 mm	Căşaru et al. 2024
<i>Thymus vulgaris</i>	N. A	36.5°C, 24 h	N. A	Bovine mastitis	MIC <sub>80</sub> = 625µg/mL	Schlemper et al. 2023
<i>Thymus vulgaris</i> L.	Leaves	37±1°C, 24h	0.39-50mg/mL	Clinical mastitis cases	MIC range (small to large value) =0.39-1.56mg/mL	Lopes et al. 2023
<i>Thymus vulgaris</i>	N. A	N. A	1-3%	Mastitic cows	Inhibit bacterial growth	Albuquerque et al. 2023
<i>Zingiber officinale</i>	N. A	N. A	N. A	Mastitic cows	Strong inhibition	Kouaho et al. 2024
<i>Zingiber officinale</i>	N. A	37°C, 24 h	N. A	Cows with Subclinical mastitis	MIC=4.605–36.84	Munive Nuñez et al. 2024
Cinnamon oil	N. A	37°C, 24h (incubation)	0.4-6%	Mastitic cows and buffaloes	MIC=0.5%	Mishra et al. 2024

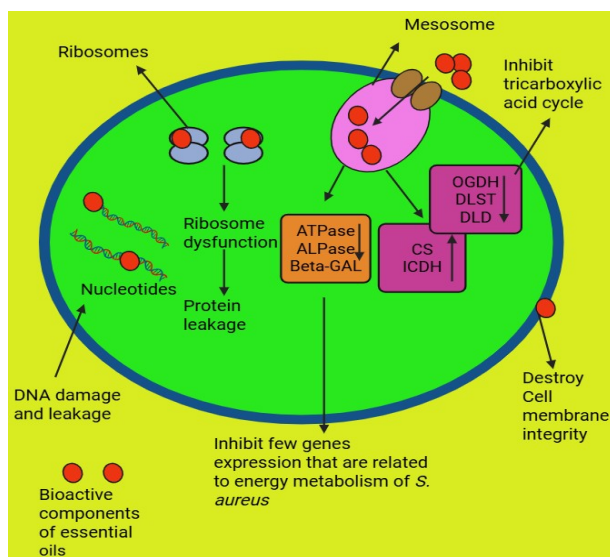
representing that thyme essential oil is more effective than geranium. According to another study by Munive Nuñez et al. (2024), *Ocimum basilicum* (mean MIC=1.561±0.223) and *Citrus aurantium bergamia* (mean MIC=2.782±0.228) essential oils showed higher antibacterial activity than *Copaifera reticulata* (mean MIC=6.541±1.705) and *Zingiber officinale* (mean MIC=18.58±2.138) (Munive Nuñez et al. 2024). In another study, essential oils of *Eugenia brejoensis*, *E. gracillima*, *E. stictopetala*, and *E. pohliana* leaves showed good antibacterial activity against *S. aureus* at MIC ranges of 64-256, 64-1,024, 64-512, and 64-256µg/mL, respectively (da Silva et al. 2024). Further, Moliva et al. (2023) tested 2 types of essential oils EO1 and EO2 of *Minthostachys verticillata* stem and leaves, against *S. aureus* and concluded that both essential oils inhibit the growth of this bacterium. MIC ranges of EO1 and EO2 were 13.7-114.6mg/mL and 288-2307mg/mL, respectively. While, according to Mishra et al. (2024), Cinnamon essential oil also showed antibacterial activity against *S. aureus* at 1-3%. Moreover, Lopes et al. (2023) investigated the antibacterial activity of *Cymbopogon citratus* and *Thymus vulgaris* L. leaves' essential oils against *S. aureus* and concluded that *Cymbopogon citratus* oil (MIC range small to large value=0.39-1.56mg/mL) and *Thymus vulgaris* (MIC range small to large value=0.39-1.56mg/mL) show better antibacterial activities. Leaves of *Eucalyptus grandis* (MIC range small to large value=6.35 - 50mg/mL), *Lavandula dentata* (MIC range small to large value=12.5-25mg/mL), and *Mentha piperita* (MIC range small to large value=3.12-12.5mg/mL) oils also exhibited antibacterial activities but less than *C. citratus* and *T. vulgaris*. Moreover, *E. grandis*, *L. dentata*, and *M. piperita* essential oils did not exhibit bactericidal activities, but only showed bacteriostatic. Optical density results showed that the essential oils of *C. citratus* and *L. dentata* had MICs of 50-0.39 and 50-6.35mg/mL, respectively. These highly variable standard deviations may be due to the effects of essential oils on bacterial growth patterns (Lopes et al. 2023).

Apart from single plant essential oil, combination of essential oils of different plants also exhibits the best antibacterial activity against *S. aureus*. Like, in one study by Schlemper et al. (2023), a combination of thyme and geranium essential oils lowered the MIC=312/625µg/mL. This reduction in MICs was 50 and 75%, respectively, for both oils (Schlemper et al. 2023). In another study, a mixture of essential oils of *T. serpyllum*, *C. zeylanicum*, *S. aromaticum*, and *M. piperita* exhibited antibacterial activities by forming a ZOI of 11 mm against *S. aureus* (Căşaru et al. 2024). Furthermore, when combined with antibiotics, essential oils show higher antibacterial activity at lower concentrations against *S. aureus* (Buldain et al., 2022). Like the *Melaleuca armillaris* essential oil and erythromycin exhibited synergistic antibacterial activity against *S. aureus* under specific conditions. Like, at pH=7.4, MIC (µLmL<sup>-1</sup>/µgmL<sup>-1</sup>) range (from small to large value) of essential oils/ erythromycin was 6.25 /0.125-64 (Buldain et al. 2022). According to another study, a combination of *Melaleuca armillaris* essential oil/rifaximin also showed better antibacterial activities under different conditions. Like, at pH=7.4, 6.5 and 5, MIC values (µLmL<sup>-1</sup>/µgmL<sup>-1</sup>) of this combination were 6.25/0.002, 3.1/0.008, and 1.6/0.004, respectively (Buldain et al. 2020). These results showed that the combined effect of essential oil and antibiotics enhanced their antibacterial activity at a lower concentration, against this bacterium (Buldain et al., 2022). Moreover, the same plant essential oil in different studies also showed different antibacterial activities against *S. aureus*, as shown in Table 1.

Plant essential oils exert their antibacterial effects against *S. aureus* by decreasing metabolic activity and diminishing energy production through altering the membrane potential, which results in an impairment of the proton equilibrium, insufficient ATP production, and inconsistent membrane functioning, as shown in Fig. 1. By damaging cell membrane integrity, these oils also result in leakage of DNA, RNA and cytoplasmic material that



eventually leads to bacterial cell lysis. Lack of structural proteins and essential enzymes, impaired bacterial growth and cell death. Essential oils also promote metabolic collapse and growth reduction by down-regulating the key genes associated with energy metabolism ( $\beta$ -GAL, ATPase, ALPase), cell wall formation (murB, PBP), DNA repair and duplication (RecN, RecF, holA), and the downstream processes of the tricarboxylic acid cycle (OGDH=oxoglutarate dehydrogenase, DLST=dihydrolipoamide succinyl transferase, and DLD=dihydrolipoamide dehydrogenase). At last, all these combined outcomes result in significant membrane rupture, hampered metabolism, protein breakdown, diminished DNA processes and eventually *S. aureus* lysis and cell death (Wang et al. 2020).



**Fig. 1:** Possible antibacterial mechanism of action of plant essential oils against *Staphylococcus aureus*.

### 1.3. Limitations

Although plant essential oils have shown better antibacterial activities against *S. aureus*, they have some limitations (Tomanić et al. 2022; Cășaru et al. 2024). Like, the chemical composition of different essential oils depends upon plant species, harvest time, extraction methods, conditions, cultivation, and storage, which makes it difficult to use them as a standard treatment because of these variations (Bakkali et al. 2008; EFSA CEF Panel 2010; JECFA 2020; National Institutes of Health 2020). Furthermore, most studies are in vitro, making it difficult to conclude that these essential oils can be used safely and effectively as a therapy (Tomanić et al. 2022). Moreover, some components of essential oils (e.g., cinnamaldehyde, thymol, carvacrol, and eugenol) can be harmful at high concentrations or with long-term use. That's why their toxic effects on animals and in milk for human use should be carefully evaluated (Guénette et al. 2007). Their mechanism of action is variable, complex, and not well defined (e.g., membrane disruption), which makes their optimization and regulatory approval difficult (Posadzki et al. 2012; Lee et al. 2018). As essential oils are volatile, they can oxidize rapidly, thereby decreasing their potential (Guénette et al. 2007; Michiels et al. 2008; Caneschi et al. 2023). Some essential oils do not show their bactericidal activities against *S. aureus* (Munive Nuñez et al. 2024). Like, *Foeniculum vulgare* did not cause mortality of *S. aureus*, even at high concentration. It may also contain biofilm-forming genes (Munive Nuñez et al. 2024). That's why future studies must focus on investigating the long-lasting effects of essential oil treatment, including appropriate dosage, application techniques, and potential drug or treatment combinations. It is essential to ensure that these compounds are safe and effective for managing bovine mastitis without negatively impacting human health or milk quality (Munive Nuñez et al. 2024).

## 2. CONCLUSION

Plant essential oils have shown excellent antibacterial activity against *Staphylococcus aureus* isolated from mastitic cows and buffaloes. Their activities against this bacterium vary with different concentrations and conditions. Moreover, these essential oils exhibit greater antibacterial activity against *S. aureus* than antibiotics. That's why they can be used as an alternative treatment for bovine mastitis. Despite these advantages, plant essential oils also have some limitations. The chemical composition of different essential oils depends on various factors (plant species, conditions, cultivation, harvest time, extraction methods, and storage), which makes it

challenging to use them as a standard treatment. Moreover, most studies are in vitro, which makes it difficult to assess their safety and efficacy. Furthermore, their mechanism of action cannot be well-defined due to their high complexity and variability. The efficacy of these essential oils is also decreased because they are volatile and can oxidize quickly. That's why more studies should be conducted to determine their extract dose, safety, and mechanism of action, especially in vivo.

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