

## Bovine Mastitis: Causes and Phytotherapies

Diptimayee Acharya<sup>1,2</sup>, Puspanjali Parida<sup>2</sup>, Himansu Sekhara Mohapatra<sup>3</sup>,  
Santi Lata Sahoo<sup>4</sup> and Jyoti Ranjan Rout<sup>5\*</sup> 

<sup>1</sup>Department of Zoology, Sri Sri Bayababa College, Mahakalpara, Kendrapara, Odisha, India

<sup>2</sup>Department of Zoology, Maharaja Sriram Chandra Bhanja Deo University, Baripada, Odisha, India.

<sup>3</sup>Department of Botany, Kendrapara Autonomous College, Kendrapara, Odisha, India.

<sup>4</sup>Department of Botany, Utkal University, Vani Vihar, Bhubaneswar, Odisha, India.

<sup>5</sup>School of Biological Sciences, AIPH University, Bhubaneswar, Odisha, India.

### Abstract

Mastitis is a highly frequent chronic ailment with inflammation in the udder of the milking cows. The causative agents are mostly microbes. It is economically prominent contamination of lactating cows resulting in reduced milk production. The disease is diagnosed by chemical, physical and nutritional changes in the milk and pathological changes in the milk glands. Prevention measures for the disease can be taken by proper and timely sanitation of the cowshed through and time again disinfection of the teat, mechanized milking process, etc. The application of bactericidal drugs generates resistant varieties of microbes that cross the allopathic boundary. In this regard, an attempt is taken to focus the plant-based pharmacopoeia. Medicinal plants are traditionally used to cure various diseases as they are comparatively accessible to administer orally in different forms and can be along with fodder. Keeping the above facts in view, the present review deals with different types of mastitis, causative pathogens, detection and diagnosis, and effective plant-based treatment process available to date.

**Keywords:** Bovine Mastitis, Causative Agents, Lactating Cows, Medicinal Plants

\*Correspondence: routjr@aiph.ac.in

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## INTRODUCTION

Dairy cows are considered the mother of human society. Fresh and hygienic milk is very much popular due to abundantly available nutraceutical.<sup>1</sup> As all the macro and micronutrients, vitamins, minerals growth factors and some other nutritional factors are there in the milk for which it is the most balanced and natural diet obtained universally.<sup>2</sup> Poor production of milk is always less than the requirement due to various factors. Some constraints are inappropriate management, mismatched genetics, nutritional deficiency, unhealthy reproductive condition, and frequently infected teat along with the udder, internally and externally. These factors contribute adversely to insufficient production of milk by lactating cows.<sup>3</sup> It is one of the most frequent diseases of cattle and capable of causing serious damage to dairy herds worldwide.<sup>4</sup> The symptoms of the disease are unusual increment in the number of somatic cells and deterioration in the quality of milk.<sup>5</sup> High somatic cell count (SCC) in mastitic cows is detected by the fluoro-optoelectronic method.<sup>6</sup> SCC approximately 200,000 cells/ml is the threshold value for mastitis.<sup>7</sup> Mastitis also can transmit chronic diseases like tuberculosis brucellosis, leptospirosis, etc. through contaminated milk to consumers.<sup>8</sup> The disease differs from other diseases of cattle as a plethora of mostly bacterial pathogens invades udder tissue. These stubborn pathogens enter the udder, navigate, infect and produce the toxin which inflamate the udder along with the teats which were more susceptible. The outcome of which is a drastic change in milk quality and quantity.<sup>9</sup> The cost-effective benefits of the poor farmers were adversely affected due to contaminated and unsafe dairy products.<sup>9-11</sup> The total economic estimation towards loss is approximately ₹ 7824 in one month per cow.<sup>10</sup>

### Types of Mastitis

Depending on the infection pattern, mastitis is of different types; contagious and environmental with a broad spectrum of microbes. Besides the above types, it can be subclinical or clinical.<sup>12,13</sup> Contagious mastitis is generated from inflected ones and contaminated to healthy cows by the time of milking through hands. It can also

spread through improper sanitization of milking machines or by cloths used during milking but in environmental mastitis, the causal pathogens originate from cowsheds, unhealthy filthy water for preparation of udder prior to milking, flies, mud holes.<sup>13</sup> Intramammary infections (IMIS) were diagnosed as SCM. However, it is difficult to identify SCM as initially it appears inside without any visible inflammation or redness in the udder or teat but an increase of somatic cells in the milk act as an indicator.<sup>14</sup> The occurrence of SCM was ~32.48% and considerably lowers in clinical mastitis 9.4%.<sup>15</sup> SCM could not be noticed so easily but is always associated with loss of milk in the dairy herd.<sup>16</sup> Traditional and advanced therapies are applicable for the control of mastitis. The treatments were through bacteriocins, nanoparticle based, vaccination, antibiotics, and phytotherapy. These therapies are not effective because multiple factors (host, pathogen, and environment) are responsible for the disease.<sup>17</sup> To date for curing mastitis, the broad-spectrum antibiotics were used as the common therapy. However, the consequence of this antibiotic therapy is the drug resistance microbes. Substitutes for this type of therapy were explored by many researchers.<sup>18-20</sup> Thus, herbal therapy for mastitis treatment is an accurate alternative to be replaced for synthetic drug.<sup>21</sup>

### Causative Pathogens

Many mastitis pathogens are identified to dates, such as *Staphylococcus aureus*, *Streptococcus agalactiae* and other *Streptococcus* spp.<sup>22-26</sup> *Streptococcus dysgalctiae* is a major causal microbe reported in the case of SCM in bovine herds followed by *Clostridium perfringens*, *Mycobacterium*, *Mycoplasma*, *Prototheca*, *Pasturella*, *Nocardia asteroides*, *Pseudomonas auriginosa*, *Staphylococcus aureus* and yeasts.<sup>27</sup> *Actinomyces* spp., *Staphylococcus* spp. and *Streptococcus* spp. are some pathogenic bacteria isolated from bovine mastitis.<sup>28</sup> *Staphylococcus aureus*, *Streptococcus agalactiae* and *Mycoplasma* spp. are contagious bacteria causing contagious mastitis.<sup>13</sup> Microorganisms that cause mastitis are categorised into three categories; 1. Contagious (*Corynebacterium bovis*, *Staphylococcus aureus*, *Streptococcus agalactia*, *Mycoplasma* sp.) 2. Environmental (*Enterobacter aerogenes*,

*Escherichia coli*, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Streptococcus uberis*, *Streptococcus bovis*, *Streptococcus dysgalactiae*, *Citrobacter* sp. and *Serratia* sp.) and 3. Other (Coagulase-negative *Staphylococci* sp., *Arcanobacterium pyogenes*, *Candida* sp., *Nocardia asteroides*, *Pseudomonas aeruginosa*, *Prototheca* sp. and *Serratia* sp.<sup>29</sup> *Staphylococcus aureus* is predominant in mastitis samples as reported by many workers.<sup>30-33</sup> *Escherichia* spp. (22.16%), *Klebsiella* spp. (1.47%), *Pasteurella* spp. (2.45%), *Pseudomonas* spp. (0.45%) *Staphylococcus aureus* (20.19%), *Streptococcus* spp. (13.3%), *Streptococcus agalactiae* (12.8%) and *Streptococcus dysgalactia* (0.5%) are the most common pathogens isolated from mastitis milk sample.<sup>34</sup> *Staphylococcus*, *Aerococcus*, *Streptococcus*, *Enterobacter*, *Macroccoccus*, *Corynebacterium*, *Acinetobacter*, *Psychrobacter*, *Ignavigranum*, and *Atopostipes* are the bacteria species which are found to be causative pathogen for bovine mastitis. *Staphylococcus* spp. and

*Streptococcus* spp. are maximum contaminants amongst all these.<sup>35</sup> *Escherichia coli*, *Klebsiella* spp. and *Staphylococcus aureus* are isolated from the mastitis milk sample.<sup>36</sup> *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus uberis*, *Streptococcus dysgalactiae*, and *Staphylococcus aureus* are isolated and identified from samples collected from mastitis cow.<sup>5</sup> The most predominant bacterial pathogen responsible for the high prevalence in both subclinical and clinical mastitis was *S. aureus* followed by *E. coli* and *S. agalactiae*. The frequency of mastitis due to fungi and yeast was found to be very less as compared to the bacterial pathogens.<sup>37</sup> Overuse of antibiotics and poor sanitation contributes to yeast mastitis.<sup>34</sup>

#### Detection and Diagnosis

Mastitis is easy to detect and diagnose clinically, in which the type phenotypical observations are quite visible like red and swollen



**Figure.** Images showing cow affected with mastitis during the sample collection. A: Filthy cowshed; B: Sample collection; C: Infected udder; D: Swollen udder

udder and fever in dairy herds (Figure). The texture of milk is different with flakes and clots than the normal type.<sup>35,39</sup> It was also notified that during infection an uncountable group of white blood cells (leukocytes) invade the mammary gland.

### **Risk Factors**

Some incidental factors for pathogens of mastitis are parity, stage and lactation, quantity production, hygienic nutrition and breeds.<sup>40-42</sup> Several risk factors like body weight, age, milk yield, udder type, teat size, floor and bedding, season, parity and duration of lactation are associated strongly with the pathogens of mastitis.<sup>15</sup> Other factors are inherent like susceptibility of mammary gland for intramammary infections (IMI), breed type, previous mastitis history and lactation stage are also countable for the prevalence of mastitis.<sup>43</sup> Due to the pathogenic load in lactating animals milk stasis (hindrance to milking) also enhances the density of disease. Various factors concerned with this disease are discussed below.

### **Age**

Older cows having more body weights are susceptible to mastitis.<sup>15</sup> There is a correlation between the age of the lactating cow and mastitis due to the relaxed sphincter muscles of teat.<sup>44</sup> Stress associated with old age makes the animal prone to a weak immune system. Hence the older cow's infection rate is persistent and ultimate loss is more due to the incidence of mastitis.<sup>15</sup>

### **Breed**

Cross breed jersey (70.41%) is more susceptible to mastitis in comparison to HF (16.33%) and local breeds (13.27%).<sup>15</sup> In India, Jersey-Holstein cross breed cows are more prone (94.54%) to mastitis than indigenous breeds (31.25%).<sup>16</sup> Maximum report of mastitis was observed in cross breed herd than local indigenous one as reported.<sup>45</sup>

### **Species**

Buffaloes are less susceptible to mastitis than cows.<sup>46,47</sup> Buffaloes are highly resistant as they have tightly closed teat orifices.

### **Milk Yield**

Increased incidence of mastitis in high

yielders than low yielders stated by many workers. This may be due to variable immune response of cow against infection.<sup>15,48,49</sup> Cross breeds are high yielders and have heavy body weight than indigenous cows. So, exposure to more body surface area also invites environmental microbes to generate mastitis. The body area and weight of the hybrid bovines have more physiological stress. Due to the higher production of milk opening of teat sphincters for a continuous period invites the colonization of microbes for mastitis.<sup>15</sup>

### **Parity**

The highest incidence of mastitis was seen in 5<sup>th</sup> parity followed by 4<sup>th</sup>, 3<sup>rd</sup>, 2<sup>nd</sup> and 6<sup>th</sup> with the lowest incidence in 8<sup>th</sup> one. The attributable reason is that IMI remains persistently up to 5<sup>th</sup> parity stage and gradually declines.<sup>50,51</sup>

### **Stage of Lactation**

Onset of lactation also sometimes generates contagious disease in milking animals. First month of lactation and last months were more critical for mastitis than the in between period.<sup>15,52</sup> Lactation also harbours microorganisms due to lack of sanitation or other factors.<sup>53</sup> Accordingly, first month of lactation is prominent for mastitis and gradually diminishes towards the onset of dry periods i.e., the last months of milk formation. The frequency of mastitis is more in first two months of lactation and 2-3 weeks from the beginning of dry period.<sup>54</sup> Sometimes cows at the lactating period of early maximum days are more prone to environmental contaminants as reported by.<sup>55,56</sup> Generally, first day of lactation is more susceptible (62.7% for mastitis might be due to the hypersensitivity of mammary glands). It is also observed that gradually it declines towards the late stage (11.2%).<sup>52</sup> One of the prime factors of mastitis during the early lactation period is that oxidative stress is more and could not be neutralised by antioxidative defence.<sup>43</sup>

### **Herd Size**

There are significantly more (46.6%) mastitis reports with larger herd size than smaller one.<sup>15,48</sup> The population of herds in the dairy industry is also a prominent factor for the maintenance of healthy lactating animals. Improper management of sanitation, hygiene,

maintenance of the floor and bedding area are contributing significantly to the proliferation and transmission of mastitis microbiota.<sup>15</sup>

### Season

Highest incidence of mastitis is observed in the rainy season (62.24%) followed by the summer (26.53%) and the winter (4.08%).<sup>15</sup> It seems that breakout of mastitis is more in low temperature. This depicts that the bacterial growth is more suitable in low temperature climate.<sup>57,58</sup>

### Floor Type of the Shed

Cowshed matters much for the origin of mastitis. Earthen floor of shed makes the dairy animals maximum (48.98%) susceptible than brick floor (38.78%) and concrete floor (12.24%). Maximum occurrence of the disease is reported in the earthen floors might be due to improper cleaning and dampening.<sup>15</sup> Poorly designed facilities increase the incidence of environmental mastitis.<sup>41</sup> Cowsheds are mostly in unhygienic condition due to obvious reasons, which plays a major role in harbouring environmental pathogens for mastitis. Due to the humid climate basement of cowshed mostly remains muddy or swampy favouring the growth of mastitis.<sup>43</sup>

### Udder Type Teat Wise Prevalence

Prevalence of mastitis is highest in cows with cup shaped udder (48.98%), then bowl shaped udder (30.61%) and round shaped udder (20.41%).<sup>15</sup> Cup or pendulous udder (depending on the depth) is closer to filthy ground in unhygienic condition in a suitable basement for mastitis.<sup>44</sup> Occurrence of mastitis is comparatively less in fore quarter 42.85% than hind (57.14%). Accordingly, the right hind teat is more prone (40%) than the left hind (17%), right fore 19% and left fore 24%.<sup>15</sup> Teat wise or udder wise contamination is more in hind part and the reason could be milk yield potential followed by contaminated hind legs and relaxed teat sphincters.<sup>59</sup> Generally, in dairy cattle prevalence of mastitis in hind quarters were comparatively more due to the exposure of that part to dung and urine.<sup>60</sup>

### Method of Milking

Occurrence of mastitis is more frequent

in hand milking process than the mechanised ones.<sup>15</sup> Mechanised milking process is always advantageous to obtain safe and healthy milk.<sup>61</sup>

### Teat Injury

Prime pathway for entry of mastitis is the teat canal. Hence attention should and must be focused on the maintenance of a healthy and clean teat. Most important is to avoid teat injury, which is a risk factor for contamination of the intra- mammary glands.<sup>62</sup>

### Use of Teat Disinfectant

Disinfectants are always advisable particularly for teat and udder which lowers a load of contaminants of mastitis proper sanitation is mandatory during the lactation period to control the spread of microbial infections and their colonial growth.<sup>63</sup>

### Habitat

Incidence of the disease is more in rural habitats than that of urban. Urban areas were more facilitated as regards general awareness of the disease, proper veterinary services and immediate attention to the animals as and when required. In contrast, rural areas have slow pace lifestyle, sluggish and careless towards the dairy herds. Negligence in sanitation, hygiene and hospital service in rural areas make the destitute cows more susceptible to microbes for mastitis or any other disease.<sup>15</sup>

### Dry Period

There is an increased risk of clinical mastitis with longer dry period >40 days.<sup>64</sup> The frequency of infection increases during the two weeks prior to calving and two weeks following drying.<sup>65</sup> However, the intramammary infection is 2 to 12 times higher during the dry period.<sup>66</sup>

### Transition Period

A transition phase during parturition is more critical for any contagious disease in dairy cows. This phase is 4 weeks before the birth of calf and continues up to 4 weeks after.<sup>67</sup> This is due to physiological stress associated with other factors like intensive growth of mammary glands, onset of overflowing synthesis and secretion of milk with higher energy requirement. To meet

the energy, the need of oxygen is improved to a higher level.<sup>68</sup> It is also experienced that during the peripartum duration alteration in defence mechanism is an inevitable change may be due to hormonal imbalance or any type of stress.<sup>69</sup>

### Milking Interval

Due to irregular intervals for milking and the pre, post milking treatment of the teat and teat canals mostly the time interval in lactating cows are neglected seriously. As the time gap is more than 12 hours per day, which allows the bacteria to colonize at the teat ends.<sup>70</sup>

### Blood Group

In Red Danish dairy cattle, the blood group is correlated with the emergence of mastitis during the lactation period. It was observed that bovines with the M blood group were more susceptible to bacterial infection as compared to the cattle herds lacking it.<sup>71</sup>

### Control and Treatment

In spite of application of all the synthetic and strong antimicrobial agents, the microorganisms put their inherent quality of resistance by altering their genome structure.<sup>72</sup> The outcome of this is drug resistance, which is sometimes considered a progressive evolutionary trend. This type of system generates resistant microbes which can survive, reproduce and stabilize themselves along with the microbiocidal drugs.<sup>73</sup> The above facts forcibly paved the pathway in biological science to develop effectively the alternate branch of drug from the living world in which lower and higher groups of plants occupied the prime position in livestock health research.<sup>74</sup> It was also observed that supplementation of vitamin D also reduces the bacterial load. Ancient literatures already established the fact that (Vanaspati), the plants were utilised in Ayurveda, Siddha and Unani for curing different ailments of living world.<sup>75</sup> According to the ethnomedicinal branch of science most or all plants possess an array of secondary metabolites otherwise known as natural products like terpenes, phenolics and nitrogen containing metabolites, alkaloids glucosinates, cyanogenic glycosides, exhibit significant defensive activity for their pests and most of the microbes.<sup>76,77</sup>

### Medicinal Plants used against Mastitis

The pharmacopoeia database evidenced the ethnomedicinal property of plants against various contamination created by the pathogenic organism. The significant aspect of application of plant-based therapeutics is the minimal side effects.<sup>78,79</sup> Several plants (whole or part) and plant extracts were traditionally applied for the treatment of mastitis in dairy cows. The most frequently reported plant species are *Amomum subulatum*, *Allium sativum*, *Capsicum annum*, *Centratherum anthelmisticum*, *Citrus limon*, *Citrullus colocynthis*, *Curcuma longa*, *Cuminum cyminum*, *Lepidium sativum*, *Nigella sativa*, *Peganum harmala*, *Rosa indica*, *Sesamum indicum*, *Triticum aestivum*, and *Zingiber officinale*.<sup>21</sup> Extracts of *Artemisia absinthium*, *Baccharis dracunculifolia*, *Cymbopogon nardus*, *Senna macranthera*, with different solvents exhibited antibacterial activity against *Staphylococcus aureus* strains isolated from mastitic cows.<sup>80</sup> Methanolic extracts of *Abutilon indicum*, *Brachiaria* sp., *Cenchrus ciliaris* and *Coccinia grandis* showed conspicuous antimicrobial activities against bovine mastitis pathogens.<sup>81</sup> Antibacterial activity and chemical profiling of *Punica granatum* was effective against pathogens isolated from cows with mastitis.<sup>82</sup> *In vitro* bactericidal properties of some selected wild medicinal plants used to cure the most frequent disease of lactating bovine, the mastitis.<sup>83</sup> Methanolic extracts of *Abutilon indicum*, *Asteracantha longifolia*, *Brachiaria* sp and *Trichodesma indicum* displayed antimicrobial activity against *Staphylococcus aureus* isolated from Bovine mastitis.<sup>77</sup> Distilled water extracts of *Aloe barbadensis*, *Annona squamosa*, *Azadirachta indica*, *Curcuma longa*, *Macrotyloma uniflorum*, *Phyllanthus niruri* and *Terminalia chebula* were found to be effective in treatment of Bovine mastitis.<sup>36</sup> Traditionally used ethnoveterinary herbs in northwest Pakistan like *Oryza sativa*, *Triticum aestivum*, *Bunium persicum* and *Allium sativum* were therapeutically active against most common microbial pathogen. Out of the above-mentioned plants, alkaloid of two (*B. persicum* and *A. sativum*) strongly inhibits the growth of mastitis causing bacteria.<sup>84</sup> Mastitis could be treated with intramammary infusion of extract of *Rheum officinale* and *Angelica dahurica*.<sup>85</sup>

Extracts of *Allium sativum*, *Zingiber officinale* and *Capsicum annuum* were highly effective against multidrug resistant *Staphylococcus aureus* and *Streptococcus pyogenes* isolated from buffalo mastitic milk.<sup>86</sup> Bactericidal property of *Artemisia nilagirica* was found to be effective against *Bacillus subtilis*, *Candida albicans*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, *Salmonella typhi*, *Staphylococcus aureus* and *Yersinia enterocolitica*.<sup>87</sup> Plant extracts of *Artemisia herba-alba* and *Jasonia montana* were found efficacious to counter bacterial flora of clinical or sub clinical mastitis.<sup>34</sup> Extracts of *Combretum mole* and *Xanthium strumarium* manifested good *in vitro* antibacterial effect against *Streptococcus agalactiae* and *Staphylococcus aureus* isolated from cows with mastitis.<sup>88</sup> Leave extracts of *Syzygium cumini*, *Millingtonia hortensis* and *Zizyphus mauritiana* have a strong antimicrobial effect against *Staphylococci* (Coagulase-negative) collected from lactating cows having mastitis.<sup>89</sup> Hexane extract of *Artemisia nilagirica* has a low MIC value against *Bacillus subtilis*, *Enterobacter aerogenes*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *Shigella flaxneri* and *Yersinia enterocolitica*. *Artemisia nilagirica* also proved to be an active agent for treating related microbial diseases.<sup>90</sup> Acetone solvent extract of *J. montana* plant showed antibacterial activity against *S. agalactiae*, *E. coli*, *S. aureus*, *Klebsiella* spp and coagulase-negative *Staphylococci*.<sup>34</sup> Crushed roots of *Asparagus racemosus* were with about 100gms of fruits of *Trigonella foenum-graecum*, *Foeniculum vulgare*, *Terminalia chebula*, *Terminalia bellirica*, *Piper nigrum*, *Elettaria cardamomum*, flower buds of *Eugenia caryophyllu* and 200gm of *Alium cepa* blended with water and further a small amount of jaggery was added to that, these formulations were administered orally to cure mastitis.<sup>91</sup> Essential oil of *Thymus serpyllum* and *T. vulgaris* in different formulations were tested against the microbes of clinical and sub clinical mastitis, which can be a substitute for the common antibiotics in the near future.<sup>92</sup> Acetone extracts of *A. nilotica* bark and *Tetradenia riparia* flowers showed the best activity against mastitis causing bacteria.<sup>93</sup> Similarly, the alcoholic extracts of marigold, absinthe wormwood, essential oils of oregano, lavender, and rosemary could be effective against

mastitis.<sup>6</sup> Methanolic and ethanolic extracts of *Laggera alata* showed antibacterial activity against *Streptococcus agalactiae* and *Staphylococcus aureus* isolated from bovine mastitis.<sup>94</sup> Methanolic extracts of *Gymnema sylvestre*, *Holarrhenaanti dysenterica*, *Vernonia anthelmintica*, *Enicostemma littorale*, *Momordica charantia*, *Swertia chirata*, *Azadirachta indica* and *Caesalpinia bonducella* showed more or less antibacterial activity against mastitis pathogens. Among these *Azadirachta indica* showed the most promising antibacterial properties.<sup>95</sup> Bactericidal properties of extracts (aqueous and methanolic) of *Tridax procumbens* were applied against *Staphylococcus aureus*. It was noticed that the extracts were quite effective against the causal pathogens of mastitis in lactating animals.<sup>96</sup> The therapeutic property of herbal raw materials as a treatment for mastitis can be used as an appropriate dose at the initial stage is a cost effective one. Above all to enhance the resistance of the body, contamination and inflammation of the teat and udder it is mandatory to follow the maintenance of aseptic environment around habitat of the lactating animal. Moreover, the efficacy of proper and balanced fodder helps a lot for the reduction of contamination.

## CONCLUSION

The rising dairy market and its versatile products throughout the globe are very lucrative and significant. As the consumers are of all the age groups like infants to super senior citizens, so care must and should be taken for the deliverance of healthy dairy products. With the increasing trend of antibiotic resistance, the frequent occurrence of bovine mastitis thrust a stake and stress on the dairy industry, particularly with more milking breeds. Attempts should be taken to reduce the prevalence of microbial contamination at the udder and the associated glands or tissues. Besides antibiotics, alternative therapies in the form of herbal application or supplementation in fodder can help in the treatment of mastitis.

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#### CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

#### AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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#### DATA AVAILABILITY

The data generated during the current study are available from the corresponding author on reasonable request.

#### ETHICS STATEMENT

Not applicable.

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