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Evaluation of *Emblica officinalis* (Amla) fruit for antibacterial activity and therapeutic potential in bovine sub-clinical mastitis

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Abstract
The present study was conducted for evaluation of *Emblica officinalis* (Amla) fruit for antibacterial activity and therapeutic potential in bovine sub-clinical mastitis. The fruit juices were extracted aseptically and the antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* was studied revealing zone of inhibition of 19.7± 0.05mm and 18.81±0.07 mm by working dilution of *E. officinalis* (90/10 v/v) and the MIC and MBC of fruit juice of *E. officinalis* against *S. aureus* were observed to be 5.62% and 11.25% respectively and against *E. coli* were observed to be 22.5% and 45% respectively. *In vivo* study involved specific subclinical mastitic affected lactating cows divided into 2 groups (n = 6), Group 1 cows served as control administered with placebo treatment (wheat bran), cows of Group 2 were treated with *E. officinalis* deseeded fruits @ 250 gm total dose P.O., divided in two parts morning and evening for 5 days. The elimination of infection in *E. officinalis* treatment vs. control was statistically non-significant ($p>0.05$) with χ^2 (01 df, N=24) = 0.974, p= 0.324 however the therapy resulted significant decline on day 21 for California Mastitis Test score, Somatic Cell Count, electrical conductivity and pH of the milk. Therapy resulted significant increase in lactose on day 7 and day 21, however non-significant effect on fat, SNF and protein was observed. Therapy caused significant decline in percentage of mean neutrophil and significant improvement in the mean lymphocyte percentage on day 21.

Keywords: Amla, *Emblica officinalis*, subclinical mastitis, *in vitro* antibacterial activity, alternative therapy

Introduction
State of Maharashtra stands 7th in livestock population and 5th in cattle population. According to 20th Livestock Census the total cattle population in the state was 13.9 million in 2019 and the milk production in the state during 2018-2019 was 2354.32 lakh MT with per capita availability of 394 grams/day. As per 2019 census total Bovine population (Cattle, Buffalo, Mithun and Yak) of India is 302.79 Million, which include 192.49 million cattle mostly comprised of cows (145.12 million). Mastitis is a multi-etiological disease characterized by inflammation of mammary gland along with physical, chemical and mostly bacteriological changes in milk and pathological changes in glandular tissues (Radostitis *et al*., 2007). Udder being a productive organ of dairy animals needs to be healthy and due to its anatomical position is subjected to outside influences that can lead to inflammatory and non-inflammatory conditions (Sharma and Vohra, 2011) [1]. Clinical mastitis usually an individual health problem is characterized by inflammation of udder and gross abnormality in quantity and quality of milk, while as, sub-clinical mastitis is a herd problem, having no observable clinical signs or changes in milk quality/quantity. The subclinical mastitis is 15 to 40 times more prevalent than the clinical mastitis and is difficult to detect, reduces milk production and adversely affects milk quality (Seegers *et al*., 2003) [2]. Mastitis is globally most important disease of economic importance responsible for loss of milk production, milk rejectiondue to undesirable changes in the milk composition and high cost of treatment and control strategies (Bardhan, 2013) [3]. Annual economic losses to mastitis were calculated to be Rs.7165.51 crores in India, out of which Rs. 4151.16 crore loss (57.93%) is attributed to subclinical mastitis (PDADMAS, 2011).
Due to huge economic importance of bovine mastitis, early diagnosis and treatment is of utmost importance which largely depends on administration of antibiotics to the mastitic animal.

The greatest risk to antibiotic use is the emergence of resistant bacteria (Van Hoek *et al.*, 2011) [4] and presence of antibiotic residues in the milk that pose a threat to public health. For this reason, nowadays attention has been given to adoption of non-antibiotic approaches based on improving the natural defense mechanism of the animal by using non-specific immune-modulators such as plant products. The herbal medicine has attained significance due to possessing lesser toxicity, lower side effects and being organic in naturedoes not require milk withdrawal period as is mandatory for antibiotic use. Possessing antibacterial and anti-inflammatory action and also not altering the milk quality herbal therapy has been found superior in many ways.

Embilica officinalis or *Phyllanthus emblica* (Syn: Amla, Indian Gooseberry) an evergreen tree highly prized in tropical Asia is natural to tropical Southeast Asia, particularly in Central and South India is commonly cultivated in gardens and grown commercially as a medicinal fruit. It is among the most important medicinal plants in the Ayurveda Materia Medica and widely used in Indian medicines for the treatment of various ailments (Poltanov *et al.*, 2009) [5]. *E. officinalis* is known to possess potent antibacterial activity against *Staphylococcus aureus* (Dhale and Mogle, 2011; Varghese *et al.*, 2013) [43, 42], *Escherichia coli*, *Klebsiella pneumoniae*, *K. ozaenae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella typhi*, *S. paratyphi A*, *S. paratyphi B* and *Serratia marcescens* (Saeed and Tariq, 2007) [45]. Amla is an excellent antioxidant and free radical scavenger (Hazara, 2010) [44]. Vitamin C in *E. officinalis* accounts for approximately 45-70% of the antioxidant activity (Scartezzini *et al.*, 2006) [46]. Various investigators have reported that the fruits of *E. officinalis* have immunomodulatory (Srikumar *et al.*, 2007) [47], anti-inflammatory (Santosh Kumar *et al.*, 2013; Golechha *et al.*, 2014) [61, 48] and hepatoprotective effect (Pramyothin *et al.*, 2006) [49]. Furthermore numerous other actions of amla have been reported by different researchers such as; analgesic (Perianayagam *et al.*, 2004) [50], anti-tussive (Nosalova *et al.*, 2003) [51], anti-atherogenic (Jeevangi *et al.*, 2013) [52], adaptogenic (Rege *et al.*, 1999) [53], gastroprotective (Rehaily *et al*., 2002) [62], nephro-protective (Yokozawa *et al.*, 2007) [54], memory enhancer (Vasudevan and Parle, 2007) [55] and anti-carcinogenic (Madhuri and Pandey, 2008) [56]. Fresh amla fruit for lactating cows can be used as a feed additive in dairy cow diets to improve antioxidant capacity, protein efficiency, butter quality and to produce more desirable milk fatty acid profiles (Tilahun *et al.*, 2022) [57]. Limited studies have been conducted to explore the efficacy of *E. officinalis* against bovine mastitis and to explore its potential biological properties in the animal health sector.

Materials and methods

In vitro antibacterial activity of leaf extract

Preparation of whole fruit juice and different working dilutions

The fruits of the *E. officinalis* were collected from the local market, washed with tap water and vinegar and then allowed to air dry. Fruits were sterilized with 70% alcohol and cut using a knife sterilized with 70% alcohol followed by passing over flame. Whole fruit juice was prepared in the electric grinder after removing the seeds. The contents were squeezed through muslin cloth and filtered using Whattmann filter paper into the container. For carrying out the antibacterial activity of the whole fruit juices different working dilutions were prepared using distilled water. Using a 1000 µl micropipette, five dilutions were prepared at different proportions of each substance to a final volume of five milliliters (5 ml).These working dilutions were filtered through a membrane filter of 25 µM pore size and stored in refrigerator at 4°C for further use.

10% (v/v) Whole Fruit juice + 90% (v/v) Distilled water (100 µl WFJ + 900 µl DW)
25% (v/v) Whole Fruit juice + 75% (v/v) Distilled water (250 µl WFJ + 750 µl DW)
50% (v/v) Whole Fruit juice + 50% (v/v) Distilled water (500 µl WFJ + 500 µl DW)
75% (v/v) Whole Fruit juice + 25% (v/v) Distilled water (750 µl WFJ + 250 µl DW)
90% (v/v) Whole Fruit juice + 10% (v/v) Distilled water (900 µl WFJ + 100 µl DW)

Preparation of antimicrobial discs from different working dilutions

Antimicrobial discs of 6mm of diameter were made using Whattmann filter paper no. 3. These discs were sterilized under ultraviolet light for 2hrs then submerged in different working dilutions of whole fruit juices (90/10, 75/25, 50/50, 25/75 and 10/90%v/v respectively) for 30 minutes such that each disc absorbed a quantity of about 20 µl of prepared mixture (Teke *et al.* 2019).

Determination of antibacterial activity of the different working dilutions

The antibacterial activities of the different working dilutions of whole fruit juice were determined using Agar disc diffusion test. A quantity of 100µl of suspension of the test microorganisms was spread on Muller Hinton agar plates. Prepared filter paper discs of whole fruit juices along with standard antibiotic discs such as amoxycillin-clavulanate (10 mcg), ceftriaxone (30 mcg) and ciprofloxacin (5 mcg) were inoculated on the agar plates using sterile forceps. After 30 minutes of applying the discs, the culture plates were inverted and incubated aerobically at 35°C for 24 hours for bacterial growth. The *in vitro* antibacterial activity was determined as percent inhibition of bacterial growth calculated from the zones of inhibition (measured in millimeters) produced by the different concentrations of whole fruit juices and the standard antibiotics used as per the method suggested by Vidyasagar *et al.* (2002) [58]. Eight replicates for each bacterial isolate were made and the final values were taken as mean ± S.E of the recorded observations.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

MIC of the whole fruit juices (dilution showing max. antibacterial activity) was determined against *S. aureus* and *E. coli* isolated from mastitis positive animals as per the procedure (Shafi *et al* 2020) [14]. For the MBC determination, all the clear test tubes indicating no visible sign of microbial growth or turbidity in the MIC assay were further sub-cultured on sterile Muller Hinton agar plates by streak plate method. The plates were incubated at 35 °C for 24 hours for bacterial growth if any. The least concentration that did not show growth of test organism was considered as the MBC.

In vivo therapeutic potential of herbs

For *in vivo* studies cows found positive for specific

subclinical mastitis in at least one of the quarters were selected and randomly assigned into treatment and control groups taking into consideration specific physiological data such as calving, lactation stage and milk yield etc. Control group (n= 06) received placebo (wheat bran) and the treatment group (n= 06) received *E. officinalis* fruit (cut into pieces, deseeded) @ 250 gm total dose P.O., divided in two parts morning and evening × 5 days (Bilal *et al.* 2009; Dilshad *et al.* 2010) [60, 59].

Sampling and parameters studied

To assess the quarterhealth status, milk quality and immune status of udder, quarter foremilk (QFM=10 mL) and Cow composite milk (CCM=40 mL) samples were collected pre-treatment (d 0), and post-treatment d 7 and d 21 and were analyzed for various parameters. Isolation and identification of bacteria from QFM samples was performed as per the standard microbial procedures of National Mastitis Council (1990). California Mastitis Test (CMT) was performed as per the method described by Pandit and Mehta (1969) [6], Milk pH was determined by an electronically operated single electrode Pen type digital pH meter, the electrical conductivity of milk was measured by MK-509 digital conductivity meter, Somatic Cell Count (SCC) was determined as described by Schalm *et al.* (1971) [7], biochemical composition parameters of the milk i.e. fat, SNF, protein and lactose were analyzed by Milk analyzer, Differential leukocyte count in milk was assessed as per Dulin *et al* (1988) [8].

Statistical analysis

The data was processed using the statistical package for social science (SPSS version 16.0 for windows) using ANOVA, followed by Duncan's multiple range test and the data on elimination of intramammary infections was analysed using Chi square test. Significance level was set at P≤0.05.

Results and Discussion

The *in vitro* antibacterial activity of fruit juices of deseeded *E. officinalis* were recorded against *S. aureus* and *E. coli* isolated from the mastitis affected animals. The zones of inhibition produced by *E. officinalis* and the antibacterial activity in terms of percent inhibition in comparison to ceftriaxone, ciprofloxacin and amoxycillin-clavulanate against *S. aureus* and *E. coli* are given in Table 1. No marked antibacterial activity was noted for working dilutions 25/75 and 10/90% v/v of whole fruit juices against *S. aureus* and *E. coli* however marked antibacterial activity was noted for working dilutions of 90/10, 75/25 and 50/50 v/v (Fig. 1, 2, 3 and 4).

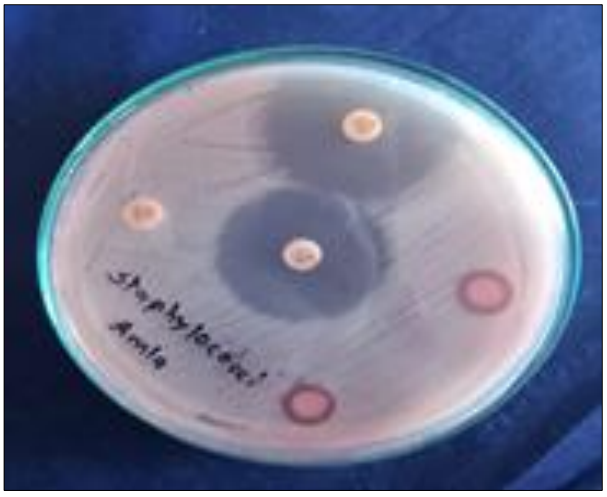

Fig 1: Antibacterial effect of *E. officinalis* fruit juice against *S. aureus*

Fig 2: Antibacterial effect of *E. officinalis* fruit juice against *S. aureus*

Fig 3: Antibacterial effect of *E. officinalis* fruit juice against *E. coli*

Fig 3 and 4: Antibacterial effect of *E. officinalis* fruit juice against *E. coli*

Different researchers have studied the antibacterial activity of *E. officinalis* and have found rewarding results (Kanthimathi and Soranam 2013, Varghese *et al.* 2013, Dharajiya *et al.* 2015, Shafi *et al.* 2016, Shafi *et al.* 2018, Haraoui *et al.* 2020, Shafi *et al.* 2020, Solanki *et al.* 2022) [9, 42, 10, 13, 15, 11, 14, 16]. Sudha *et al.* (2017) [17] determined antimicrobial activities of aqueous extract of amla fruit and observed maximum activity against *K. pneumonia* (16.50±0.342 mm), followed by *E. coli* (14.33±0.33 mm), *S. aureus* (14.33±0.42 mm), *P. aeruginosa* (13.33±0.33mm) and *C. albicans* (10.33±0.21mm). Shah and Malik (2019) studied antibacterial activity of aqueous *P. emblica* fruit extract against eight pathogenic cultures (*S. typhi*, *E. coli*, *S. aureus*, *V. cholerae*, *S. paratyphi A*, *S. paratyphi, B*, *Shigella spp*., and *B. cereus*) using different concentrations of APE (25%, 50%, 75%, and 100%, v/v). The average zone of inhibition against the culture for the concentration of 25%, 50%, 75% and 100% (v/v) of APE were 12.2±1.48 mm, 15.4±1.6 mm, 17±1.7 mm and 19±2.0 mm respectively.

Table 1: *In vitro* antibacterial activity of *E. officinalis* (90/10 v/v) in terms of MIC, MBC and% inhibition of bacterial growth

Pathogen	MIC (%)	MBC (%)	Zones of inhibition		% of inhibition	Zones of inhibition		% of inhibition	Zones of inhibition		% of inhibition
			E. officinalis	CTR		*E. officinalis*	Cipro		*E. officinalis*	AMC	
S. aureus	5.62	11.25	19.7±0.05	23.1±0.07	85.29%	19.7±0.05	22.2±0.10	88.74%	19.7±0.05	Resistant	-
E. coli	22.5	45	18.81±0.07	28.11±0.04	66.92%	18.81±0.07	21.2±0.14	88.73%	18.81±0.07	Resistant	-

The MIC and MBC of fruit juice of *E. officinalis* against *S. aureus* were observed to be 5.62% and 11.25% respectively and against *E. coli* were observed to be 22.5% and 45% respectively. Shah and Malik (2019) [18] studied antibacterial activity of aqueous *P. emblica* fruit extract against eight pathogenic cultures (*S. typhi*, *E. coli*, *S. aureus*, *V. cholerae*, *S. paratyphi A*, *S. paratyphi, B*, *Shigella spp*., and *B. cereus*) using different concentrations of APE (25%, 50%, 75%, and 100%, v/v).MIC was observed in the range of 12.5% - 50% (v/v)and the MBC values indicated that a concentration of 50% (v/v) APE could kill 75% (6/8) test cultures. Varghese *et al.* (2013) [42] investigated the *in vitro* antibacterial activity of aqueous, ethanolic and acetone extracts of fruits of *E. officinalis* against *S. aureus* and *E. coli*. MIC for ethanol and aqueous extracts for *S. aureus* and *E. coli* were 0.3 and 1.0 µg and 1.5 and 3.75µg, respectively. Mayachiew *et al.* (2008) [19] studied antimicrobial and antioxidant activities of Indian gooseberry and Alpinia galangal extracts were investigated against *S. aureus*. Theminimum inhibitory concentration (MIC) values of Indian gooseberry and galangal extracts were found to be 13.97 and 0.78mg/ml and the minimum biocidal concentration (MBC) values were 13.97 and 2.34mg/ml, respectively.

The therapeutic management with *E. officinalis* could eliminate only 9/16 of intramammary infections at d21 post-treatment in comparison to control group (5/19) that was statistically non-significant ($p>0.05$) with χ^2 (01 df, N=24) = 0.974, p= 0.324 (Table 2). Contrary to our finding several researchers have studied different herbs/non-antibiotic preparation against intra-mammary infections that were observed having beneficial effects against bovine mastitis. Sharma *et al.* (2010) [50] observed the effect of *P. emblica* and amoxicillin sulbactum combination on the bacterial growth of milk and observed no bacterial growth on day 30 of therapy as compared to heavy bacterial growth on day 0.

Table 2: Effect of therapy on elimination of Intra-mammary infections (IMIs)

Infection Status of the Quarters	Control Group		*E. officinalis* Group	
	Present at 0d	Eliminated at 21d	Present at 0d	Eliminated at 21d
No. of quarters with IMIs	19	5	16	9
Overall	19	5 (26.31%)	16	9 (56.25%)*

Figures in parentheses indicate percentage

Non-significant differences existed in elimination of IMI between *E. officinalis* treatment as compared to control [$*\chi^2$ = 0.974 (01df, N=24)]

Khan *et al.*, (2018) compared the therapeutic efficacy of *E. officinalis* fruit extract and procaine penicillinin the treatment of subclinical mastitis in dairy buffaloes and observed quarter based bacteriological cure rate of 64.7% with amla fruit extract and concluded that amla fruit extract is an inexpensive alternative to procaine penicillin for treatment of subclinical mastitis in dairy animals. Gao *et al.*, (2018) [21] studied that *E. officinalis* fruit extract could effectively alter the oral microbiome and decrease the total bacterial count. Pandey *et al.*, (2020) [26] evaluated three different dosage regimens (@ 250 gram, 200 gram and 150 gm of crushed deseeded fresh *E. officinalis* in cattle affected with subclinical mastitis and observed that supplementation significantly decreased total bacterial count as compared to non-supplemented group in which increase in somatic cell count and total bacterial count and decline in milk yield was recorded. Rizwan *et al.*, (2021) [22] studied comparative therapeutic efficacy of procaine penicillin, *P. emblica* fruit extract and *Cocos nucifera* oil against subclinical mastitis and reported bacteriological cure rate of sub- clinically mastitic teats by *P. emblica* fruit extract as 40% and 50% at 7th and 14th day respectively.

The values of SCC, CMT, pH and EC are given in Table 3 and it can be observed that significant decline in their values was seen on day 21 thereby indicating decreased udder inflammation and improvement in the udder health and quality of milk. Similarly various researchers have reported beneficial role of *E. officinalis* therapy or other non-antibiotics herbal preparation in terms of reducing udder inflammation and improving milk parameters such as SCC, CMT, EC and pH (Das *et al.* 2003, Sharma *et al.* 2010, Ibrahim *et al.* 2016, Shafi *et al.* 2016, Dutta *et al.* 2020, Pandey *et al.* 2020, Patel and Gupta 2020, Rathour *et al.* 2020, Shafi *et al.* 2020, Keshamoni *et al.* 2021, Siyal *et al.* 2017, Martinez *et al.* 2022) [24, 50, 23, 13, 63, 26, 14, 29, 21, 30]. Khan *et al.*, (2021) [64] observed that aqueous fruit extract of *P. emblica* significantly decreased udder inflammation and ceruloplasmin concentration, an acute-phase protein in udder inflammation in bovine mastitis. Rizwan *et al.* (2021) [22] studied comparative therapeutic efficacy of procaine penicillin, *P. emblica* fruit extract and *Cocos nucifera* oil against subclinical mastitis and reported 40% and 50%

bacteriological cure rate of sub-clinically mastitic teats by *P. emblica* fruit extract on 7th and 14th day respectively and also significant reduction in CMT score was observed.

Therapy with feeding of *E. officinalis* fruit caused significant decline in percentage of mean neutrophil on day 7 and also on day 21 and significant improvement in the mean lymphocyte percentage on day 21 (Table 3). Dhakal and Kapur (1992) [33] reported that neutrophils are the predominant in mastitis milk followed by lymphocytes, epithelial cells and monocytes and observed decreased percentage of lymphocytes while as increased percentage of neutrophils in the milk leukocytes of mastitis affected animals. Pragati *et al.* (2015) [34] studied comparative efficacy of *E. officinalis* and *T. cordifolia* on hemato-biochemical profile of Murrah buffalo calves fed @ 250 mg/kg body weight once a day orally for 28 days and observed significant increases in total erythrocyte count, total leukocyte count and lymphocyte neutrophil ratio in calves fed *E. officinalis*; lymphocyte count was found significantly elevated in calves fed *E. officinalis* and *T. cordifolia* while as significantly decreased level of neutrophil and monocyte count calves fed *E. officinalis*. Leukocytes recruited early in the infection due to their ability to phagocytose or eliminate intra mammary infections are vital for host innate immune response and due to this fact polymorphonuclear leukocytes predominate in the mammary secretions. The activation of polymorphonuclear leukocytes especially lymphocytes by herbal therapy is indication of immunomodulation a mechanism important for elimination of intra-mammary infections and subsiding of udder inflammation that has been supported by the findings of various researchers (Gupta and Pachauri 2001, Acharya *et al.*, 2002, Mukherjee *et al.*, 2005, Shafi *et al.*, 2016, Shafi *et al.*, 2018 and Shafi *et al.*, 2020) [35, 36, 12, 15, 14].

Table 3: Effect of *E. officinalis* (G2) therapy on inflammatory markers of the udder as compared to control group (G1)

Parameters	Group	Days after initiation of treatment (AT)		
		Day 0	Day 7	Day 21
CMT point score	G1	2.17 ± 0.30^{1a}	2.00 ± 0.25^{1a}	1.67 ± 0.21^{1a}
	G2	2.00 ± 0.25^{1a}	1.33 ± 0.33^{1a}	0.33 ± 0.33^{2b}
SCC ($\times10^3$ /ml)	G1	749.17 ± 66.60^{1a}	628.83 ± 60.68^{1ab}	518.67 ± 29.55^{1b}
	G2	679.67 ± 60.29^{1a}	495.83 ± 84.28^{1a}	253.17 ± 59.53^{2b}
EC (mƱ)	G1	63.51 ± 5.51^{1a}	60.76 ± 0.95^{1a}	66.15 ± 1.77^{1a}
	G2	54.16 ± 1.05^{1a}	52.26 ± 0.82^{2a}	46.03 ± 0.56^{2b}
pH	G1	7.02 ± 0.07^{1a}	6.98 ± 0.06^{1a}	7.0133 ± 0.05^{1a}
	G2	6.95 ± 0.06^{1a}	6.82 ± 0.05^{12ab}	6.68 ± 0.06^{2b}
Neutrophil (%)	G1	55.33 ± 2.56^{1a}	48.83 ± 2.72^{1a}	49.00 ± 2.43^{1a}
	G2	51.33 ± 2.37^{1a}	44.17 ± 2.84^{1b}	26.83 ± 1.51^{1c}
Lymphocyte (%)	G1	16.00 ± 2.42^{12a}	15.17 ± 1.07^{1a}	16.17 ± 0.83^{1a}
	G2	14.17 ± 0.47^{1a}	17.83 ± 0.79^{2b}	19.50 ± 0.76^{2b}

Superscripts in each row (a, b, c) and each coloumn (1,2) differ significantly ($p < 0.05$)

Therapy with *E. officinalis* resulted in significant increase in lactose on day 7 and day 21 however non-significant effect on fat, SNF and protein was observed (Table 4). Milk composition in the terms of percentage of fat, SNF, protein and lactose were important parameters in measuring the milk quality and subclinical mastitis affected animals were seen with decreased percentage of these parameters, however, therapy with fruits of *E. officinalis* showed beneficial effect in terms of increasing the percentage of lactose only. Similarly various researchers have reported beneficial role of *E. officinalis* therapy or other non-antibiotics herbal preparations in terms having positive effect on fat, SNF, lactose and protein and thereby having potential of improving the milk quality (Shafi *et al.* 2016, Shafi *et al*. 2018, Harjanti *et al.* 2019, Pandey *et al.* 2020, Patel and Gupta 2020, Shafi *et al.* 2020, Singla *et al.* 2021) [13, 15, 37, 26, 27, 14, 40]. Tilahun *et al*., (2022) [57] studied effect of feeding fresh amla fruits in lactating cows on milk fat, protein and lactose content of milk and observed animals receiving 600 g/d achieved higher milk yields than the animals receiving 200, 400 g/d and control and also animals receiving 400 g/d and 600 g/d led to greater milk protein yield ($P < 0.01$), milk protein content ($P < 0.01$), and milk nitrogen efficiency ($P < 0.01$) compared to cows fed 200 g/d FAF or control. Rizwan *et al.* (2021) [22] studied comparative therapeutic efficacy of procaine penicillin, *P. emblica* fruit extract and *Cocos nucifera* oil against subclinical mastitis and its effects on milk composition of 30 lactating beetal goats, divided into three groups (GA, GB and GC), each having 10 goats. The group receiving *P. emblica* fruit extracts showed marked increase in milk fat, protein, specific gravity as well as total milk yield increased with time from zero day to 14th day ($p < 0.05$).

Table 4: Effect of *E. officinalis* therapy (G2) on milk composition compared to G1

Parameters	Group	Days after initiation of treatment (AT)		
		Day 0	Day 7	Day 21
Protein (%)	G1	2.89 ± 0.12^{1a}	2.75 ± 0.15^{1a}	2.97 ± 0.18^{1a}
	G2	2.79 ± 0.05^{1a}	2.79 ± 0.10^{1a}	2.98 ± 0.05^{1a}
Fat (%)	G1	2.70 ± 0.48^{1a}	2.56 ± 0.20^{1a}	2.52 ± 0.21^{1a}
	G2	2.82 ± 0.41^{1a}	2.90 ± 0.32^{1a}	3.19 ± 0.22^{12a}
SNF (%)	G1	7.98 ± 0.30^{1a}	7.65 ± 0.23^{1a}	8.01 ± 0.13^{1a}
	G2	7.59 ± 0.30^{1a}	7.90 ± 0.20^{1a}	7.97 ± 0.31^{1a}
Lactose (%)	G1	3.48 ± 0.62^{12a}	4.30 ± 0.09^{1a}	4.41 ± 0.09^{1a}
	G2	4.16 ± 0.06^{1a}	4.40 ± 0.10^{1ab}	4.56 ± 0.15^{1b}

Superscripts in each row (a,b,c) and each coloumn (1,2) differ significantly (P<0.05).

Conclusion

Although *E. officinalis fruit* therapy showed non- significant effect on elimination of intra mammary infections, however, significant decline in the values of SCC, CMT, pH, EC, neutrophils and significant increase in the values of lymphocytes and lactose on day 21 suggest its beneficial role in thery of bovine mastitis in terms of minimizing the udder inflammation and improvement of udder health and quality of milk.

Conflict of Interest

The authors declare no conflict of interest.

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