SHORT COMMUNICATION

In Vitro Efficacy of *Curcuma longa* in Combating *Staphylococcus aureus* Subclinical Mastitis in Buffalo

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Abstract

Subclinical mastitis (SCM) being hidden, goes unnoticed until diagnosed in the lab and is therefore the most studied type of mastitis engaging researchers to look for efficient therapeutic or preventive modalities. The *in-vitro* efficacy of aqueous, methanolic and hydro-methanolic extracts of *Curcuma longa* was studied against isolates of *S. aureus* from SCM cases of buffaloes. The stock solution at the concentrations of 50 (HM-50), 100 (HM-100), 150 (HM-150), 200 (HM-200) and 250 (HM-250) mg/mL was used to prepare the disc. Enrofloxacin disc was used as standard. The highest zone of inhibition (8.2 mm, and 10.1 mm) was noted with the concentration 250 mg/disc of aqueous and methanolic extracts of *Curcuma longa,* respectively. The hydro-methanolic extract failed to yield zone of inhibition against *S. aureus* at all five concentrations. It can be concluded that the antimicrobial potential of aqueous and methanolic extract of *Curcuma longa* can be used in the treatment or prevention of mastitis.

Key words: Buffalo, *Curcuma longa,* Subclinical mastitis.

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Introduction

Mastitis is one of the most widespread disease of milking animals and is categorized as an endemic disease worldwide (Halasa *et al*., 2007). Subclinical mastitis is considered as economically the most important type of mastitis because of its higher prevalence and long-term devastating effects as compared to clinical mastitis (Mungube *et al*., 2005). Usually caused by bacterial pathogens, it is the most cost intensive production disease in dairy industry (Schroeder, 2010). *Staphylococcus aureus* is the most prevailing pathogen known to be associated with various forms of mastitis (Rainard *et al*., 2018). *S. aureus* do not cause abnormalities or fatality but it produces degradative enzymes and toxins that irreversibly damage the lactiferous tissue, ultimately decreasing milk production (Vasudevan *et al*., 2003). The most practiced treatment modality of *S. aureus* infections is the use of antibiotics. However, Rainard *et al*. (2018) demonstrated that antibiotic is not an efficient method due to resistance developed by the pathogen against β-lactam antibiotics and the ability of *S. aureus* to produce biofilm and adapt to host environment. Among *S. aureus*, methicillin-resistant strains (MRSA) have recently emerged as a serious threat (Raina *et al*., 2013). This menace of resistance has led to frequent treatment failures and growing demand for safe, effective and natural antimicrobial, focusing attention on alternate approach of treatment (Singh *et al*., 2013).

In this scenario alternative herbal therapy holds prime importance. Plants produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection (Samie *et al.,* 2010). *Curcuma longa* is a yellow rhizome commonly known as turmeric, a spice ingredient containing a variety of active polyphenolic compounds, found to have anti-inflammatory, antioxidant, antimicrobial, analgesic, wound healing and skin regeneration properties (Cheppudira *et al*., 2013; Moghadamtousi *et al*., 2014). Therefore, the present study was planned to evaluate the *in vitro* efficacy of *Curcuma longa* against *Staphylococcus aureus* in subclinical mastitis cases in buffaloes.

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Materials and Methods

Collection of Sample

Total 180 non-descript buffaloes under different stages of lactation were screened for subclinical mastitis using field tests namely Whiteside test (WST) and California mastitis test (CMT). Ten mL of milk samples showing strong positive reaction were collected following asceptic precautions, in a sterile container for isolation of *S. aureus* by standard procedure.

Processing and Testing of *Curcuma longa*

Curcuma longa rhizomes were dried and then ground into powder form. Powder was soaked in hydro, alcoholic and hydro-alcoholic solvent, sieved by several layers of muslin cloth and finally filtered by Whatman Filter Paper No.1 for a clear solution of *Curcuma longa*. The filtrate was concentrated in incubator at 45°C to obtain the final solid extract. Lastly, sterilized blank discs were soaked in different concentration of hydro, alcoholic and hydro-alcoholic extract of *Curcuma longa* and antimicrobial sensitivity test was carried out with Enrofloxacin as standard antibiotic. The dried material was finally stored in an air tight colored bottles at refrigerated temperature at 4°C. Hydro-extract of *Curcuma longa* was yellowish in colour with very sticky consistency, whereas, both Methanolic and Hydro-methanolic extracts were in dry powder form. Methanolic extract was dark yellowish brown in colour and Hydro-methanolic extract was yellowish brown in colour.

Disc Preparation

A stock solution of each types extract was prepared by dissolving 50 mg, 100 mg, 150 mg, 200 mg, and 250 mg of extract with one mL of their respective solvents (sterile distilled water or 99.9% dimethyl sulfoxide). The final concentrations of 50 mg/mL, 100 mg/mL, 150 mg/mL, 200 mg/mL, and 250 mg/mL were then used to impregnate in sterilized blank discs (Bauer *et al*., 1966). Distilled water and dimethyl sulfoxide-loaded discs were used as negative controls for aqueous and ethanolic extracts, respectively. All impregnated discs were ensured to be fully dried in 45 °C in incubator for 18 to 24 h prior to the application on bacterial lawn (Zaiden *et al.,* 2005). The standard antibiotic disc of Enrofloxacin was used as positive control.

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The lowest concentration of the antibacterial agent at which no turbidity was seen was taken as the MIC value for that set of analysis. The minimum bacterial concentration (MBC) was determined by subculture from each tube showing no growth on nutrient agar plate without any antimicrobial agent. The method described by Lorian (1996) was used to determine MIC and MBC.

Results and Discussion

Out of total 180 buffaloes screened through CMT and WST, 89 (49.44%) were found positive for subclinical mastitis.

The prevalence of *E. coli*, *S. aureus* and *Streptococccus spp*. was 28.08% (25/89), 53.93% (48/89) and 17.98% (16/89), respectively.

In-vitro Efficacy of *Curcuma longa*

In-vitro efficacy of discs infused with aqueous, methanolic and hydromethanolic extracts at different concentrations, *viz.,* 50, 100, 150, 200 and 250 mg/mL of *Curcuma longa* was studied against *S. aureus* (Fig. 1). Curcumin inhibits the growth of both Gram-positive and Gram-negative bacteria (Tyagi *et al*., 2015*). S. aureus* is one of the Gram-positive strains that is susceptible to curcumin-mediated inhibition. The zones of inhibition recorded against these isolates were as shown in Table 1.

No zone of inhibition was observed for aqueous extract of *Curcuma longa* at 50 mg/mL (H-50) and concentration having 100 mg/ mL (H-100), 150 mg/ mL (H-150), 200 mg/ mL (H-200), 250 mg/ mL (H-250) resulted in an average of 6.9±1.79, 7.1±1.96, 7.3±1.41 and 8.2±2.29 mm of zone of inhibition, respectively, against isolates of *S. aureus* (Table 1, Fig. 2). Our findings are in agreement with Keskin & Ceyhan-Guvensen, (2019). The aqueous extract of *Curcuma longa* against *S. aureus* showed the highest inhibition 8.2 mm with the concentration 250 mg/ mL.

For methanolic extract of *Curcuma longa* no zone of inhibition was observed at 50 mg/ mL (M-50) but concentration of 100 (M-100), 150 (M-150), 200 (M-200), 250 (M-250) mg/ mL resulted in an average of 7.9±2.51, 8.6±2.41, 8.6±2.41, 8.6±2.41, 10.1±2.92 mm of zone of inhibition, respectively, against isolates of *S. aureus* (Table 1, Fig. 3). Napolean *et al.* (2009) and Bukar *et al.* (2010) also reported antimicrobial activity of *Curcuma longa* against *S. aureus* at concentration of 200 mg/ mL

Table 1: *In-vitro* antimicrobial sensitivity pattern of extracts of *Curcuma longa* against *S. aureus*

Disc Code	Concentration of *Curcuma longa* (mg/mL) & Zone of Inhibition (mm)				
	50	100	150	200	250
H- Aqueous extract of *Curcuma longa*	Nil	6.9±1.79	7.1±1.96	7.3±1.41	8.2±2.29
M- Methanolic extract of *Curcuma longa*	Nil	7.9±2.51	8.6±2.41	8.6±2.41	10.1±2.92
HM-Aqueous-Methanolic extract of *Curcuma longa*	Nil	Nil	Nil	Nil	Nil

The hydro-methanolic extract failed to yield zone of inhibition against *S. aureus* at all five concentrations tested (Table 1, Fig. 4).

Determination of MIC and MBC

Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC) values of aqueous extract (AE) of *Curcuma longa* calculated were 25 µg/mL each. MIC and MBC values of methanolic extract (ME) of *Curcuma longa* were 12.5 µg/mL each, and those of aqueous-methanolic (AME) extract of *Curcuma longa* were 50 µg/mL and 25 µg/mL, respectively.

Our values of MIC and MBC for methanolic extract of Curcuma *longa* against *S. aureus*, are in agreement with the Ogbonna and Ozgor (2021). Keskin and Ceyhan-Guvensen (2019) recorded MIC and MBC values of *Curcuma longa* aqueous extract for *Staphylococcus aureus* as 2 mg/mL and for methanolic extract as 16 mg/mL. Hegde *et al*. (2012) reported MBC of aqueous extract of *Curcuma longa* for *S. aureus* at 750, which is much higher than our values. Niamsa and Sittiwet (2009) also recorded MBC at higher concentration (32 mg/mL) for aqueous extract of *Curcuma longa* against *S. aureus,* but for MIC, it was only 6 mg/mL.

From the present findings it is concluded that among the three extracts of *Curcuma longa* aqueous and methanolic extracts of *Curcuma longa* at 250 mg/mL concentration showed the highest zone of inhibition against *S. aureus*. This study highlights important microbial properties of *Curcuma longa* which can have potential clinical application. Different concentration of extract of *Curcuma longa* with Enrofloxacin may yield 100 % control over subclinical mastitis.

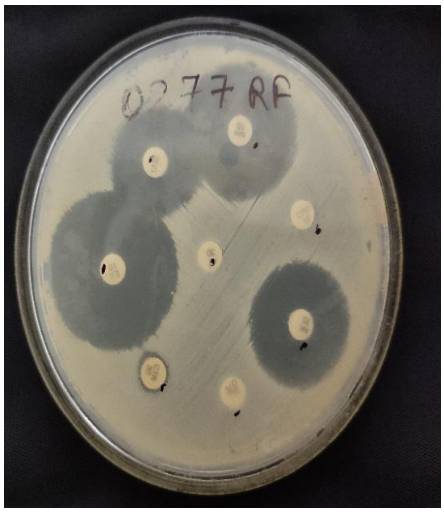

Fig. 1: *In-vitro* efficacy of Antibiotics against *S. aureus*

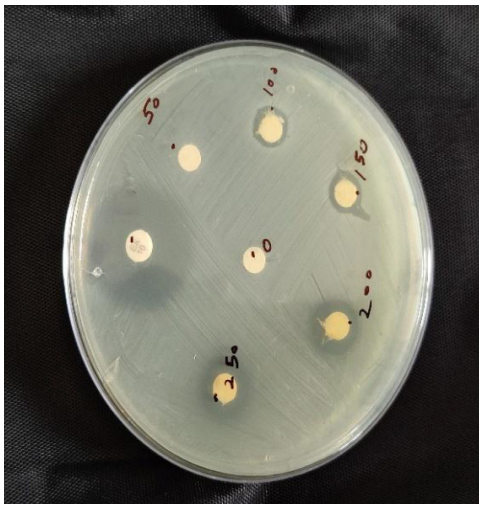

Fig. 2: *In-vitro* efficacy of aqueous extract of *Curcuma longa* against *S. aureus*

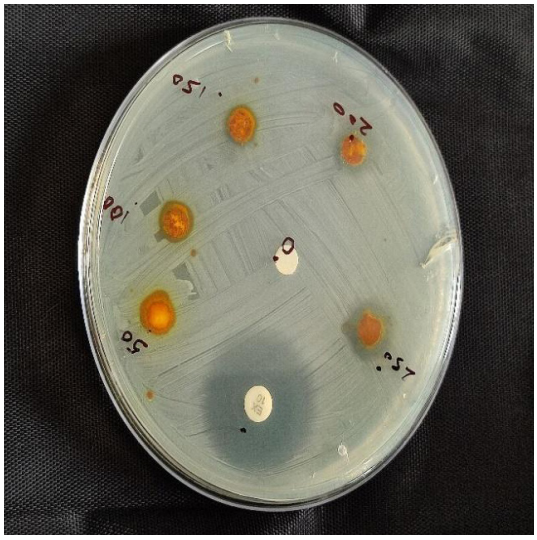

Fig. 3: *In-vitro* efficacy of methanolic extract of *Curcuma longa* against *S. aureus*

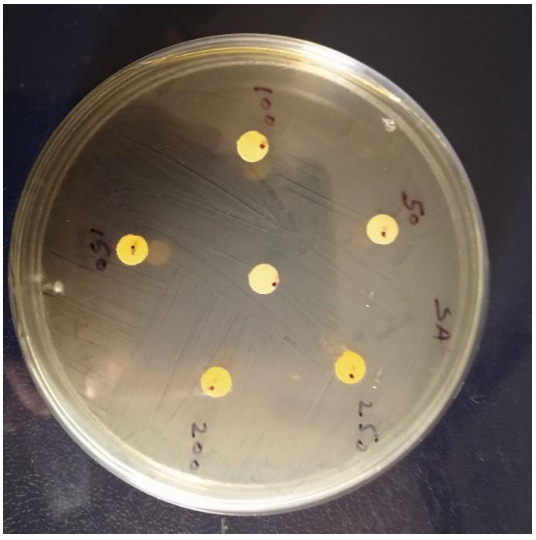

Fig. 4: *In-vitro* efficacy of hydro-methanolic extract of *Curcuma longa* against *S. aureus*

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