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Antimicrobial Potential of *Vetiveria zizanioides* Root Oil against Common Pathogens of Sub Clinical Mastitis in Dairy Cattle

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ABSTRACT

This study was conducted during October 2020 to June 2021 by screening 200 cows of subclinical mastitis by using California mastitis test and White side test, and anti microbial efficacy of Ushir (*Vetiveria zizanioides*) (Linn.) was studied against mastitis pathogens. In vitro efficacy of Vetiveria root oil was studied against positive control, i.e. antibiotic Ciprofloxacin. In the experimental study, the minimum inhibitory concentration of *E. coli* and *S. aureus* was recorded at 12.5% while the minimum bactericidal concentration against *E. coli* was recorded at 12.5%. The maximum area of inhibition was recorded against S. aureus whose the mean diameter was 10.67 0.81. and the lowest zone of inhibition was recorded against E. coli which had a diameter of 10 0.89. In conclusion antimicrobial efficacy of Vetivera root oil was found to be effective in controlling sub clinical mastitis.

Keywords Sub clinical mastitis, Cattle, *In vitro*, *Vetiveria ziziniodes*, Antimicrobial.

INTRODUCTION

Mastitis is a complex disease of multiple etiological origins which is result of interaction of multiple co-related factors. Clinical form of mastitis represents various visible symptoms whereas sub-clinical form of mastitis does not reveal any visible symptoms (Cobirka *et al*. 2020). The sub-clinical form of mastitis is important because this form of disease is 15 to 40 times more dominant than clinical mastitis (Seegers *et al*. 2003). Mastitis is commonly treated by administration of intramuscular or intravenous injection, intramammary infusion of antibiotics such as penicillin, amphicillin, streptomycin, cloxacillin etc. (Bhosale *et al*. 2014). Pathogen have acquired

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resistance due to huge use of drugs, so many studies are focused on treatment of animal by use of an alternative methods (Kalinska *et al*. 2019).

Ushir (*Vetiveria zizanioides*) (Linn.) Nash, is a perennial grass containing aromatic properties (Singh *et al*. 2013). Aromatic properties and multiple use of Vetiver, its also used in traditional medicine in pest control, and as fragrant materials (Devi *et al*. 2010). The antibacterial activity of oil also represents significant action against *Staphylococcus aureus, B. subtilis, P. aeurogenosa* and moderate activity against *S. pyogens, E. coli* and *Corynebacterium ovis*. Ethanolic extract of *Vetiveria zizanioides* is known exert antimicrobial activity (David *et al*. 2019 and Devi *et al*. 2010). Antimicrobial property of Vetiver oil may be due to its complex composition of lipid constituents, complex polysaccharides with extra cellular and soluble proteins which is found to be effective antimicrobial substance against a wide range of microorganisms. The presence of tannins in the roots of *Vetiveria zizanioides* is responsible for in vitro antibacterial activity (Devi *et al*. 2010). The minimum inhibitory concentrations of different samples of Vetiver root oil exhibited a relatively strong antimicrobial ability against *Staphylococcus aureus* (David *et al*. 2019). The present study aim to evaluate the in vitro antimicrobial efficacy of Ushir (*Vetiveria zizanioides*) (Linn.) oil against common pathogen of Sub clinical mastitis in cow.

MATERIALS AND METHODS

Screening of Animals

The present study was conducted following ehical approval of Institutional Animal Ethics Committee (IAEC) during October 2020 to June 2021. 200 Animals were screened by California Mastitis Test (CMT) and White Side Test (WST) and the physical examination of udder health was performed for presence of any abnormality of udder and teat (Schalm *et al*. 1971) in 4 blocks and 8 villages of Gonda and Basti districts of Uttar Pradesh. In-vitro efficacy of Ushir (*Vetiveria zizanioides*) oil was studied against common mastitis causing organism.

Vetiveria Zizanioides root oil was collected from nature care India, Lucknow. Farm produces vetiver oil from CIMAP approved varieties like KS-1 and Sugandha. All the procedure was done at the cytoGene research and development biotechnology, Sahara state, Lucknow, Uttar Pradesh

Preparation of Disc and Antimicrobial activity test

Different concentrations of the Vetiver oil were used that is 25 %, 12.5 %, 6.25 %, 3.125 % and 1.5625 % by performing serial dilution method. The Vetiver oil was used as it is for the test, the oil was loaded over the disc made with Whatman filter paper and allowed to soak thoroughly.

The Antibiotic susceptibility testing was performed by Kirby-Bauers disk diffusion method (Bauer *et al*. 1966) on Mueller Hinton agar (Hi Media, Mumbai, India) as per the CLSI guidelines (Clinical Laboratory Standards Institute 2014). Sample extract used for the test was prepared for the concentration of 1 mg/ml or 1000 µg/ml in DMSO (Dimethyl sulfoxide) solvent. The discs were placed at respective position. Plates had two discs other than sample, one of the positive control, that is antibiotic Ciprofloxacin at 500ppm concentration and 100% DMSO as a negative control were used. Bioassay was carried out in triplicate and experiments were repeated thrice (Mahida and Mohan 2007).

Determination of Minimum Inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC).

MIC is defined as the lowest concentration of antimicrobial or drug that inhibit the visible growth of bacteria after overnight incubation (Levison 2004), while MBC is the lowest concentration of antibacterial agent required to kill a particular bacterium (Wiegand *et al*. 2008).To determine the MIC value of all 3 sample extracts, Broth micro-dilution method was applied (Wagenlehner *et al*. 2006). For this first of all, for each isolate 5 different concentrations of the Vetiver oil were used that is 25 %, 12.5 %, 6.25 %, 3.125 % and 1.5625 %. Each of the tubes were loaded with 250 µl of the sterile nutrient broth medium and then the tube labelled as 25 % concentration was loaded with 250 µl of the Vetiver oil. From the tube with 25

% concentration a serial dilution was performed to reach the concentration of 1.5625 %, this concentration gradient was prepared for each bacterial isolate (*S. aureus, S. agalactiae* and *E. coli*). After this, the tubes containing the samples were loaded with 500 µl of 0.5 Macfarland suspension of bacterial isolated in each well, hence final volume in each tube was 1ml. Based on this observation, the minimum concentration of the sample at which there was no visible growth that is no turbidity in the tube was taken as the MIC value of that sample and then100 µl aliquot from these tubes was inoculated on the Nutrient agar media plates. The minimum concentration at which no colony appeared on the media plate was taken as the MBC value for that sample.

The relative percentage inhibition of the test extract with respect to positive control was calculated by using the following formula by Paluri *et al*. (2012): RPI= 100 (X-Y)/Z-Y. Where, X= Total area of inhibition of test extract; Y= Total area of inhibition of solvent and Z= Total area of inhibition of standard drug.

RESULTS AND DISCUSSION

Total 200 cattle were screened from two districts of eastern Uttar Pradesh 132 (66%) animals were found positive for sub clinical mastitis. Among these 49.2% animals showing sub clinical mastitis in Gonda district and 50.75% animals in Basti district.

Antimicrobial efficacy of *vetiveria zizanioides* root oil against common pathogens of mastitis-

Minimum inhibitory concentration of *Vetiveria zizanioides* root oil was recorded lowest for *E. coli* and *S. aureus* at same concentration value i.e., 12.5 % followed by 25% for *S. agalactiae*. While the minimum bactericidal concentration of *Vetiveria zizanioides* root oil was recorded lowest against *E. coli* at the concentration of 12.5% followed by *S. aureus* and *S. agalactiae* at similar concentration of 25% (Table 1). Similar type of result was obtained by Hammer *et al*. (1999) By using *Vetiveria zizanioides* oil against *S. aureus* and *E. faecalis* recorded MICs were 0.06 to 0.12% (v/v). Luqman *et al*. (2007) also observed recognisable antibacterial activity hexane extracts of the roots of *Vetiveria Zizanioides* against the drug resistant strains of *M. smegmatis* and *E. coli*. Similar results were obtained from various other studies about antimicrobial potential of vetiver (Putiyanan *et al*. 2005, Barad *et al*. 2013, Soni and Dahiya 2015).

In a study David *et al*. (2019) observed that *Vetiveria zizanioides* oil extracted by different methods of extraction have variable antimicrobial potential against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli* at different concentration. The MIC of HDVO oil was 39 µg /mL, by IVDVO oil was 78 µg /mL, by CXEVO oil was 78 µg /mL, by SFEVO oil was 78 µg /mL against *S. aureus*, while the MIC obtained by HDVO oil was 312.5µg/mL, by IVDVO oil was 312.5 µg /mL, by CXEVO oil was 312.5 µg /mL, by SFEVO oil was 625 µg /mL for *E. coli* spp. In a study conducted by Devi *et al*. (2010) EEVZ oil showed better growth inhibition against *S. aureus, P. aeurogenosa* and *E. coli* 25 mm, 18 mm, 20 mm respectively at 750 µg. Another study was conducted by Derya Efe (2019) which resulted the MIC value for *E. cloacae, E. faecalis, E. coli* as 15.63 µg /ml., 31.25 µg /ml., 15.63 µg /ml. and 15.63 µg /ml.

Relative Percentage of Inhibition

In this study the maximum Relative Percent Inhibition was observed in *S. agalactiae* (52.02 %), followed by *S. aureus* (33.19 %) and least RPI is observed in *E. coli* (29.79 %) (Table 2).

Maximum zone of inhibition by selected Vetiver oil was seen against *S. aureus* bacteria (10.67 ± 0.81)

Table 1. MIC and MBC value of *Vetiveria zizanioides* root oil.

Sl. No.	Bacterial Isolate	MIC value	MBC value
1	*E. coli*	12.5%	12.5%
2	*S. aureus*	12.5%	25%
3	*S. agalactiae*	25%	25%

Table 2. RPI of *Vetiveria zizanioides* root oil against all three bacterial isolates.

Sl. No.	Strain	RPI
1.	S. agalactiae	52.02 %
2.	S. aureus	33.19 %
3.	E. coli	29.79 %

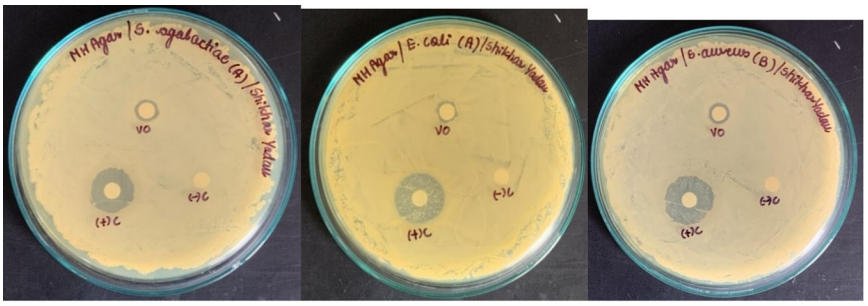

Fig. 1. Showing antibacterial activity of *Vetiveria zizanioides* against *S. agalactiae, E.Coli, S. aureus* respectively.

and least against *E. coli* (10 ± 0.89) shown in Fig. 1 and Table 3.

The maximum zone of inhibition of Vetiver oil observed in the case of *S. agalactiae* was 10.33 mm ±0.81 while the maximum zone of inhibition achieved by ciprofloxacin loaded positive control disk was 15 mm±0.89. The maximum zone of inhibition of Vetiver oil observed in the case of *S. aureus* was 10.67 mm ± 0.81 while the maximum zone of inhibition achieved by ciprofloxacin loaded positive control disk was 18.5 mm ± 1.04. The maximum zone of inhibition of Vetiver oil observed in the case of *E. coli* was 10 mm ± 0.89 while the maximum zone of inhibition achieved by ciprofloxacin loaded positive control disk was 18.33 mm ± 0.81.

Burger *et al*. (2017) conducted a study on 8 gram-positive and 12 gram-negative bacterial strains (µg/mL), and on two *Candida* species and found notable growth inhibition activity of *Vetiveria zizanioides* EOs obtained on SARM (*Staphylococcus aureus* resistant to methicillin) with MICs comprised between 500 and 2000 µg/mL, (i.e., between 0.5 and 2 µL/mL or 0.05 to 0.2% v/v). different extracts and essential oil of *Vetiveria zizanioides* promising antibacterial effect against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa, Enterococcus faecalis, Klebsiella pneumoniae, Salmonella typhi, Salmonella aureus, Acinetobacter* spp.

Table 3. Average zone of inhibition achieved against common mastitis pathogen. V.O. = Vetiver oil, C+ =Positive control, C- = Negative control.

	S. agalactiae	*S. aureus*	*E. coli*
V.O	10.33±0.81	10.67±0.81	10±0.89
C+	15±0.89	18.5±1.04	18.33±0.81
C-	Nil	Nil	Nil

Vetiver has traditionally been used as medicinal and aromatic plants in manycountries, especially in Asia. To estimate antimicrobial activity of Vetiver oil against *E. coli, S. aureus* and *S. agalactiae*, 12.5% /ml, 12.5% /ml, 25% /ml respectively. Maximum zone of inhibition shown by Vetiver oil for *E. Coli* followed by *S. Agalactiae, S. aureus*. Which is most common cause of mastitis so it can be used as traditional medicine to treat Mastitis. At ends with the discussion on the main objective of planting vetiver, environmental implication, socio-economic aspects, and industrial potentials. As a campaign to go‘back to nature’ is everywhere, the utilization of vetiver as a medicinal plant to produce pharmaceutical products on a commercial scale has great potential for development. Furthermore explore to full potential of Vetiver oil for its antimicrobial activity and other clinical applications, molecular characterization and Pharmadynamics and Pharmacokinetics studies are needed.

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