Evaluation of the Root Oil of Vetiveria Zizanioides or Subclinical Mastitis in Dairy Cattle

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ABSTRACT
Between October 2020 and June 2021, 200 cows were screened for subclinical mastitis using the California mastitis test and the White Side test, and Ushir (Vetive-Ria zizanioides) (Linn.) was tested for its antimicrobial activity against mastitis pathogens. A positive control, namely the antibiotic ciprofloxacin, was used to compare the in vitro effectiveness of Vetiveria root oil. In the experimental investigation, the lowest bactericidal concentration against E. coli was recorded at 12.5%, whereas the minimum inhibitory concentration of E. coli and S. aureus were both observed at this level. The largest inhibitory zone was measured against S. aureus, whose lowest zone of inhibition was measured against, which had a mean diameter of 10.67 ± 0.81 mm. E. coli had a mean diameter of 10.89 mm. As a result, the antimicrobial efficacy of Vetivera root oil was found to be effective in controlling subclinical mastitis.

Keywords: subclinical mastitis, cattle, *in vitro*, *Vetiveria ziziniodes*, antimicrobial.

INTRODUCTION

Mastitis is an inflammatory disease with several etiological causes and is brought on by the interaction of many relevant elements. In contrast to the subclinical form of mastitis, which shows no apparent symptoms, the clinical form of mastitis exhibits a variety of visual signs (Cobirka et al. 2020). Because it is 15 to 40 times more prevalent than clinical mastitis, the subclinical form of the disease is significant (Seegers et al. 2003). Intramammary infusions or intramuscular injections of antibiotics such as penicillin, amphicillin, streptomycin, cloxacillin, etc. are frequently used to treat mastitis (Bhosale et al. 2014). Numerous studies are focusing on treating animals with alternative techniques because pathogens have developed resistance due to the widespread use of medications (Kalinska et al. 2019). Ushir (*Vetiveria zizanioides*) (Linn.) Nash is a perennial grass containing aromatic properties (Singh *et al*. 2013). Aromatic properties and multiple uses of vetiver are also used in traditional medicine for pest control and as fragrant materials (Devi *et al*. 2010). The antibacterial activity of oil also represents significant action against *Staphylococcus aureus, B. subtilis, and P. aeurogenosa* and moderate activity against *S. pyogens, E. coli,* and *Corynebacterium ovis*. The ethanolic extract of *Vetiveria zizanioides* is known to exert antimicrobial activity (David *et* al., 2019; Devi *et* al., 2010). The antimicrobial properties of Vetiver oil may be due to its complex composition of lipid constituents and complex polysaccharides with extracellular and soluble proteins, which are found to be effective antimicrobial substances against a wide range of microorganisms. According to Devi et al. (2010), the tannins found in the roots of Vetiveria zizanioides are what give them their in vitro antibacterial properties. Staphylococcus aureus was relatively sensitive to the minimum inhibitory doses of several species of vetiver root oil (David et al. 2019). The objective of the current study is to assess Ushir (Vetiveria zizanioides) (Linn.) oil's in vitro antibacterial effectiveness against the typical pathogen causing subclinical mastitis in cows.

Materials and Methods

Screening of Animals

From October 2020 to June 2021, the current study was carried out with the Institutional Animal Ethics Committee's (IAEC) ethical permission. In 4 blocks and 8 villages in the Gonda and Basti districts of Uttar Pradesh, 200 animals were screened for mastitis using the California Mastitis Test (CMT) and the White Side Test (WST), and udder health was examined physically to check for any abnormalities of the udder and teat (Schalm et al. 1971). Ushir (Vetiveria zizanioides) oil's in vitro effectiveness against typical mastitis-causing microorganisms was investigated. *Vetiveria zizanioides* root oil was collected from Nature Care India, Lucknow. The farm produces vetiver oil from CIMAP-approved varieties like KS-1 and Sugandha. All the procedures were done at the Centre for Gene Research and Development in Biotechnology, Sahara State, Lucknow, Uttar Pradesh. The study aimed to evaluate the effectiveness of Ushir oil against common mastitis-causing organisms. The root oil used in the study was obtained from Nature Care India, a farm in Lucknow that produces vetiver oil from CIMAP-approved varieties like KS-1 and Sugandha.

Preparation of the disc and antimicrobial activity test

Vetiver oil was used in various strengths. It is 25%, 12%, 6%, 3%, and 1.5%. using the serial dilution technique. The test was conducted using the vetiver oil exactly as it is; the oil was applied to the Whatman filter paper disc and allowed to soak completely. According to the CLSI recommendations (Clinical Laboratory Standards Institute, 2014), the Kirby-Bauer disk diffusion method (Bauer et al., 1966) was used to assess the antibiotic susceptibility on Mueller Hinton agar (Hi Media, Mumbai, India). The sample extract utilized in the test was made in DMSO (dimethyl sulfide oxide) solvent at a concentration of 1 mg/mL or 1000 g/mL. The discs were positioned where they belonged. In addition to the sample, the plates had two discs, one of which was the positive control, which was the antibiotic Ciprofloxacin at 500 ppm concentration, and the other was 100% DMSO as the negative control. Bioassay was carried out in triplicate, and experiments were repeated three times (Mahida and Mohan 2007).

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

MIC is defined as the lowest concentration of antimicrobial or drug that inhibits 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 (Wie-Gand *et al*. 2008). To determine the MIC value of all three sample extracts, the broth micro-dilution method was applied (Wagenlehner *et al*. 2006). First of all, for each isolate, 5 different concentrations of the vegetable oil were used: 25%,

12.5%, 6.25%, 3.125%, and 1.5625%. Each of the tubes was loaded with 250 µl of the sterile nutrient broth medium, and then the tube labeled as 25% concentration was loaded with 250 µl of the Vetiver oil. From the tube with a 25% concentration, a serial dilution was performed to reach a 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 bacteria isolated in each well; hence, the final volume in each tube was 1 ml. Based on this observation, the minimum concentration of the sample at which there was no visible growth or turbidity in the tube was taken as the MIC value of that sample, and then a 100-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 the positive control was calculated using the following formula by Paluri *et al*. (2012): RPI = 100 (X-Y)/Z-Y. Where X = Totalarea of inhibition of the test extract; Y = total area of inhibition of the solvent; and Z = total area of inhibition of the standard drug.

Results and discussion
The results showed that the test extract exhibited significant antimicrobial activity, as indicated by the absence of colonies on the media plates at a minimum concentration. The calculated relative percentage inhibition (RPI) of the test extract compared to the positive control further confirmed its effectiveness in inhibiting microbial growth. These findings suggest that the test extract has potential as a natural antimicrobial agent. A total of 200 cattle were screened from two districts of eastern Uttar Pradesh. 132 (66%) animals were found positive for subclinical mastitis. Among these, 49.2% of animals showed subclinical mastitis in Gonda district and 50.75% in Basti district. These results indicate a high prevalence of subclinical mastitis in the cattle population of both districts. It is important to implement effective control measures to prevent the spread of this disease and minimize its impact on milk production and animal health.

Antimicrobial efficacy of *Vetiveria zizanioides* root oil against common pathogens of mastitis

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

Sr.No.	Bacterial isolates	MIC value (%)	MBC value (%)
1	*E.coli*	12.5	12.5
2	*S. aureus*	12.5	25
3	*S. agalctiae*	25	25

The minimum inhibitory concentration of *Vetiveria ziza-nioides* root oil was recorded as the lowest for *E. coli* and *S. aureus* at the 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 a concentration of 12.5%, followed by *S. aureus* and *S. agalactiae* at a similar concentration of 25% (Table 1). A similar type of result was obtained by Hammer *et al*. (1999). By using *Vetiveria zizanioides* oil against *S. aureus* and *E. faecalis, the* recorded MICs were 0.06 to 0.12% (v/v). Luqman *et al*. (2007) also observed recognizable antibacterial activity in 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 the antimicrobial potential of vetiver (Putiyanan *et* al., 2005; Barad *et* al., 2013; Soni and Dahiya, 2015). These studies provide strong evidence for the antimicrobial properties of Vetiveria zizanioides oil and its potential use in combating drug-resistant bacterial strains. The consistent findings across multiple studies highlight the effectiveness of vetiver as a natural alternative to conventional antibiotics. In a study, David *et al*. (2019) observed that *Vetiveria zizanioides* oil extracted by different methods of extraction has variable antimicrobial potential against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *E. coli* at different concentrations. 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 at* 25 mm, 18 mm, and 20 mm, respectively, at 750 µg. Another study was conducted by Derya Efe (2019), which resulted in the MIC values for *E. cloacae, E. faecalis, and E. coli* as 15.63 µg/ml, 31.25 µg/ml, 15.63 µg/ml, and 15.63 µg/ml. The study conducted by Devi et al. (2010) demonstrated that EEVZ oil exhibited stronger growth inhibition against S. aureus, P. aeurogenosa, and E. coli at 25 mm, 18 mm, and 20 mm, respectively, when tested at a concentration of 750 µg. Similarly, in another study conducted by Derya Efe (2019), the MIC values for E. cloacae, E. faecalis.

Relative Percentage of Inhibition

Fig.1. Showing antibacterial activity of *Vetiveria zizanioides* root oil against *S. agalactiae, S. aureus* and *E coli*

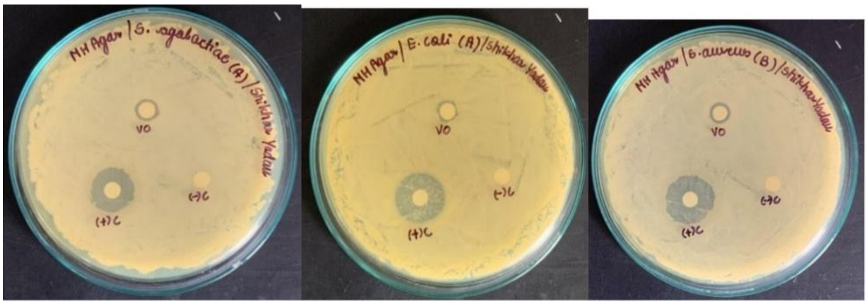

Table.2. RPI of *Vetiveria zizanioides* root oil against all three bacterial isolates.

Sr.No	Strain	RPI (%)
1	*Ecoli*	52.02
2	*S. aureus*	33.19
3	*S. agalctiae*	29.79

Table.3. Average zone of inhibition achieved against common mastitis pathogens 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

In this study, the maximum relative percent inhibition was observed in *S. agalactiae* (52.02%), followed by *S. aureus* (33.19%), and the least RPI was observed in *E. coli* (29.79%) (Table 2). The maximum zone of inhibition by selected Vetiver oil was seen against *S. aureus* bacteria (10.67 ± 0.81) and least against *E. coli* (10 ± 0.89), as 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 the 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 the 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 the 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 oils of *Vetiveria zizanioides have* promising antibacterial effects against *Staphylococcus aureus, Escherichia* coli, *Pseudomonas aeruginosa, Enterococcus faecalis, Klebsiella pneumoniae, Salmonella typhi, Salmonella aureus, and Acinetobacter* spp. These findings suggest that Vetiveria zizanioides extracts and essential oils have a broad spectrum of antibacterial activity against various pathogens. Further research is needed to explore their potential as alternative treatments for bacterial infections.

Conclusion:

Vetiver has traditionally been used as a medicinal and aromatic plant in many countries, especially in Asia. To estimate the 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 tra- ditional 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 prod- ucts 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.

Barad R, Atodariya U, Bhatt S, Patel H, Upadhyay S, and Upadhyay U (2013) Antibacterial and preliminary cytotoxic activity of Vetiver oil against various pathogens has been reported in several studies. However, further research is required to fully understand its antimicrobial properties and explore its potential as a natural alternative to conventional antibiotics. Additionally, conducting molecular characterization studies can provide valuable insights into the chemical composition of Vetiver oil and its potential therapeutic benefits.

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