Proceedings of the Pakistan Academy of Sciences: Part B
Life and Environmental Sciences 59(2): 71-78 (2022)

ISSN (Print): 2518-4261; ISSN (Online): 2518-427X
http://doi.org/10.53560/PPASB(59-2)686

Pakistan Academy of Sciences

Research Article

Antibacterial Potential of *Aloe vera* against *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from Mastitic Milk

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Abstract: The extensive use of antibiotics has developed antibacterial resistance and also may cause toxic effects (hepatotoxicity, nephrotoxicity) on vital organs. To overcome this problem, Scientists gain attention towards medicinal plants. Pure *Aloe vera* (AV) is a common alternative antimicrobial medicine, hence, the current study was conducted to explore its antibacterial potential and compared it with a commonly used antibiotic amoxicillin. During this study, clinically positive mastitis milk samples (n=50) were collected from buffaloes, after microbial culture analysis. Various concentrations (C1=40, C2=20, C3=10, C4=5, C5=2.5, C6=1.25, C7= 0.62, C8=0.31, C9=0.15, C10=0.07 and C11=0.03µl) of pure AV and amoxicillin (µg/µl) were used to evaluate antibacterial activity through minimum inhibitory concentration (MIC) against Gram-positive organisms including *Staphylococcus aureus* and *Streptococcus agalactiae.* The MIC was evaluated based on turbidity and transparency of the medium. Prevalence of *S. aureus* was recorded at 25 (50 %) whereas, 15 (30 %) positive samples for *S. agalactiae* and 10 (20 %) positive samples were found in mixed bacterial colonies from milk samples. The mean values of MIC at 10 µl of pure AV showed 50% sensitivity against *S. aureus* whereas, at 5 µl of pure AV showed 52.5 % sensitivity against S. agalactiae. While amoxicillin inhibited the growth of *S. aureus* and *S. agalactiae* at 2.5 µg/µl and 1.25 µg/µl concentrations showed 52.5 %, and 55 % sensitivity respectively. A significant ($P < 0.05$) difference was noticed between both tested groups. It has been concluded that pure AV possessed antibacterial potential and can be used as a safe and economic alternative against infections caused by *S. aureus* and *S. agalactiae.*

Keywords: *Aloe vera*, Amoxicillin, *Staphylococcus aureus*, *Streptococcus agalactiae*

1. INTRODUCTION

Mastitis is an inflammation of the mammary gland of dairy animals caused by fungi, bacteria and likely viruses [1, 2]. Antimicrobial agents have been used to treat mastitis [3]. Amoxicillin, a broad-spectrum beta-lactam antibiotic commonly administered to treat clinical and subclinical mastitis in cows caused by Enterobacteriaceae, *Escherichia coli, Klebsiella* spp. *Streptococcus agalactiae* and penicillin-sensitive *Staphylococcus aureus* [4, 5]. Since the antibiotic resistance of pathogenic bacteria is increasing rapidly day by day. Scientists get attention towards herbal plants to minimize the antibacterial resistance, among that *Aloe vera* (AV), a medicinal plant that has been used therapeutically for hundreds of years. The center of the AV contains mucilaginous tissue designated as a gel that has been conventionally used for the treatment of a number of ailments such as acne, nourishment for the hairs, gastrointestinal tract disorders, wounds, and sunburn. So far, more than 75 active compounds have been recognized from the AV gel. The gel comprises of 98-99 % water and the remaining 1-2 % containing the active ingredients. It contains a lot of amino acids, antibacterial substances (aloin, polysaccharides, fumaric acid, anthraquinones), vitamins, minerals, enzymes, saponins, sterol, natural sugar, and many other biologically active compounds.

Hence, AV possessed pharmacological

Received: November 2021; Accepted: June 2022
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properties including antimicrobial, anti-inflammatory, antiallergic, antidiabetic, antioxidant, anthelmintic, antifungal, antiseptic, antitumor, protection against radiation, laxative, anti-aging, nephroprotective, and immune stimulation effects [6-8]. The efficacy of crude AV extract has shown a broad range of activity against G+ve and G-ve bacterial organisms. It either kills or inhibits the growth of Streptococcus pyogens, *S. aureus*, *S. agalactiae*, *E. coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Salmonella typhi, Propionibacterium acne*, and *Helicobacter pylori* [9, 10]. AV has been reported to use as a teat-dip or an ointment in lactating cows by intra-mammary administration to treat mastitis or high somatic cell counts [11]. Looking at the scanty information on comparative antibacterial potential, this study was designed to assess the antibacterial activity of AV against *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from mastitis milk of buffaloes and compared it with amoxicillin.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of 50 mastitis milk samples of buffaloes were randomly collected (3 & 4 lactating animals) under the aseptic condition in bijous bottles from the buffalo dairy farms in the locality [13].

2.2 Bacteriological Isolation and Identification

The milk samples found positive after the California Mastitis test were examined microbiologically and cultured on Nutrient agar, Blood agar, Edward's agar and MacConkey agar plates. All bacterial growth was identified and recorded after 24 and 48 h of incubation. For the standard, centrifugation, and incubation methods, *S. aureus* were identified by hemolytic pattern, Gram-staining characteristics, positive catalase reaction, positive mannitol agar reaction, coagulase test, Baird-Parker medium culture test, DNase test, Voges-Proskauer test, coagulase test, and annitol fermentation test in positive tube [12]. On the blood agar colony characteristics of *S. agalactiae* were identified morphology appearance of gray to whitish-gray colonies with, beta hemolysis while on Chrome agar it was colony was identified as characteristic light blue color. These findings were confirmed by the growth on the selective media and the positive reaction (Arrow-head formation) to the Christie, Atkins, and Munch-Peterson test.

2.3 Extraction of Gel from *Aloe vera* Leaves

Aloe vera (AV) fresh leaves as shown in figure 1 were collected from a local plant nursery, cleaned with 70 % alcohol, and incised and the gel was separated with the help of a sterile knife. The gel was made homogenous by the process of blending, filtered with muslin cloth and autoclaved at 121 ºC at 15 lb pressure for 15 mins for sterilization. Then, sterilized stock solution (100 % concentration) was used to evaluate antibacterial activity. Sterilized filtrate was diluted as 40, 20, 10, 5, 2.5, 1.25, 0.62, 0.31, 0.15, 0.07 and 0.03 µl [13].

2.4 Preparation of Antibiotic Stock Solution

Amoxicillin stock solution was prepared by adding 15 mg of Amoxicillin powder in 15 ml distilled water then dissolved thoroughly. The solution was sterilized and was kept under refrigeration at 4 ^{0}C until further use. Different concentrations of amoxicillin i.e. 40, 20, 10, 5, 2.5, 1.25, 0.62, 0.31, 0.15, 0.07 and 0.03 µg/µl were used to assess its minimum inhibitory concentration (MIC) [14].

2.5 Determination of Antibacterial Minimum Inhibitory Concentration

For determination of minimum inhibitory concentration (MIC) through microdilution methodology as recommended by Clinical and Laboratory Standard Institute (CLSI) methods with some modifications on Muller-Hinton medium [15]. MIC results were interpreted according to CLSI [16]. 96 well plates containing test microorganisms at the concentration of 2x10^6 CFU/ml [26]. The 96 well plates were incubated at 37 ^{0}C overnight. The break, where the bacterial multiplication was inhibited, was recorded by turbidity/cloudy appearance in cultured wells. These turbidity/ cloudy appearances were recorded as the MIC for pure *Aloe vera* extract and amoxicillin as well.

2.6 Statistical analysis

Data were analyzed by Statistical Package Social Science (SPSS) version 8.1. The experimental results were expressed as mean ± standard error of the mean (SEM). Groups were compared by analysis

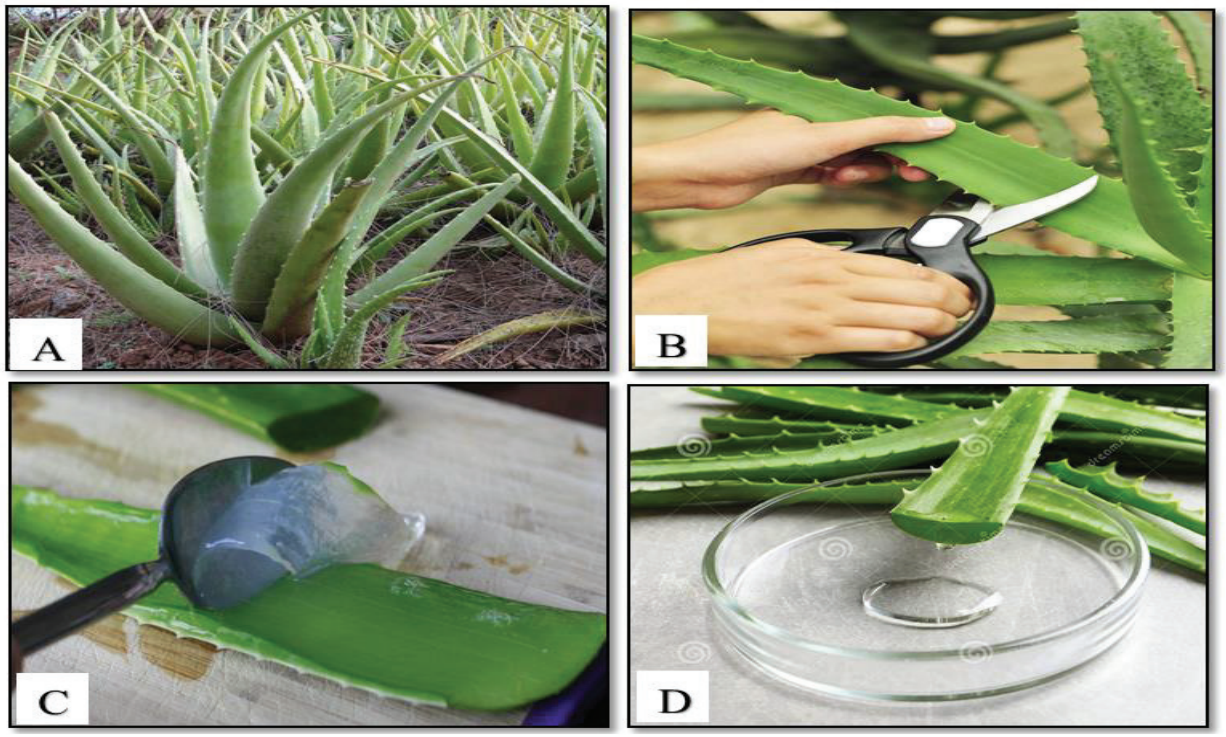

Fig. 1. (A) *Aloe vera* plant, **(B)** Cutting of *Aloe vera* leaves (Source: Google), **(C)** Removal of *Aloe vera* gel from leaves, **(D)** Collection of pure *Aloe vera* gel in petri dishes for antimicrobial activity (Source: Google)

of variance using one-way ANOVA. p-value <0.05 was considered statistically significant.

3. RESULTS

In this study, a total of 50 mastitis milk samples were collected and examined the number and percent prevalence of isolated organisms were recorded. Out of 50 mastitis milk samples, 25 (50 %) were found positive for *Staphylococcus aureus*, 15 (30 %) were positive for *Streptococcus agalactiae* and 10 (20 %) were noticed in mixed colonies.

3.1 Sensitivity of isolated bacteria against pure *Aloe vera* Concentrations

The various concentrations (C1=Control, C2=40, C3=20, C4=10, C5=5, C6=2.5, C7=1.25, C8= 0.62, C9=0.31, C10=0.15, C11=0.07 and C12=0.03 µl) of pure *Aloe vera* (AV) were used for determining the sensitivity of isolated bacterial organisms. It was observed that *Staphylococcus aureus* obtained sensitivity at 40 µl (100 %), 20 µl (87.5 %), and 10 µl (50 %) concentrations, while lower concentrations of pure AV were found resistant against *Staphylococcus aureus*. Whereas Streptococcus agalactiae's growth halted at 40 µl (100 %), 20 µl (100 %), 10 µl (92.5 %) and 5 µl (55 %) concentrations. while lowered concentrations of AV showed resistance against *Streptococcus agalactiae* (Figure 2).

3.2 Sensitivity of Isolated Bacteria against Amoxicillin Concentrations

The various concentrations (C1=Control, C2=40, C3=20, C4=10, C5=5, C6=2.5, C7=1.25, C8=0.62, C9=0.31, C10=0.15, C11=0.07 and C12=0.03 µg/µl) of amoxicillin were used for evaluating its sensitivity against isolated organisms. It was found that *S. aureus* exhibited sensitivity at 40 (100 %), 20 (100 %), 10 (100 %), 5 (80 %) and 2.5 (52.5 %) µg/µl concentrations. Whereas it indicated resistance to amoxicillin below 2.5 µg/µl concentration. While *S. agalactiae* presented sensitivity at 40 (100 %), 20 (100 %), 10 (100 %), 5 (100 %), 2.5 (90 %) and 1.25 (55 %) µl/µg concentrations. But found resistance below 1.25 µg/µl concentrations of amoxicillin (Figure 3).

3.3 Comparative MIC of pure *Aloe vera*, Amoxicillin against Isolated Organisms

The various concentrations (C1=Control, C2=40,

C3=20, C4=10, C5=5, C6=2.5, C7=1.25, C8= 0.62, C9=0.31, C10=0.15, C11=0.07 and C12=0.03µl) of pure AV and amoxicillin were used for halting the growth of isolated organisms. *Staphylococcus aureus* were stop the growth at 10 µl (50 %) concentration of pure AV and 2.5 µl (52.5%) of amoxicillin concentrations. The pure AV stopped the growth of *Streptococcus agalactiae* at 5 µl (55 %) concentrations whereas, amoxicillin exhibited its sensitivity at 1.25 µl (55 %) concentration (Figure 4).

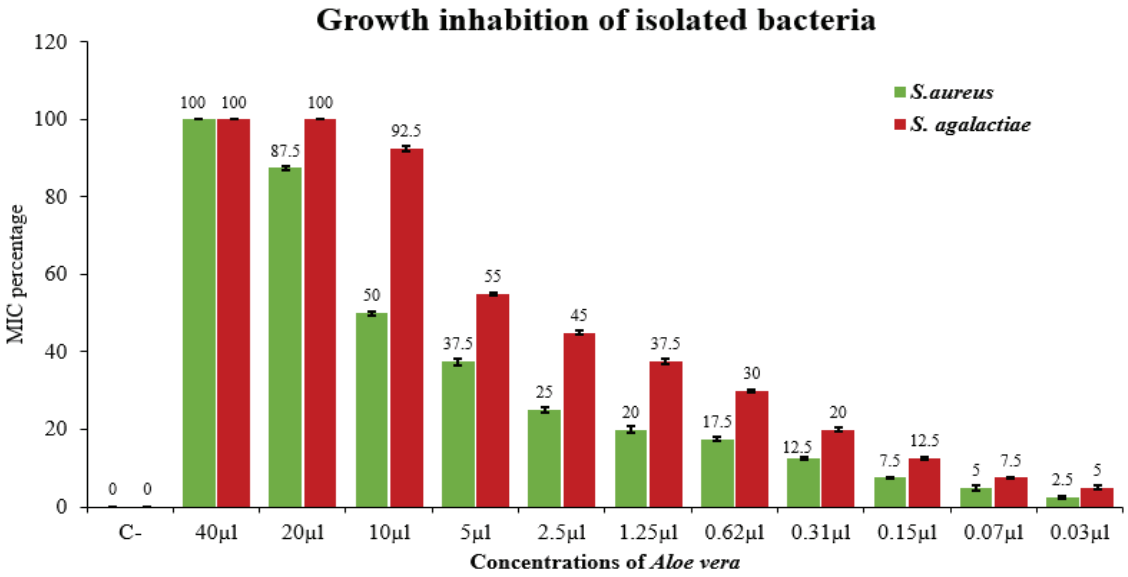

Fig. 2. Minimum inhibitory concentrations (MIC) of antibacterial activity of various concentrations of pure Aloe vera against *S. aureus* and *S. agalactiae*. Significant (P<0.05) difference was determined between both groups.

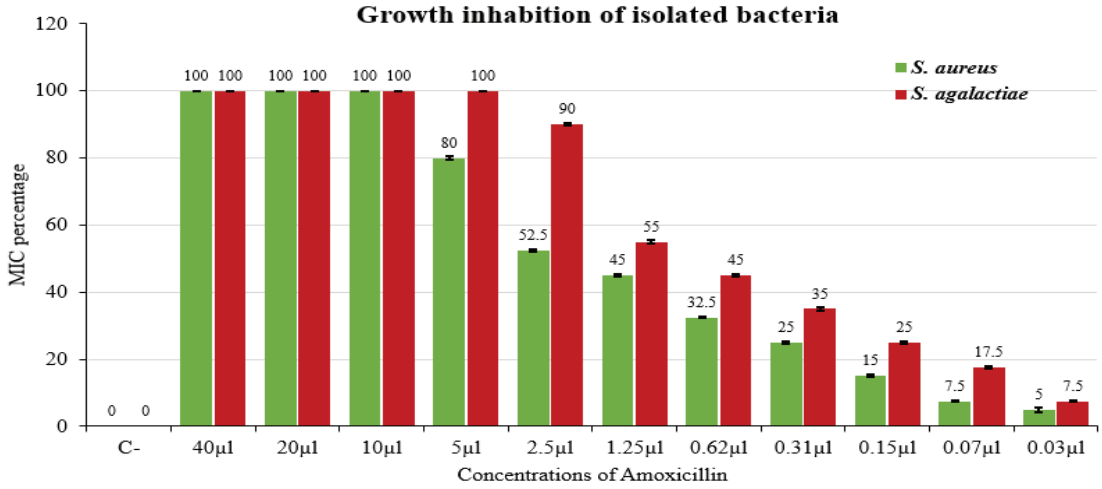

Fig. 3. Minimum inhibitory concentrations (MIC) of antibacterial activity of various concentration of amoxicillin against *S. aureus* and *S. agalactiae*. Significant (P<0.05) difference was determined between both groups

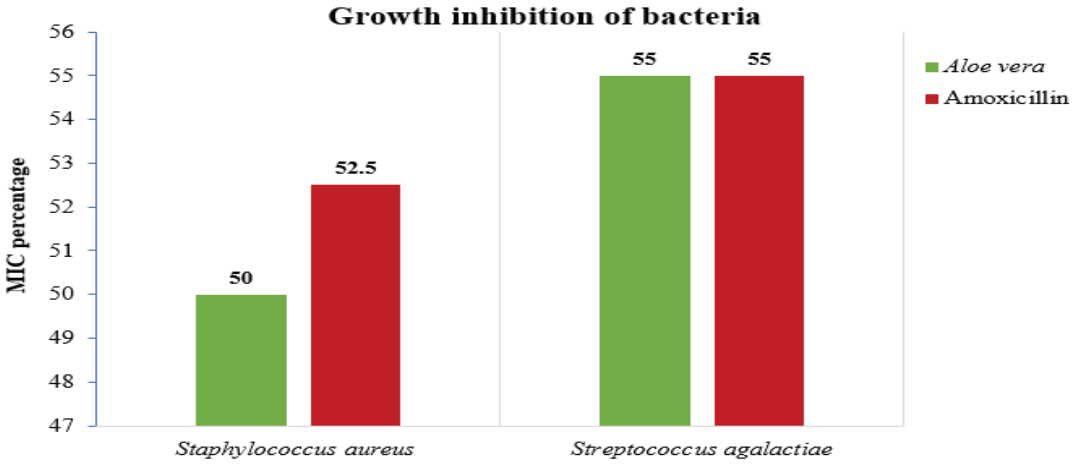

Fig. 4. Comparative minimum inhibitory concentrations (MIC) of isolated organisms against pure *Aloe vera* and amoxicillin. Significant (P<0.05) difference was determined between both groups.

4. DISCUSSION

In the current study, various concentrations of pure *Aloe vera* (AV) and amoxicillin were used to determine their antibacterial activity against *Staphylococcus aureus* and *Streptococcus agalactiae* through the Micro broth dilution method was used to examine minimum inhibitory concentration (MIC) of AV and Amoxicillin against isolated organisms. It was determined that the MIC at which AV inhibited the growth of *S. aureus* and *S. agalactiae* were 20 µl and 5 µl respectively.

The current study showed agreement with previous studies where it was also found that AV extract possessed broad-spectrum antimicrobial activity due to its inhibitory and bactericidal effect against mastitis-causing organisms including *S. aureus*, *S. agalactiae*, *E. coli, P. aeroginosae, P. vulgaris, E. faecalis, S. epidermididis,* and *Bacillus subtilis* [17-19]. The present result is in accordance with previous findings in which crude extract of AV showed antibacterial potential against Gram-positive and Gram-negative bacterial organisms such as *S. aureus*, *S. agalactiae* (Fig. 2) [10, 20]. In these studies, it was observed that the AV gel and leaf possessed antibacterial activity as they inhibited the growth of the above-mentioned bacterial organisms. The AV exhibited antibacterial properties due to the presence of various biologically active ingredients such as carboxy peptidase, emodin, magnesium lactate, salicylate, polysaccharides, anthrone, C-glucosyl chromone, anthraquinones, allantoin dithranol, and chrysarobin [21]. It has also been reported that due to the presence of polysaccharides in the structure of AV, which retained antibacterial activity via the stimulation of phagocyte leucocytes to halt the growth of bacterial organisms [9]. AV also contains the active ingredient anthraquinones which possessed structural similarity with tetracycline antibiotics. Anthraquinone acts like tetracyclines by blocking the 30S ribosomal unit at the accepter side by preventing the transferase enzyme rather than the peptide site. Hence, its mode of action is similar to that of the tetracyclines (where it prevents the access of aminoacyl tRNA to the acceptor site on the mRNA ribosomal complex subsequently, inhibiting protein synthesis of the bacterial organism). It has been reported that the bacterial organisms could not grow in a media containing AV extract [17].

Amoxicillin is a semisynthetic cell wall synthesis inhibitor, it acts by inhibiting the peptidoglycan which is the main polymer of the bacterial cell wall and it is still being administrated continually as a drug of choice within its class [22, 23]. This antibiotic is active against various pathogenic organisms including *Staphylococcus* spp., *Streptococcus* spp., *Clostridium* spp., *Klebsiella* spp., *Shigella* spp., *Trueperella* spp., *Proteus* spp., *Salmonella* spp., *Escherichia* spp. and *Pasteurella* spp. [24]. In the present study, different concentrations of amoxicillin were used to detect the susceptibility of *S. aureus* and *S. agalactiae* isolated from mastitis milk samples of buffaloes (Fig. 3). It was observed that the MIC of amoxicillin for *S. aureus* and *S. agalactiae* was noticed at 10 and

2.5 µg/µl respectively. Furthermore, it was also noticed that *S. aureus* was less susceptible to amoxicillin in comparison to *S. agalactiae* which showed susceptibility even at lowered concentration. This may be owing to selective pressure exhibited in the environment from where the isolates were obtained or it may carry resistant genetic characteristics that showed less susceptibility to amoxicillin concentration.

The present results demonstrated that amoxicillin possessed antibacterial activity against *S. aureus* which is supported by previous findings [4, 25]. However, the present study agreed with the previous study [13] comparison of the various concentration of gentamycin with *Aloe vera* crude extract. The MIC results showed the additive effect with antibiotics, in which growth of E. coli and K. pneumoniae was inhibited at concentrations 1.25 µg/µl and 0.0390625 µg/µl respectively. The present study is also in line with the previously studied comparative study of antibacterial activity of essential oils with antibiotics showed superior effect against the isolated organisms [26]. The present findings are also comparable with previous studies in which antibacterial susceptibility of streptococcus spp. isolated from clinical mastitis samples in dairy cows were reported. It showed that *S. agalactiae*, *S. dysgalactiae, S. uberis* remained susceptible to amoxicillin, while similar findings were noticed by Ikiz *et al*. (2013) and Maia *et al.* (2018) [27, 28]. *S. agalactiae* was found more susceptible as compared to *S. aureus* in the current study, it might be due to less resistance developed in *S. agalactiae* than in *S. aureus* and because of the location of these bacterial organisms in mammary cells. Whereas *S. aureus* inhabits deeper in mammary cells while *S. agalactiae* is localized on the side of the mammary cell. Consequently, during mastitis treatment, antibiotics achieved lowered concentration in deeper mammary cells due to the efflux mechanism that existed in pathogenic organisms. Hence, *S. aureus* received a sub-therapeutic concentration than *S. agalactiae* which would shift the spectrum of activity to an antibiotic to higher concentrations. The bacterial organism may develop resistance through the efflux mechanism, at the target site, which would lower the therapeutic concentration of antibiotics making them less susceptible to the bacterial organisms [29].

5. CONCLUSION

It has been concluded from the current study that the *Staphylococcus aureus* and *Streptococcus agalactiae* are more prevalent in mastitis milk samples in buffaloes. Moreover, both organisms showed susceptibility at slightly higher concentrations against pure AV at different concentrations i.e. 10 and 5 µl but at lowered concentrations then this did not affect the growth of isolated organisms when examined through MIC. On the other hand, amoxicillin retarded the growth of isolated organisms at lowered concentrations than AV. Additionally, amoxicillin also halted the growth of *S. agalactiae* even at the reduced concentration in comparison to *S. aureus* which was inhibited at a higher concentration of the used antibiotic. However, amoxicillin exhibited better inhibition results than pure *Aloe vera* but because of developing antibiotic resistance against commercially available antibiotics and their increased expenses, AV can be used as a safe and economic alternative to amoxicillin against isolated pathogenic organisms.

6. CONFLICT OF INTEREST

The author(s) declared no potential conflicts of interest concerning research, authorship, and/or publication of this article.

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