Effect of Pure and Ethanol Extract of Aloe Vera and Moringa against Streptococcus Agalactiae and Staphylococcus Aureus Isolated from Mastitis Milk of Buffalo

Muhammad Abdullah[1], Hamid Ali Khan[2], Abdul Kabir[3], Muhammad Jawad Ullah[4,5], Faiza Rashid[5], Abbas Khan[5], Rizwan Khan[5], Hammad Khan Khushmir[5], Sayab Khan[6], Muskan Saleem[5], Zeeshan Ahmad[6], Junaid Ahmad[6], Shubana Hayat[6], Asia Hayat[7],Shamsuddin Bughio[1]

[1]Department of Veterinary Pharmacology Sindh Agriculture University Tandojam
[2]Bacha Khan University Charsadda Khyber Pakhtunkhwa, Pakistan
[3]Department of Veterinary Microbiology Sindh Agriculture University Tandojam
[4]Department of Allied Health Sciences Iqra National University Peshawar
[5]Center of Biotechnology and Microbiology University of Peshawar.
[6]Department of Microbiology Hazara University Manshera, KPK, Pakistan
7Department of Biochemistry Hazara University, Manshera, KPK, Pakistan
Corresponding author Junaid Ahmad, Email address:ofidai116@gmail.com
Co-Corresponding author Shubana Hayat, Email address:Shubanahayat@gmail.com

Abstract

Among medicinal plants, *Aloe vera* and Moringa are used commonly due to their various therapeutic effects. Hence, this research aimedto see whether there's any antibacterial activityof pure and ethanol extract of *Aloe vera* and Moringa for some pathogenic organisms isolated from mastitis milk. During the current study, 50 mastitis milk samples were obtained from different dairy farms of the nearby Tandojam under aseptic conditions and brought to the Department of Veterinary Pharmacology for microbial culture analysis. After isolation and identification of the organisms, the disc diffusion method did the antibacterial activity. For this purpose, 30µg/µl and 60 µg/µl of *Aloe vera* and Moringa and their ethanol extracts were used against *Staphylococcus aureus* (*S. aureus*) and *Streptococcus agalactiae (Strep. agalactiae).*Results revealed that out of 50 mastitis milk samples analyzed, 25 (50%) were found positive for *S. aureus,and* 20(40%) were positive for *Strep. agalactiae* and 5 (10%) were detected positive for mixed colonies. The mean growth inhibition of *S. aureus* against 30 µg/µl of pure *Aloe vera* was recorded(2.83mm), and for *Strep. Agalactiae* (2.36 mm) and on 60µg/µl, both organisms reacted more sensitive and exhibited inhibitory zone of (8.03mm) and (7.1mm), respectively. At 30µg/µl, ethanol extract of Aloe vera yielded (7.9mm) sensitivity for S. aureus and *Strep. agalactiae* (6.46mm), and on 60µg/µl, the isolated organisms remained highly sensitive and showed an inhibitory zone of (16.66mm) for *S. aureus* and (13.26mm)for *Strep. agalactiae*. However. Pure Moringaat 30µg/µl, inhibition zone for *S. aureus* recorded was (2.37mm) and *Strep. agalactiae* showed (2.36 mm) sensitivity while, on 60µg/µl, the organisms responded more sensitive and presented an inhibitory zone of (8.03mm) and (7.1mm), respectively. Though, 30µg/µl of ethanol extract of Moringa showed (7.9mm) sensitivity on *S. aureus* and *Strep. agalactiae* showed (6.46mm) sensitivity, but on 60µg/µl of ethanol extract, it persisted as highly sensitive against isolated

organisms and produced an inhibitory zone of (14.43mm) for *S. aureus* and(12.56mm) for *Strep. agalactiae*. Thus, it has been concluded that all treatments hampered the growth of isolated organisms, but pure *Aloe vera* and its ethanol extract exhibited better inhibition zones than Moringa.

Keywords:*Aloe vera,* Moringa against *Streptococcusagalactiae,Staphylococcusaureus* and mastitis milk

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1. INTRODUCTION

Buffalo is the ultimate popular milk yielding huge ruminant of the Bovidae family containing more than 34 million heads in the dairy systems of Pakistan[1]. In milk-producing countries, Pakistan is the ranking 4 in the world. Milk production increased upto 48 million tons in 2018-19, where 67% of milk is produced by buffalos (Economic Survey of Pakistan 2018-19). Livestock as an agriculture segment pays 57% of its worth accumulation and nearly 11% ofnational GDP[2]. Buffaloes are mostly used for meat and milk purposes and are known as "black gold"[3]. Bovine mastitis is an important common infection in buffaloes, producing economic losses, decreasing milk production, increasing treatment prices, and forcing the animals to cull [4]. Infectious pathogens cause dairy production loss; *S. aureus* alone counted for about 88 %, and other pathogenscause mastitis at 6%[5].Bovine mastitis mainly results from infection in mammary glands and is a microorganism found in udders, such as coliform, staphylococci, and streptococci species. Mastitis appears when the udder is inflamed due toa bacterial attack of the teat canal. The organisms multiply and produce their toxins, injuring the milk-producing tissue and leading to physical damage and chemical irritation[6]. According to World Health Organization, more than 80 % of the international population relies on traditional medications to treat various ailments. With the increasing resistance of multidrug-resistant bacteria to antibiotics, seeking alternatives has become a serious issue[7]. Herbal plants play an essential role in protecting the body systems against dangerous chemicals that cause oxidative stress and variation in antioxidant enzyme and non-enzyme systems[8]. Among other plants, *Aloe vera* has gained popularity in medicine; this plant contains two different products, yellow latex, referred to as Aloe juice, and leaf pulp, which is the innermost portion of the leafthat contains the gel[9]. Most herbal medicines are present for different infections and diseases. *Aloe vera* is one of them and is well known for its beneficial medicinal properties. This plant is the richest natural source of human health[10]. It has been used for many centuries; over 75 inside gel's active constituents have been discovered for its curative properties[11]. The polysaccharides contained in the inner leaf tissue are responsible for many of the therapeutic activities of Aloe leaf extracts. It has been reported that the polysaccharides in *Aloe vera* gel have medicinal properties such as healing of the wound, immune stimulation, anti-inflammatory effects, promotion of radiation damage repair, antibacterial, anti-viral, anti-fungal, anti-diabetic, and anti-neoplastic activities, and stimulation of hematopoiesis and antioxidant effects[12]. In traditional popular medicine, *Moringa oleifera (MO)* is a good curative plant. Numerous pharmacological studies have revealed the ability of this plant to exhibit anti-inflammatory, analgesic, antipyretic, antioxidant, anticancer, nootropic, hepatoprotective, gastroprotective, cardiovascular, anti-ulcer, anti-obesity, antiasthmatic, antiepileptic, antidiabetic, anti-

urolithiasis, diuretic, local anesthetic, anthelmintic, anti-allergic, wound healing, immunomodulatory, antibacterial and antidiarrheal properties. *Moringa oleifera* has pharmacological and wide traditional uses in various disease conditions[13].These studies showed that the pure *Aloe vera, Moringaoleifera* and their ethanol extracts possessed antibacterial effects in treating various bacterial infections. To the best of our knowledge, till now, few studies have been carried out to determine the antibacterial effect of pure *Aloe vera, Moringaoleifera*and their ethanol extracts against those bacterial organisms isolated from mastitis animals. Hence, considering the importance of economic losses produced by mastitis in buffaloes, this work has been planned to compare the antimicrobial effect of pure *Aloe vera, Moringaoleifera*and their ethanol extracts against S*treptococcus agalactiae* and *Staphylococcus aureus* isolated from mastitis milk of buffaloes.

2. MATERIALS AND METHODS

2.1 Collections of samples

An overall of 50 clinical mastitis milk samples was collected from the buffaloes under hygienic conditions from the diary forms of the nearby Tandojam. It was carried to the postgraduate research laboratory of the DepartmentofVeterinaryPharmacology, SindhAgricultureUniversity Tandojam. The different primary culture was done onto nutrient agar for primary culture. Isolates were purified by culturing on Nutrient agar and blood agar afterward incubated at 37ºC for 24 hrs. Further purification was done by subculture on a blood agar medium. Bacterial organism identification (*Streptococcus agalactiae* and *Staphylococcus aureus*) was completed through culture on different agars. To identify the staining reaction, Gram's staining was performed.

2.2 Culture of bacteria

Samples were cultured on nutrient agar for primary culture. Isolates were purified by subculture onto Nutrient agar and Blood agar and then incubated for 24 hours at 370°C. After 24 hours, pure colonies from nutrient and blood agar were selected for sub-culture using a sterilized wire loop, and this process was repeated until pure colony growth was attained. The cultural, morphological, and biochemical features of microorganisms were used to confirm them. The pure colonies of the bacterial organisms were then transferred aseptically onto nutrient agar and for 24 hours of incubationat 37°C before being stored in a refrigerator at 4°C until further usage.

2.3 Isolation and identification of *Staphylococcus aureus* and *Streptococcus agalactiae*

To identify *Staphylococcus aureus* from cultured plates. Yellow golden colonies were observed and further confirmation was done by biochemical reactions(Catalase Test, Oxidase Test and Coagulase Test) and gram staining.To detect*Streptococcus agalactiae* from mastitis, milk samples were sub-cultured on blood agar. After that, Petri-dishes were observed for smooth, non-pigmented convex colonies with the entire margin surrounded by a zone of β-hemolysis and more validation was done via Gram staining and chemical reaction.

2.4 Extraction of gel from *Aloe vera*

Tandojam's local plant nursery provided us with *Aloevera* plant leaves. The leaves of the plants were thoroughly cleanedand washed with tap water for 5 minutes to remove dust, dirt and soil particles and then it will be disinfected with spirits. The plant leaves were cut, and the gel was separated using a sterilized knife. The gel was then homogenized by blending it, filtering with cloth, and autoclaved for 15 minutes. The sterilized filtrate was used as a stock solution.

2.5 Ethanol extractionof *Aloe vera* gel

Matured, healthy, and newly harvested leaves were rinsed in clean water and dissected lengthwise to produce ethanol extracts from Aloe vera. Colorless parenchymatous tissue has been scraped gently with a clean knife, leaving the green fibers behind. The collected gel weighing 790g was grinded and mixed with 100ml of hot water and allowed for 24hours. In the oven, the gel was dried at 80^0C for 48 h. Then, the gel was minced using a pestle and mortar, and further, it was reduced to a fine powder using a blender.10 gm of powder was soaked in 100ml of ethanol for 24hrs. The contents will be filtered by Whatman No.1 filter paper and filtrate will disappear, leading to dryness. The extract was further minced and diluted in 10ml of distilled water and sterilization did. The resulting mixture was kept at 4°C.

2.6 Preparation and extraction of the leaves of Moringa

Fifty grams of powdered leaves were weighed and dropped into a 500ml conical flask, then filled with 400ml distilled water. The combination was maintained for 12 hours, with 30minute intervals of continual shaking. Whatman No. 1 filter paper was used to filter the extract. The extracts were kept refrigerated at 4°C until they were used.

2.7 Sample preparation and Ethanol extraction procedure of Moringa

The collected fresh leaves of *Moringa oleifera*were destalked, washed with clean water, dried at room temperature and finally grounded using an electric blender. The powdered plant material was Extraction by weighing 50g of the powder plant sample into 300ml of ethanol. The extracts were filtrated using Whatman No.1 filter paper, then kept in disinfected bottles and stored in the Fridge until further process.

2.8 Antibiotic susceptibility Test

In this study, the pure and EE of Aloe vera and Moringa oleifera for Streptocoocus agalactiae and Staphylococcus aereus were usedto conduct an antibacterial susceptibility test via discs diffusion way.The Mueller-Hinton agar plate's dry surface was created and dry via incubation at 37 ° C for 30 minutes[14]. In barium chloride, pure colonies were selected and dispensed. The sterilized swab is immersed in the solution of bacteria and spread overthe agar surface using a disc dispenser; the selected pure and ethanol extract of *Aloevera* and *Moringaoleifera* discs were put over the surface of agar plates and lightly pushed

with sterile forceps to make it stick to the medium's surface. Plates were sealed, enclosed in a polyethylenebag, inverted (medium up, disc down), and incubated at 37°C for 24 hours[15].

3. RESULTS

This study was performed for comparison of antibacterial activity of pure plus EE of Moringa and *Aloe vera* against *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from mastitis milk of buffaloes.Total of 50 mastitis milk samples were tested positive for various pathogens. From 50 samples, 25 (50%) and 20 (40%) were found positive for *S. aureus* and *Strep. agalactiae* (Table 3.1). Pathogens were identified on their morphology, culture characteristics and stain reaction, and further confirmation was done with biochemical tests.

Table 3.1: Number and percentage prevalence of *Staphylococcus aureus* and *Streptococcus agalactiae* isolated from mastitis milk of buffalo.

Bacterial species	Mastitis sample	No. of a positive sample	Percentage (%)
Staphylococcus aureus	50	25	50%
Streptococcus agalactiae		20	40%
Mixed colonies		5	10%

3.1 Zone of inhibition of pure *Aloe vera* against *S. aureus* and *Strep. agalactiae*

Themean values of Zone of inhibition of pure *Aloevera* against *S.aureus* and *Strep. agalactiae* at 30 µg /µl and 60 µg /µl concentration. For *S. aureus,* the means values which is recorded (2.83±0.05 mm) at 30 µg /µl and (8.03±0.05 mm) at 60 µg /µl, respectively. While for *Strep. agalactiae* (2.0±0.05 mm) at 30 µg /µl and (7.1±0.05 mm) at 60 µg /µl were recorded in that order. The data showed an important ($p<0.05$) difference between 30µg/µl and 60µg/µl of pure*Aloe vera*against *S. aureus* and *Strep. agalactiae.*

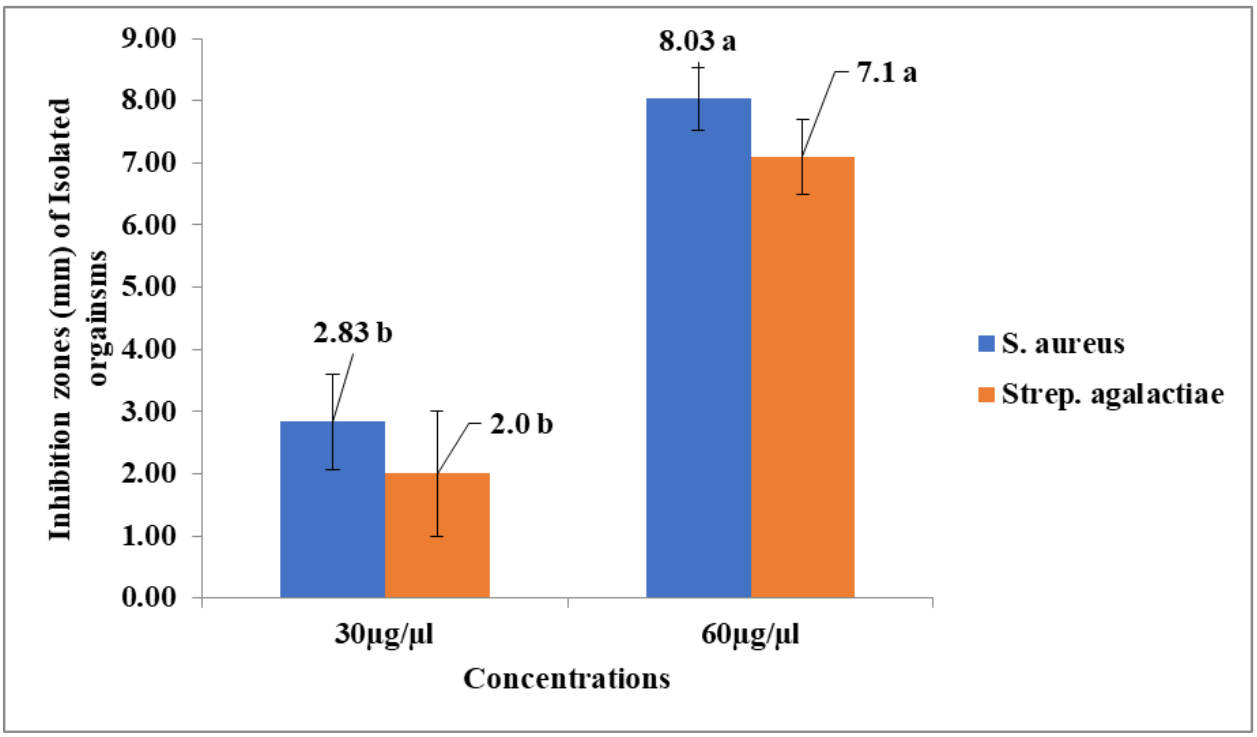

FIGURE3.1: Means bearing letters(a , b) show significant difference ($P < 0.05$)
LSD value 1.39.

Bacteria	LSD	P Value
Staphylococcus aureus	1.39	0.05
Streptococcus agalactiae	1.39	0.05

3.2 Zone of inhibition of ethanol extract of *Aloevera* against *S. aureus* and *Strep. agalactiae*

The mean values of Zone of inhibition of ethanol extract of *Aloevera* against *S. aureus* and *Strep. agalactiae* at 30 µg /µl and 60 µg /µl concentration. For *S.aureus* the means values were recorded (7.90±0.05 mm) at 30 µg /µl and (16.67±0.05 mm) at 60µg /µl, respectively. Whereas, for *Strep. agalactiae* (6.47±0.05 mm) at 30 µg /µl and (13.27±0.05 mm) at 60 µg /µl were recorded in that order. The data showed an important ($p<0.05$) difference between 30µg/µl and 60µg/µl of ethanol extract *Aloe vera*against *S. aureus* and *Strep. agalactiae.*

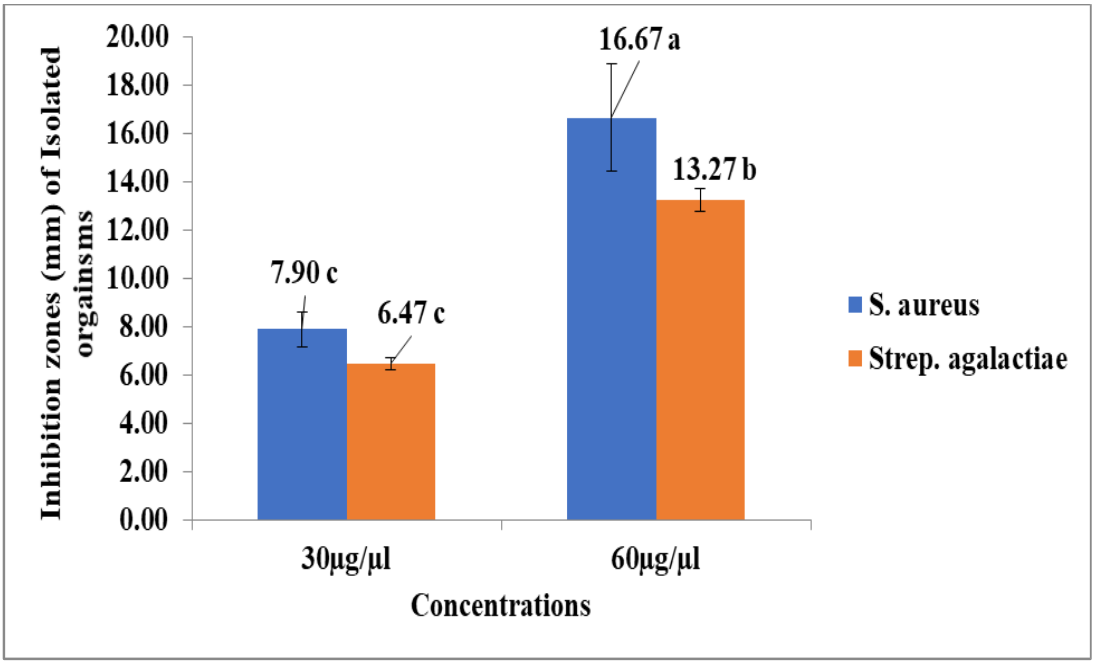

FIGURE3.2: Means bearingletters (a, b, c) indicatesa significant difference (P <0.05)
LSD value 2.27.

Bacteria	LSD	P-Value
Staphylococcus aureus	2.27	<0.05
Streptococcus agalactiae	2.27	<0.05

3.3 Zone of inhibition of pure *Moringa oleifera* against *S. aureus* and *Strep. agalactiae*

The mean values of Zone of inhibition of pure *Moringa oleifera* against *S. aureus* and *Strep. agalactiae* at 30 µg /µl and 60 µg /µl concentration. For *S. aureus,* the mean values were recorded (2.37±0.05 mm) at 30 µg/µl and (7.20±0.05 mm) at µg/µl individually. However, the zone of inhibition for *Strep. agalactiae* were noticed(1.83±0.05 mm) at 30µg/µl and (6.33±0.05 mm) at 60µg/µl recorded in that order. The data showed significant (p<0.05) differences between 30µg/µl and 60µg/µl of *Moringa oleifera* against *S. aureus* and *Strep. agalactia.*

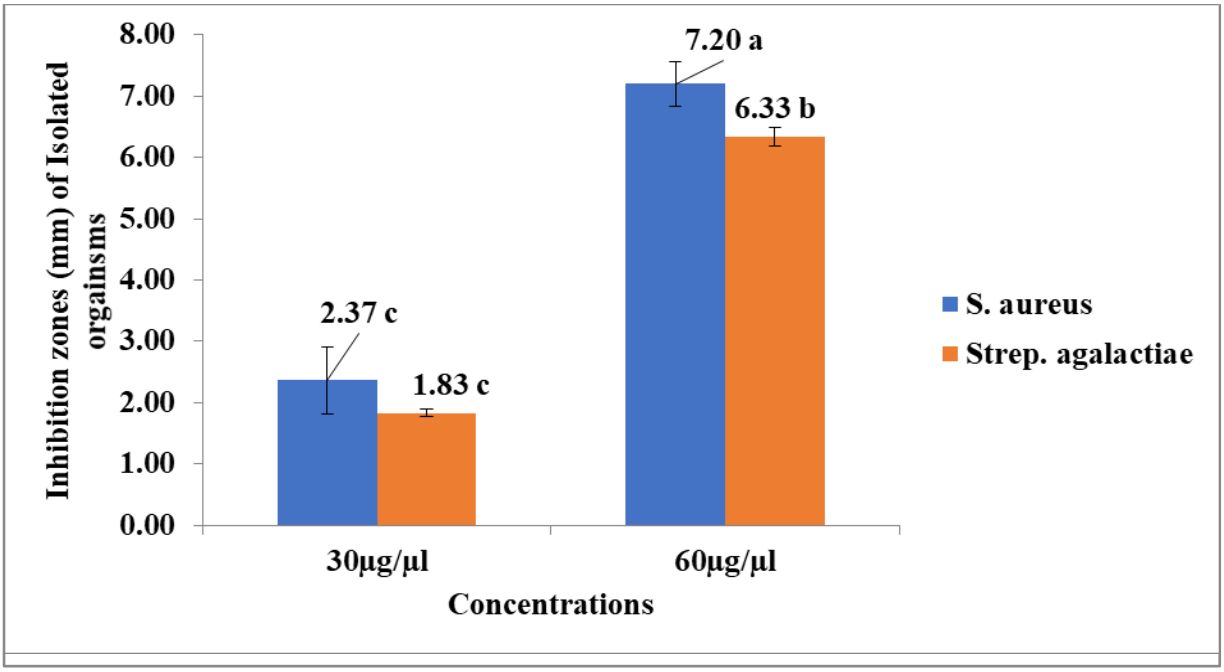

FIGURE 3.3: Means bearing letters (a, b, c) indicates a significantdifference (P <0.05)

LSD value 0.63.

Bacteria	LSD	P Value
Staphylococcus aureus	0.63	0.05
Streptococcus agalactiae	0.63	0.05

3.4 Zone of inhibition of ethanol extract of *Moringa oleifera* against *S. aureus* and *Strep. agalactiae*

Mean values of Zone of inhibition of ethanol extract of*Moringa oleifera* against *S. aureus* and *Strep. agalactiae* at 30µg/µl and 60µg/µl concentration. *S. aureus* zone of inhibition means values were observed (6.87±0.05 mm) at 30µg/µl and (14.43±0.05 mm) at 60µg/µl, respectively. Whereas, for *Strep. agalactiae* (5.50±0.05 mm) at 30µg/µl and (12.57±0.05 mm) at 60µg/µl were recorded, respectively. The data showed significant (p<0.05) differences between 30µg/µl and 60µg/µl of ethanol extract of *Moringa oleifera* against *S. aureus* and *Strep. agalctiae.*

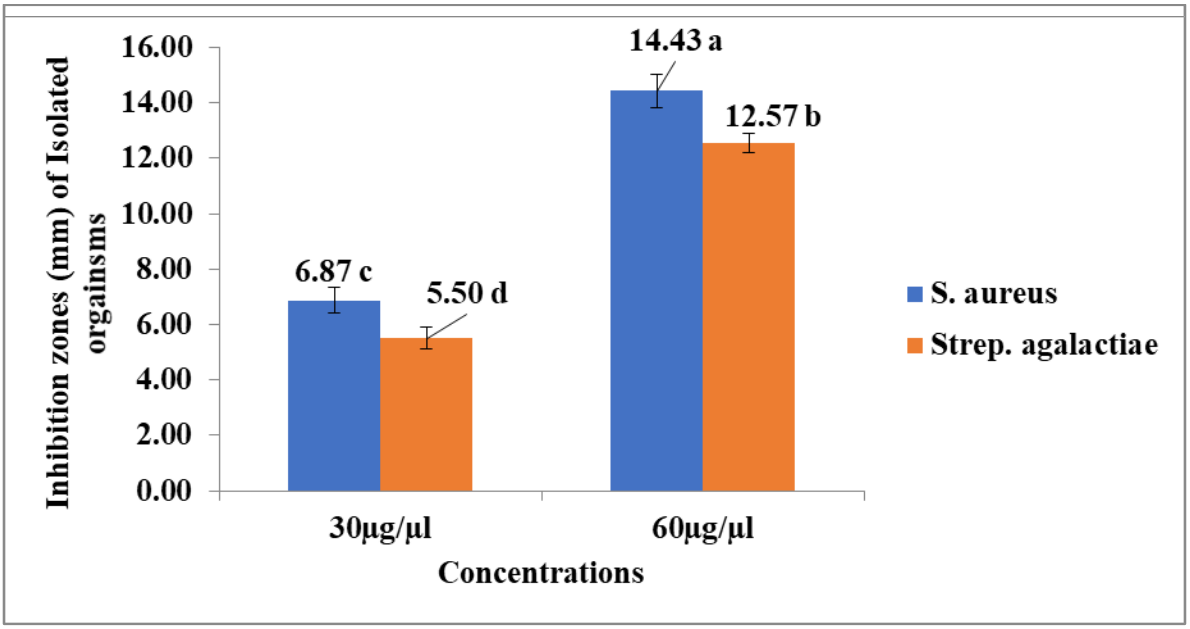

FIGURE 3.4: Means bearing letters (a, b, c, d) showa significant difference (P <0.05)

LSD value .86

Bacteria	LSD	P Value
Staphylococcus aureus	0.86	<0.05
Streptococcus agalactiae	0.86	<0.05

3.5Zones of inhibition of Pure *Aloe vera* and Pure *Moringa oleifera* and their ethanol extracts at 30µg/µl

The mean values of the Zone of inhibition of pure *Moringa oleifera* and their ethanol extract against *S. aureus* and *Strep. agalactiae* at 30µg/µl concentration. For *S. aureus,* the mean values of pure *Aloe vera* at 30µg/µl (2.83±0.05) and *Strep. agalactiae* (2.36±0.05) were recorded. Zone of inhibition of ethanol extract of *Aloe vera* against *S. aureus* and *Strep. agalactiae* were noticed (7.9±0.05) and (6.46±0.05) at 30µg/µl, respectively. For pure Moringa (2.37±0.05) and (1.83±0.05) were recorded, respectively, while for Ethanol,*Moringaoleifera* at 30 µg /µl (6.87±0.05) and (5.5±0.05) were recorded. The data showed a significant (p<0.05) difference between pure *Aloe vera,* pure *Moringa oleifera,* and their ethanol extractat 30µg/µl against *S. aureus* and *Strep. agalactiae.*

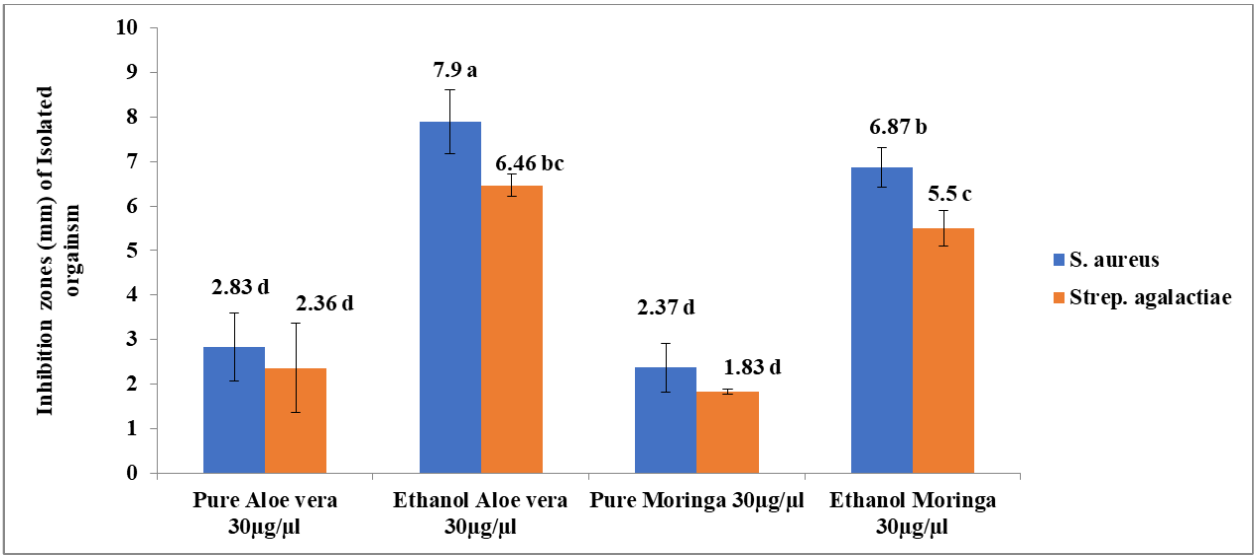

FIGURE 3.5:Means bearing letters (a, b, c, d) indicatea significant difference. (P <0.05)

LSD value 1.03.

Bacteria	LSD	P-Value
Staphylococcus aureus	1.03	<0.05
Streptococcus agalactiae	1.03	<0.05

3.6Zone's inhibition of pure *Aloevera* and pure *Moringa oleifera* and their ethanol extracts at 60µg/µl

Mean values of Zone of inhibition of pure*Aloevera,*pure *Moringa oleifera* and their ethanolextractsagainst *S. aureus* and *Strep. agalactiae* at 60µg/µl concentration. For *S. aureus,* the mean values of the zone of inhibition following the usage of pure *Aloe vera* at 60µg/µl (8.03±0.05) and for *Strep. agalactiae* (7.1±0.05) were recorded. While the ethanol extracts of pure *Aloe vera,* pure *Moringa oleifera* at 60µg/µl concentration exhibited the inhibition zone against *S. aureus* and *Strep. agalactiae* 16.66±0.05 and 13.26±0.05, respectively. Forpure Moringa (7.20±0.05) and (6.33±0.05) were recorded, respectively. And for Ethanol Moringa at 60 µg /µl (14.43±0.05) and (12.56±0.05) were recorded. The data showed a significant (p<0.05) difference between pure *Aloe vera,* pure *Moringa oleifera,* and their ethanol extractat 60µg/µl against *S. aureus* and *Strep. agalactiae*.

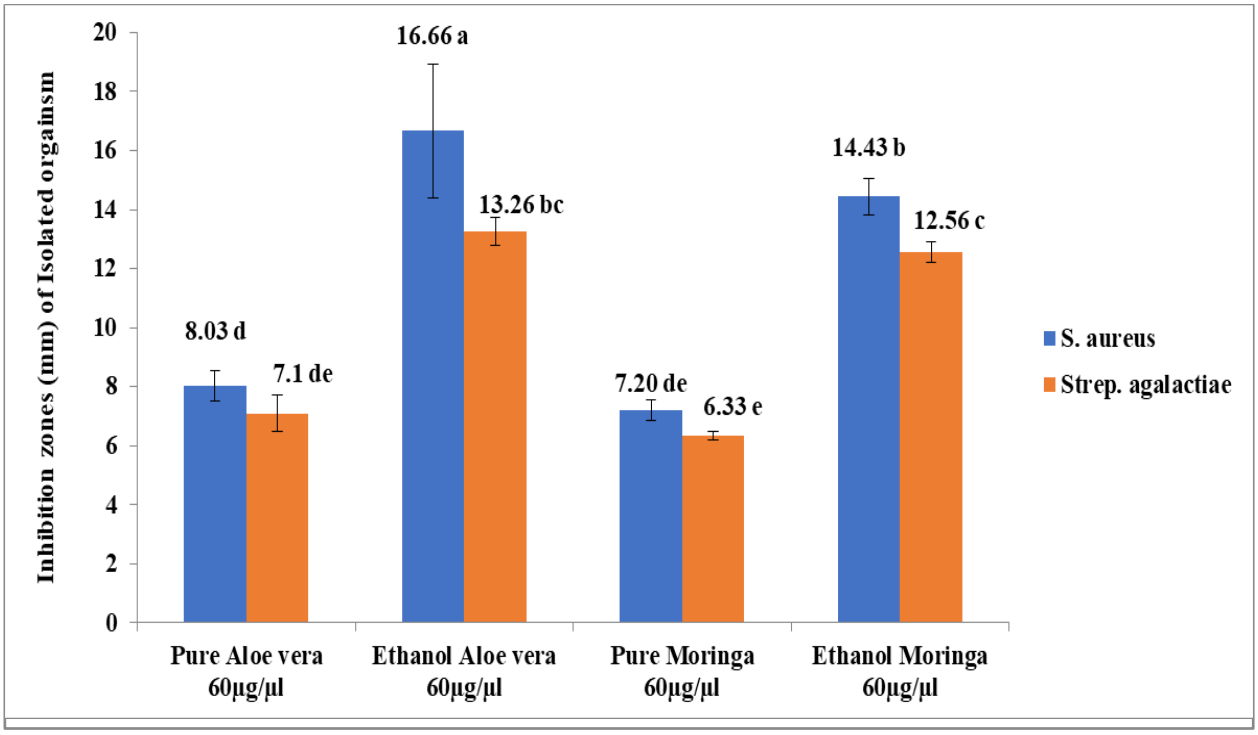

FIGURE 3.6: Means bearing letters (a, b, c, d, e) indicatea significant difference (P <0.05) LSD value 1.56

Bacterial	LSD	P Value
Staphylococcus aureus	1.56	<0.05
Streptococcus agalactiae	1.56	<0.05

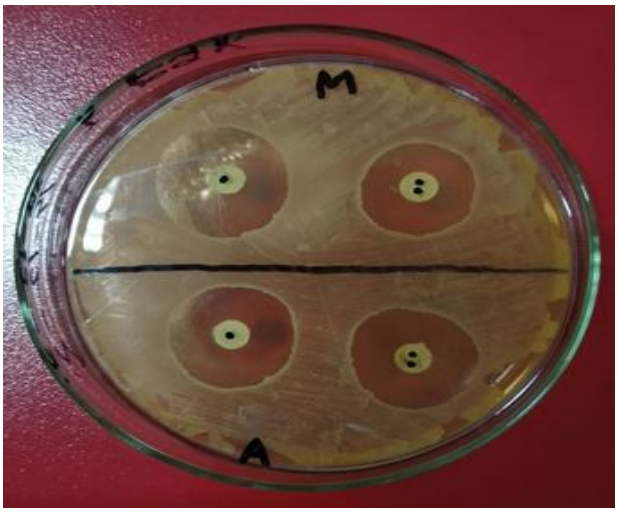

Plate 1: Photograph showing ZIs produced by ethanol extract of Moringa and *Aloevera* against *S. aureus* at 30 µg/µl.

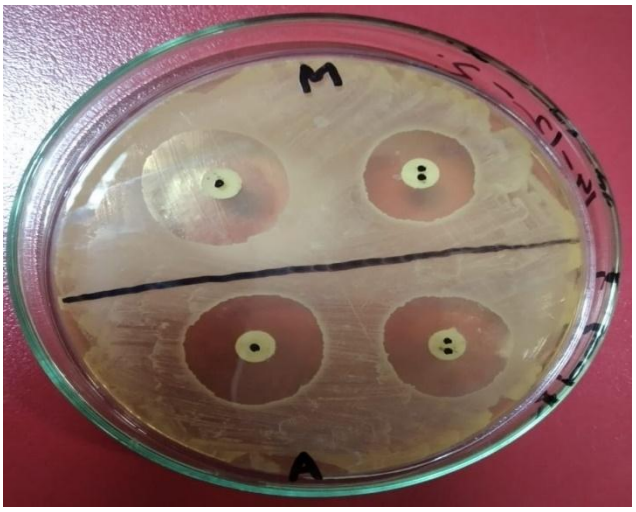

Plate 2: Photograph showing ZIs produced by by ethanol extract of Moringa and *Aloevera* against *Strep. agalactiae at* 30µg/µl.

Plate 3: Photograph showing ZIs produced by ethanol extract of Moringa and *Aloe vera* against *S. aureus* at 60µg/µl

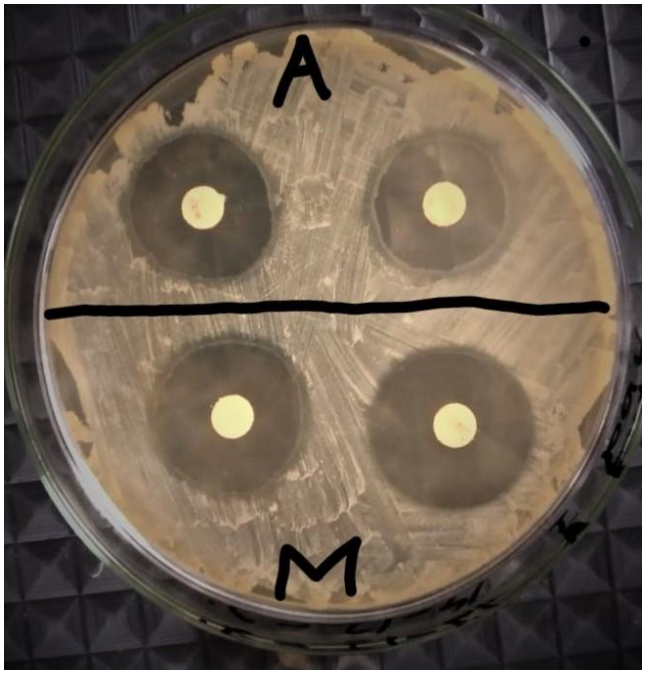

Plate 4: Photograph showing ZIs produced by by ethanol extract of Moringa and *Aloevera* against *Strep. agalactiae at* 60µg/µl

4. DISCUSSION

Aloe vera plant contains various amino acids, anthraquinones, vitamins, minerals, enzymes, saponins, sterol, raw sugar and many more bioactive compounds;; however main active constituent of AV plant extract isalone and anthraquinone, which possess an antibacterial property[16]. Due to these active ingredients *Aloe vera* plant possesses pharmacological properties such as antimicrobial, anti-inflammatory, antioxidant, anthelmintic, antifungal, and antiseptic activities [17]. Extract of *Aloe vera* produced antimicrobial activity against pathogenic bacteria, including *E.coli, S. aureus, K. pneumonia, Aspergillus niger,*and candida and against both grams positive and gram-negative bacteria by usingthe disc diffusion method[10]. The Ethanol extracts *Aloe vera* against bacterial and fungal cultures have shownthe highest activity through the disk diffusion method. *M. oleifera*is known as a “miraculous tree” due to its great nutritional and pharmacological value. Dietary inclusion of *M. oleifera* has been showing to improve antioxidant capability, health status, growth performance, milk production and meat quality in numerous livestock species. Inthe medicinal field,*M. oleifera*is used due to its pharmacologically active and substantial curative compounds. Due to these pharmacological activities,, this plant is designated as a miraculous tree and placed among high-value plants. Moringahas been used as a drug by many ayurvedic practitioners for the treatment of asthma and its pure and ethanolic extractshowed and against both G +ve and G -ve bacteria and also exhibited anthelmintic activity at various doses[18]. Hence, the current research was carried out in order to evaluate the antimicrobial effects of pure and ethanol extracts

Aloe vera and *Moringa oleifera* against *S. aureus* and *Strep. agalactiae* isolated from mastitis milk of buffaloes.

5.1 Percent prevalence of *S. aureus* and *Strep. agalactia* obtained from mastitis milk of buffaloes.

During this research, 50 clinical mastitis milk samples of buffaloes were collected under hygienic condition and samples were processed by conventional methods. To isolate, two contagious mastitis producing organism were isolated from mastitis milk samples. Out of 50 sample analyzed, 25 (50%) were found positive for *S. aureus,*20(40%) were positive for *Strep. agalactiae* and 5 (10%) were detected positive for mixed colonies. *S. aureus*was found most prevelant in current mastitis samples followed by Strep. *agalactiae* (Table 2).It has been reported that S. aureus is the most common pathogen causing mastitis in buffaloes throughout the world. High level spread of S. *aureus* commonly spread during and between two milking by milker's hands as well as usage of towels, which indicates poor milking hygiene. *S. aureus* has also got public health significance as it has been stated that entero-toxigenic strain and can cause food poisoning in humans, while *Streptococcus spp* including *Streptococcus agalactiae* tailed *Staphylococcus aureus* has been reported mastitis triggering micro-organisms. The latest investigation confirmed the findings of prior studies also reported bacterial organism's i.e, *Staphylococcus aureus* and *Streptococcus agalactiae* from mastitis animals[19]. Further, it has been observed in agreement that *Staphylococcus* and *Streptococcus* remained the predominant cause of clinical bovine mastitis. The current findings agree with those of earlier investigations in which it has been reported that examined 546 quarter of buffaloes for mastitis, out of that, 320 quarters were found culturally positive of bacterial infection. These causative organismshas been stated as *Staphylococcus aureus* and *Streptococcus agalactiae* in 140 and 13 samples respectively. It has been reported in different studies that *Staphylococcus aureus* remained the most prevalent cause of mastitis in buffaloes, whereas, *Streptococcus agalactiae's presence* was followed by *Staphylococcus aureus*[20].

5.2 Isolated organism susceptibility against different concentration of pure *Aloevera* 30 µg /µl and 60 µg /µl

In current study, Pure *Aloe vera*, at 30µg/µl and 60µg/µl concentration were used to measure the zone of inhibition against *S. aureus* and *Strep. agalactiae*. For *S.aureus* the means values recorded (2.83±0.05 mm) at 30µg/µl and (8.03±0.05 mm) at 60µg/µl respectively. Whereas, for *Strep. agalactiae* (2.0±0.05 mm) at 30µg/µl and (7.1±0.05 mm) at 60µg/µl zone of inhibition were recorded respectively. The current study showed agreement with earlier studies in which it was also found that *Aloe vera* extract possessed broad spectrum antimicrobial activity due to its inhibitory effect on *S. aureus*, *E. coli, P. aeruginosa, P. vulgaris, Enterococcus faecalis, Stap. epidermidis* and *B. subtilis* while *Aloe vera*was noticed more effective against *S. aureus*[21]. In another study it was found that *Aloe vera* showed bactericidal activity for mastitis G +ve bacteria i-e *S. aureus* and *Strep. agalactiae*, *Stap. epidermidis*. It has been stated that the active ingredient anthraquinone is also existed in the *Aloe vera* plant and it possessed antibacterial activity against various bacterial organisms. It acts like tetracycline antibiotics that it halts bacterial protein synthesis by blocking the bacterial ribosomal unit at accepter side by preventing the

transferase enzyme rather than the peptide site. Hence, its mode of action is same as that of the tetracyclines (where amino acylated tRNA enter). Hence, it has been reported that the bacterial organism could not grow in a media containing *Aloe vera* extract[22]. Additionally, tetracyclines are dose dependent antibiotics, their antibacterial activity increased with increased concentration. Similarly, it is also expected that as *Aloe vera's* mode of action is reported same as that of the tetracyclines hence it may produces its mechanism of action in dose dependent manner same as that of the tetracyclines. Due to this, in current study with increased concentration of *Aloe vera,* it yielded better zone of inhibition against the isolated bacterial organisms.

5.3 Isolated organism susceptibility against different concentration of ethanol extract of *Aloevera* on 30 µg /µl and 60 µg /µl

In present study, ethanolextract*Aloe vera*, at 30µg/µl and 60µg/µl concentration were used to measure the zone of inhibition against *S. aureus* and *Strep. agalactiae*. For *S. aureus* the mean values were recorded (7.90±0.05 mm) at 30µg/µl and (16.67±0.05 mm) at 60µg/µl respectively. While, for *Strep. agalactiae* (6.47±0.05 mm) at 30µg/µl and (13.27±0.05 mm) at 60µg/µl were noted respectively. The current study showed agreement with previous findings in which potential antimicrobial activity of ethanol extract of *Aloe vera*of inhibited gram positive bacteria *B.subtitis* and indicated 15mm, *B.megaterium* 14.5mm, *B. cereus* 13mm, and *S. aureus* 14mm, *Strep.pyogenes* 13mm zone of inhibition at concentration of 30µg/µl[23]. Another study also showed agreement with the current study, in which it was also claimed that the methanol and ethanol extract of *Aloevera* showed inhibitory potential with agar well diffusion system against *K. pneumoniae. E.coli*, *S.typhi*, *B.subtilis*, *S.aureus, B.cereus*, *Strep. pyogenes, Staph. saprophyticus, Strep.pneumoniae* and *C.albicans*. ethanolic extract of *Aloevera* shows that the maximum antibacterial activity on *L. monocytogenes* and *S. aureus* with 8 and 12mm zone respectively. Due to the existence of anthraquinone, Hydroxyanthra and Saponin, ethanolic *Aloevera*extract is responsible for antimicrobial activity[24]. It has been reported that various quantities of ethanolic extract (15, 20, 25 and30µg/µl) of *Aloe vera* root and leaf was applied, and it inhibit bacteria and fungi development via the use of diffusion method. Moreover, increased concentration exhibited enhanced zone of inhibition by yielding extreme inhibition zone with maximum concentration. Antibacterial activity of ethanolic extract of leaf and gel reportedly in comparison to *T. mentagraphytes, S. aureus, P. aeruginosa, T. schoeleinii, C. albicans* and *M. canis it* has been found that *S. aureus* was stopped by both extracts. *T. mentagrophytes* was only prevented through means of gel, but *P. aeruginosa* and *C. albicans* were inhibit with only leaf extract.It has been stated that antibacterial activity +ve bacteria (*S. aureus, B. subtilis*) and gram-ve bacteria (*P. aeruginosa, E. coli, K. pneumoniae*) against *Aloe vera* was examined in different extracts ie, petroleum ether, ethyl acetate, ethanol, and hexane. It was observed that g ethanol extract and Ethyl acetate showed better antimicrobial activity and it was (1- 9 mm) and (7- 12 mm) respectively. But the petroleum ether extract showed minimum inhibitory activity nearby 2 mm [25]. Furthermore, because to its widespread use, as demonstrated in this study, extracts of this plant could define an excellent, lesser expensive alternative to allopathic medications, as well as a better new source of antibacterial and antifungal activity. It has been reported that *Aloe vera* ethanol extract is the best extraction between

methanol and aqueous showed good antibacterial activity against the examined gram +ve and gram -ve bacterial pathogens such as *S. aureus, Strep. aglactiae, Strep. uberis, C. albicans and klebsiella spp*. and the zone of inhibition following the the usage of ethanol extract of *Aloe vera* were recorded against *S. aureus, Strep. aglactiae*as 6 mm and 5 mm via Disc diffusion method [26].

5.4 Zone of inhibition of pure *Moringa oleifera* against *S. aureus* and *Strep. agalactiae*

In current study, Pure *Moringaoleifera*, at 30µg/µl and 60µg/µl concentration were used to measure the zone of inhibition against *S. aureus* and *Strep.* a*galactiae.*. For *S. aureus* the mean values were noted (2.37±0.05 mm) at 30µg/µl and (7.20±0.05 mm) at 60µg/µl respectively. Whereas, for *Strep. agalactiae* (1.83±0.05 mm) at 30µg/µl and (6.33±0.05 mm) at 60µg/µl recorded respectively. Present study showed agreement with previous studies that the leaf aqueous extract of MO produced antibacterial activity against G+ve bacteria (*B. cereus, B. subtilis, S. aureus*)and also against Gram-negative bacteria (*Escherichia coli,Salmonella,*and acid-fast*Mycobacterium phlei*). Hence, this plant has broadly employed in the treatment of a variety of diseases as a traditional homeopathic herb. Furthermore, it has been stated that *M. oleifera* extracts revealed *in-vitro* antimicrobial activity through disk-diffusion against bacteria, yeast, dermatophytes and helminths. The leaves extract and aqueous extracts of seed prevented the growth of*S. aureus* and*P. aeruginosa* however, other bacterial organisms growth was not halted such as Candida albicans[27].Similarly, the result of a previous study in agreement with the current study determines that extract of pure *Moringa oleifera* produced a zone of inhibition (8.7mm) and (8mm) against *S. aureus* and *S. agalactiae* respectively. MO leaf water extract exhibited antibacterial activity against S. *aureus*, *Enterococcus faecalis,* and *E. coli* sensitive to leaf extracts. It has also been reported that leaf extracts showed moderate inhibition against *E. coli*, and *S. aureus.* The zone of inhibitions of the aqueous extracts of MO as inhibitory effect against *S. aureus* was found (10mm), *E. coli* (12mm) and *P. vulgaris* (10mm)[28].

5.5 Zone of inhibition of Ethanol extracts *Moringa oleifera* against *S. aureus* and *Strep. agalactiae*

In current study, antibacterial activityin terms of zone of inhibition of ethanol extracts of *Moringa oleifera* at 30µg/µl and 60µg/µl concentration was evaluated against *S. aureus* and *Strep.* a*galactiae.* For *S. aureus* the mean zone of inhibition values was noticed (6.87±0.05 mm) at 30µg/µl and (14.43±0.05 mm) at 60µg/µl respectively. Though, for *Strep. agalactiae* achieved zone of inhibition (5.50±0.05 mm) at 30µg/µl and (12.57±0.05 mm) at 60µg/µl respectively.The present study showed agreement with earlier studies, in which it was demonstrated that *Moringa oleifera* has a wide range of antibacterial properties, by using its different plant parts and different extraction techniques[29]. *Moringa oleifera* indicated antibacterial activity against *E. coli, P. multocida*, *S. aureus* and *Bacillus subtilis*In agreement with current results, it has been expressed in previous studies that ethanolextracts of Moringa produced antibacterial activity against the clinical isolates of, Shigella spp, *Enterococcusfaecalis*, *S, typhi*, *E. coli* and *S, aureus.* The *Shigella spp* was found the most susceptible organism showing higher zone of inhibition of 12.46mm but *enterococcus faecalis* is the least susceptible among the examined bacterial organisms with average zone of inhibition of 9.76mm. This study indicated that ethanol leaf extract possessed higher antibacterial

property than aqueous extract. This may be credited to better solubility and the extraction of active ingredients ie, phytochemical in ethanol extract than aqueous extract since the phytochemicals are responsible for the antibacterial properties of Moringa leaf. The leaves of *Moringa oleifera* have many phytochemical secondary metabolites of great pharmacological and bacterial properties, such as alkaloids, flavonoids, and saponins. All these metabolites have been found to have antibacterial properties[30].

5.6 Comparison of *Aloe vera* and Moringa against *S. aureus* and *Strep. agalactiae*30µg/µl and 60µg/µl

The findings of this study demonstrated that medicinal plants *Aloe vera* and Moringa showedantimicrobial activity at two different concentrations ie, 30µg/µl and on 60µg/µl. showed (2.83mm) sensitivity for *S. aureus* and for *Strep. agalactiae*(2.36 mm) and on 60µg/µl, the organisms reacted more sensitive and produces inhibitory zone of (8.03mm) and (7.1mm) respectively. Whereas, for *S. aureus* at 30µg/µl ethanol extract of *Aloe vera* showed (7.9mm) sensitivity and for *Strep. agalactiae* (6.46mm) and on 60µg/µl, the organisms highly sensitive and produced inhibitory zone of (16.66mm) for *S. aureus* and (13.26mm)for *Strep. agalactiae*. However. at 30µg/µl of pure Moringathe *S. aureus* exhibited (2.37mm) and *Strep. agalactiae* showed (2.36 mm) sensitivity and on 60µg/µl, the organisms responded more sensitive and produced inhibitory zone of (8.03mm), (7.1mm) respectively. While, at 30µg/µl ethanol extract of Moringa showed (7.9mm) sensitivity on *S. aureus* and *Strep. agalactiae* showed (6.46mm) sensitivity and on 60µg/µl, the organisms highly sensitive and produced inhibitory zone of (14.43mm) for *S. aureus* and(12.56mm). Consequently, it was noticed in the present study that either pure *Aloe vera* or its ethanol extract yielded better zone of inhibitions than Moringa or its ethanol extract. It may be because *Aloe vera* contains active ingredients like anthraquinone and others, thay act like tetracycline antibiotics and possessed good antibacterial activity in comparison to Moringa.

5. CONCLUSION

All three treatments i.e., Pure *Aloe vera,*Moringa and their ethanol extracts inhibited the growth of isolated bacterial organisms, but the pure *Aloe vera* and its ethanol extract produced better zone of inhibition than moringa.*Staphylococcus aureus* was found more prevalent than *Sreptococcus agalactiae,* in mastitis milk.*Staphylococcus aureus* was noticed more sensitive at 30µg/µl and 60µg/µl against used treatments than *Sreptococcus agalactiae.*All treatments (Pure *Aloe vera,*Moringa and their ethanol extracts)exhibited antibacterial activity at 30µg/µl and 60µg/µl against isolated organisms Ethanol extract of *Aloe vera* and Moringa produced better zone inhibition than their pure extracts.Ethanol extractof *Aloe vera*produced betterzone inhibition than ethanol extractof MoringaPure *Aloe vera*and its ethanol extract yielded better zone inhibition than Moringa.All treatments, with increasing concentration (60µg/µl) produced dose dependent effects by producing better zone inhibition of isolated organisms.

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