

Comparative Efficacy of Aloe Vera and Levofloxacin Against Corynebacteriumpyogenes and Staphylococcus Epidermidis Isolated from Camel Wound.

Muhammad Mubashir Farooq¹, Rehana Buriro¹, Abdul Kabir^{*2,} Misbah Shehzadi³, Zainab lanjar⁴, Shams Uddin Bughio¹, Faisal khan¹, Muneeb Anwar³, Asif Nawaz⁵, Safdar Aman^{6,} Ihtisham Ishfaq⁵

¹Department of veterinary Pharmacology Faculty of Animal Husbandry & Veterinary Sciences, Sindh Agriculture University, Tandojam

²Department of veterinary microbiology Faculty of Animal Husbandry & Veterinary Sciences, Sindh Agriculture University, Tandojam

³College of veterinary sciences the university of agriculture Peshawar

⁴Department veterinary Microbiology University: Shaheed benezir Bhutto University of Veterinary and Animal science.

(SBBUVAS)

⁵Livestock and Dairy Development Department (Extension) Khyber Pakhtunkhwa ⁶Department of veterinary medicine University of veterinary and animal sciences Lahore

*Corresponding author: - Abdul Kabir kabirvet32@gmail.com

*Department of veterinary microbiology Faculty of Animal Husbandry & Veterinary Sciences, Sindh Agriculture University, Tandojam

ABSTRACT

The study aimed to determine the minimum inhibitory concentration (MIC) of pure Aloe vera, its ethanol extract, and the antibiotic Levofloxacin against Staphylococcus epidermidis and Corynebacterium pyogenes isolated from camel wounds. Seventy wound samples were collected from veterinary clinics and nomadic animals near Hyderabad and Tando Allah Yar. These samples were processed at the Department of Veterinary Pharmacology, SAU Tandojam, for isolation and identification of the organisms. Different concentrations of pure Aloe vera, its ethanol extract, and Levofloxacin were tested to record the MIC against the isolated organisms. The MIC was determined by observing the turbidity and translucency of the cultured medium. Out of 70 wound samples, 50 were positive, with 29 (58%) positive for Staphylococcus epidermidis and 21 (42%) for Corynebacterium pyogenes. Staphylococcus epidermidis growth was inhibited at $5\mu g/\mu l$, $2.5\mu g/\mu l$, and $0.31\mu g/\mu l$ of pure Aloe vera, its ethanol extract, and $0.55\mu g/\mu l$ for pure Aloe vera, $4.22\mu g/\mu l$ for ethanol extract, and $0.55\mu g/\mu l$ for Levofloxacin. Corynebacterium pyogenes growth was inhibited at $5\mu g/\mu l$, and $0.15\mu g/\mu l$ of pure Aloe vera, $4.22\mu g/\mu l$ for pure Aloe vera, its ethanol extract, and $0.55\mu g/\mu l$ for ethanol extract, and $0.25\mu g/\mu l$ for Levofloxacin. respectively. The mean MIC values were $7.77\mu g/\mu l$ for pure Aloe vera, $3.33\mu g/\mu l$ for ethanol extract, and $0.25\mu g/\mu l$ for Levofloxacin. results indicated that the ethanol extract of Aloe vera had better antibacterial activity than pure Aloe vera, supporting its traditional use as an alternative to antibiotics to reduce antibiotic resistance.

INTRODUCTION

The camel (Camelus dromedarius) is a versatile desert animal that thrives in warm, chilly, and dry environments (Yagil et al., 1985). It serves multiple purposes, including providing milk, meat, wool, and transportation, and is used in racing, tourism, and agricultural work. Camel meat and hide are valuable sources of income, and its milk is gaining popularity in developing countries (Rathore and Kataria, 2012). Despite its resilience, camels are prone to skin infections caused by Staphylococci, leading to conditions like skin necrosis, dermatitis, wounds, and abscesses (Wernery, 2000).

A wound is defined as a loss or disruption of the cellular, anatomical, or functional integrity of living tissues. Wounds can be classified based on their cause, location, type of injury, symptoms, depth, and tissue loss. They are categorized as superficial (loss of epidermis), partial thickness (loss of both epidermis and dermis), or full thickness (loss of dermis, subcutaneous fat, and sometimes bone) Walker et al 2022. Wounds are also classified by duration: acute wounds occur suddenly, while chronic wounds develop when acute wounds fail to heal, often due to ulcers, decubitus, or burns (Eriksson et al 2022).

Wounds can be contaminated and worsened by physical, chemical, or microbial agents, making animals more susceptible to infections (Mohan, 2005). Bacterial contamination of wounds can impact animal health and the value of their hides (Tyler et al., 1999). Common aerobic bacteria causing superficial skin infections include Staphylococcus, Streptococcus, Pasteurella multocida, Vibrio species, Corynebacteria species, and Pseudomonas aeruginosa. Anaerobic pathogens like Clostridium species, Fusobacterium, and Bacteroides typically infect deeper wounds (Tiwari and Dhama, 2014).

Antibiotics are essential for treating these infections, with broad-spectrum antibiotics being preferred. Levofloxacin, a broad-spectrum fluoroquinolone, is particularly effective. It works by inhibiting bacterial DNA gyrase, targeting both Gram-positive and Gram-negative bacteria, and showing moderate activity against anaerobes. Its broad antibacterial action, good bioavailability, extended serum half-life, and general safety make it a valuable option for treating various infections (Randall et al., 1991).

The rise of drug resistance in conventional medicine has spurred the development of alternative treatments, including the use of plants. About 20% of the world's plants have been tested for their effectiveness against pathogens (Adnan *et al.*, 2015). Aloe vera, from the Liliaceae family, is notable for its therapeutic properties (Boudreau and Beland, 2016). It produces two main products: yellow latex (Aloe juice) and leaf pulp (gel). The gel, composed of 99.3% water and 0.7% active compounds like vitamins, minerals, and enzymes, is valued for skincare (Waithaka *et al.*, 2018). Aloe vera contains six antiseptic agents, including lupeol and salicylic acid, which inhibit fungi, bacteria, and viruses (Surjushe *et al.*, 2008). It's used to treat skin irritations, burns, eczema, and more, due to its cell regeneration, anti-inflammatory, antiviral, and moisturizing properties (Paoulomi *et al.*, 2013).

Antibiotics have been crucial for health, but rising resistance has renewed interest in plant extracts as antimicrobial agents. Aloe vera's alcoholic extracts show higher antibacterial and antifungal activity than aqueous extracts. (Choi *et al.*, 2001). Different solvent extracts of Aloe vera exhibit varying antibacterial responses against bacteria like E. coli and Salmonella typhi, with methanol extracts showing the highest activity. Ethanol extracts also demonstrate significant antibacterial effects (Lawrence *et al.*, 2009).

Previous studies have shown that Aloe vera possesses remarkable wound healing properties. However, there is limited research on organisms isolated from camel wounds. Given the significance of camels, this study aims to evaluate the antibacterial effectiveness of pure and ethanol extracts of Aloe vera against Corynebacterium pyogenes and Staphylococcus epidermidis isolated from camel wounds, comparing these results to commonly available antibiotics. The goal is to provide an affordable source of medication for camels.

MATERIALS AND METHODS

Sampling and Bacteriological Isolation

Seventy wound samples from camels were collected aseptically using transwab tubes from veterinary clinics and nomadic animals around Hyderabad and Tando Allah Yar. Samples were processed at the Department of Pharmacology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University Tandojam.

Bacterial Isolation and Identification

Samples were cultured on nutrient agar for primary isolation. Purification was achieved by subculturing onto nutrient and blood agar, incubated at 37 °C for 24 hours. Identification was based on cultural, morphological, and biochemical characteristics, including Gram staining.

Sterilization of Glassware

Glassware was washed with diluted detergent and tap water, dried in an oven, and wrapped in brown paper. Conical flasks, pipettes, and test tubes were cotton-plugged, wrapped, and sterilized in a hot air oven at 165 °C for two hours. Plastic items were autoclaved at 121 °C for 15 minutes.

Media Preparation

Nutrient Agar: Prepared by dissolving 2.8 g of nutrient agar in 100 mL distilled water, autoclaved at 121 °C for 15 minutes, and poured into petri dishes.

Blood Agar: Prepared by dissolving 4.8 g of blood agar in 100 mL distilled water, autoclaved, cooled, and mixed with 5 mL sheep blood before pouring into petri dishes.

Muller Hinton Broth: Prepared by dissolving 2.1 g of Muller Hinton broth in 100 mL distilled water, autoclaved at 121 °C for 15 minutes.

Bacterial Culture

Samples were cultured on nutrient agar for primary isolation, followed by subculturing on nutrient and blood agar. Pure colonies were identified by cultural, morphological, and biochemical characteristics, including Gram staining. Pure colonies were stored on nutrient agar slants at 4 °C.

Identification Tests

Gram Staining: Bacterial smears were stained with crystal violet, Lugol's iodine, decolorized with alcohol, and counterstained with carbol fuchsine.

Catalase Test: Bacterial colonies were mixed with 3% hydrogen peroxide; bubbling indicated a positive result. Oxidase Test: Colonies were mixed with oxidase reagent; blue coloration indicated a positive result.

Aloe Vera Gel Extraction

Aloe vera leaves were cleaned, disinfected, and the gel was extracted, blended, filtered, and sterilized by autoclaving. Ethanol extraction involved air-drying the gel, grinding it into powder, soaking in ethanol, filtering, and sterilizing.

Minimum Inhibitory Concentration (MIC)

MIC was determined using micro broth dilution on Muller-Hinton media. Aloe vera and Levofloxacin were tested against *Corynebacterium pyogenes* and *Staphylococcus epidermidis*. MIC plates were incubated at 37 °C overnight, and breakpoints were recorded.

Preparation of Antibiotic Solution

Levofloxacin stock solution was prepared by dissolving 20 mg in 20 mL distilled water, autoclaved, and stored at 4 °C. *Data Analysis*

The break points, where the bacteria stop to grow were recorded as MIC for antibiotic as well as pure Aloe vera and its ethanol extract. The obtained data were analyzed through ANOVA (micro statistical package).

RESULTS

This study was conducted to evaluate the antibacterial activity of pure Aloe vera and its ethanol extract, comparing them with Levofloxacin against Staphylococcus epidermidis and Corynebacterium pyogenes isolated from camel wounds. *prevalence of Staphylococcus epidermidis and Corynebacterium pyogenes isolated from camel wound*.

A total of 70 wound samples were examined, all of which tested positive for various organisms. Of these, 50 samples were confirmed positive, with 29 testing positive for Staphylococcus epidermidis and 21 for Corynebacterium pyogenes. These organisms were identified based on their morphological and cultural characteristics, as well as their staining reactions. (Table 1).

Table 1: Prevalence of Staphylococcus epidermidis and Corynebacterium pyogenes isolated from camel wound.

Bacterial species	Total No. of wound samples	No. of positive wound samples	Percentage
Staphylococcus epidermidis	50	29	58%
Corynebacterium pyogenes		21	42%

Susceptibility of Staphylococcus epidermidis at various concentration of pure Aloe vera, Ethanol extract and Levofloxacin

Various concentrations of pure Aloe vera, its ethanol extract, and Levofloxacin—ranging from $20\mu g/\mu l$ to $0.01\mu g/\mu l$ —were tested for their effectiveness against Staphylococcus epidermidis. The bacteria showed susceptibility to pure Aloe vera at concentrations of $20\mu g/\mu l$, $10\mu g/\mu l$, and $5\mu g/\mu l$, but not at lower concentrations. Ethanol extract was effective at $20\mu g/\mu l$, $5\mu g/\mu l$, and $2.5\mu g/\mu l$, while Levofloxacin was effective down to $0.31\mu g/\mu l$. The minimum inhibitory concentrations (MIC) were determined to be $5\mu g/\mu l$ for pure Aloe vera, $2.5\mu g/\mu l$ for ethanol extract, and $0.31\mu g/\mu l$ for Levofloxacin.

The mean inhibitory values for Aloe vera, ethanol extract, and Levofloxacin against Staphylococcus epidermidis were 8.22, 4.22, and 0.55, respectively, at which 50% of the isolated organisms ceased to grow. The results indicated a highly significant difference among the three treatments, with a p-value of .000



Figure-1 MIC of pure Aloe vera, ethanol extract and Levofloxacin against *Staphylococcus epidermidis*.

Susceptibility of Corynebacterium pyogenes at various concentration of pure Aloe vera, ethanol extract and Levofloxacin.

Various concentrations of pure Aloe vera, its ethanol extract, and Levofloxacin—ranging from 20 μ g/ μ l to 0.01 μ g/ μ l were tested to determine the minimum inhibitory concentration (MIC) against Corynebacterium pyogenes isolated from camel wounds. The bacteria showed susceptibility to pure Aloe vera at concentrations of 20 μ g/ μ l, 10 μ g/ μ l, and 5 μ g/ μ l, but not at lower concentrations. Ethanol extract was effective at concentrations from 20 μ g/ μ l to 1.25 μ g/ μ l, while Levofloxacin was effective down to 0.15 μ g/ μ l. The MICs for Corynebacterium pyogenes were 5 μ g/ μ l for pure Aloe vera, 1.25 μ g/ μ l for ethanol extract, and 0.15 μ g/ μ l for Levofloxacin.

The mean inhibitory values for Aloe vera, ethanol extract, and Levofloxacin against Corynebacterium pyogenes were 7.77, 3.33, and 0.25, respectively, at which 50% of the isolates ceased to grow. The results indicated a highly significant difference among the three treatments, with a p-value of .000, demonstrating the significant susceptibility of the species to these treatments.



Figure-2 MIC of Aloe vera, ethanol extract and Levofloxacin against Corynebacterium pyogenes.

Comparative MIC of pure Aloe vera, ethanol extract and Levofloxacin against Staphylococcus epidermidis and Corynebacterium pyogenes

Various concentrations of pure Aloe vera, its ethanol extract, and Levofloxacin—ranging from 20 μ g/ μ l to 0.01 μ g/ μ l were tested for their effectiveness against Staphylococcus epidermidis and Corynebacterium pyogenes. Staphylococcus epidermidis showed susceptibility to pure Aloe vera at 20 μ g/ μ l, 10 μ g/ μ l, and 5 μ g/ μ l. For the ethanol extract, susceptibility was observed at 20 μ g/ μ l, 10 μ g/ μ l, 5 μ g/ μ l, and 2.5 μ g/ μ l. Levofloxacin was effective at concentrations of 20 μ g/ μ l, 10 μ g/ μ l, 5 μ g/ μ l, 1.25 μ g/ μ l, 0.62 μ g/ μ l, and 0.31 μ g/ μ l.

Similarly, Corynebacterium pyogenes showed susceptibility to pure Aloe vera at 20 μ g/µl, 10 μ g/µl, and 5 μ g/µl. For the ethanol extract, susceptibility was noted at 20 μ g/µl, 10 μ g/µl, 5 μ g/µl, 2.5 μ g/µl, and 1.25 μ g/µl. Levofloxacin was effective at concentrations of 20 μ g/µl, 10 μ g/µl, 5 μ g/µl, 2.5 μ g/µl, 0.62 μ g/µl, 0.31 μ g/µl, and 0.15 μ g/µl (Figure-3).



Figure-3 Comparative MIC of Aloe vera, ethanol extract and Levofloxacin against *Staphylococcus epidermidis* and *Corynebacterium pyogenes*.

The results showed that Corynebacterium pyogenes was more sensitive to the ethanol extract of Aloe vera and Levofloxacin compared to Staphylococcus epidermidis. Both bacteria were equally sensitive to pure Aloe vera at a concentration of 5μ l. This suggests that the ethanol extract might be a better option than pure Aloe vera and Levofloxacin because it may be more effective and less likely to face resistance

DISCUSSIONS

Aloe vera is a perennial, drought-resistant, succulent plant belonging to the Liliaceae family. It has sharp, barbed, and edged leaves, and contains over 200 compounds, 75 of which exhibit biological activity. Aloe vera has been utilized for various purposes, including food and medicine, with many therapeutic effects attributed to the polysaccharides present in its gel. The plant exhibits numerous pharmacological activities, such as antimicrobial, anticancer, antioxidant, antidiabetic, antiulcer, hepatoprotective, and immunomodulatory properties (Karkala and Bhushan, 2014). This study aimed to assess the minimum inhibitory concentration (MIC) of pure Aloe vera, ethanol extract of Aloe vera, and Levofloxacin against *Staphylococcus epidermidis* and *Corynebacterium* isolated from camel wounds.

In this study, 70 wound samples were examined for the presence of Staphylococcus epidermidis and Corynebacterium pyogenes. Out of these, 50 samples tested positive, with 29 and 21 samples positive for Staphylococcus epidermidis and Corynebacterium pyogenes, respectively. This indicates a prevalence of 58% and 42% for Staphylococcus epidermidis and Corynebacterium pyogenes. The findings align with previous studies, which reported the isolation of twelve different bacterial species from camel wounds, with Corynebacterium pyogenes (20.00%) and Staphylococcus epidermidis (2.63%) being predominant (Deveraini et al., 2010). Other studies have also suggested that aerobic bacterial species (75.80%) are more prevalent in superficial skin infections compared to anaerobic organisms, which are more common in deeper wounds (Tiwari and Dhama, 2014). Staphylococcus spp. and Corynebacterium spp. have been consistently reported as prevalent among microorganisms isolated from wounds (Letowska., et al 2010). The high prevalence of these organisms is likely due to their environmental presence, allowing them to easily colonize wounds, which provide an ideal medium for growth due to the presence of pus, blood, and other debris. The MIC of Staphylococcus epidermidis and Corynebacterium pyogenes was evaluated in this study. Results showed that Staphylococcus epidermidis was susceptible to Aloe vera and its ethanol extract at concentrations of 5 μ g/ μ l and 2.5 μ g/ μ l, respectively. Corynebacterium pyogenes was susceptible at concentrations of 5 μ g/ μ l and 1.25 $\mu g/\mu l$, respectively. Both pure Aloe vera extract and its ethanolic extract demonstrated inhibitory action against a wide variety of microorganisms. It has been reported that alcoholic extracts (methanolic or ethanolic) exhibit higher antimicrobial action than pure Aloe vera extract due to more efficient extraction of biologically active compounds in alcohols. This study's results are consistent with previous findings that reported maximum antimicrobial action of alcoholic extracts against Staphylococcus epidermidis (Lawrence et al., 2009; Darioush et al., 2015).

Plant extracts are a potential source of antimicrobial compounds, especially against bacterial pathogens (Mohanasundari et al., 2007). Medicinal herbs can be considered synthetic laboratories, producing and containing many active antibacterial agents (Subramanian et al., 2006). Aloe vera gel contains potent biologically active antibacterial agents, including anthraquinones, phenols, saponin, aloin, and salicylic acid, which inhibit bacterial growth (Ro et al., 2000). Acemannan, an acetylated mannose in Aloe vera gel, forms a viscous layer around the urinary, genital, gastrointestinal, and respiratory tracts when ingested, entrapping microorganisms and preventing their entry into the system. Anthraquinones, structurally similar to tetracycline, produce antibacterial actions by hindering bacterial protein synthesis (Pandey and Mishra, 2010). Other antimicrobial agents in Aloe vera include pyrocatechol, cinnamic acid, p-coumaric acid, and ascorbic acid, which disrupt cell membranes, inhibit glucose uptake and ATP production, and denature proteins, respectively (Lawrence et al., 2009).

Fluoroquinolones, including Levofloxacin, are a new generation of quinolones with improved microbiologic and pharmacologic properties. Levofloxacin, a third-generation fluoroquinolone, is the L-isomer of ofloxacin and has high water solubility at neutral pH, allowing for high-concentration formulations (Yoshiko et al., 2012). Fluoroquinolones exert antimicrobial activity by inhibiting DNA gyrase, an enzyme involved in bacterial DNA synthesis. They are frequently prescribed for their broad antibacterial spectrum against Gram-positive, Gram-negative, mycobacterial pathogens, and anaerobes (Diren and Zeynep, 2007). Levofloxacin has shown strong antibacterial activity against various pathogens, including Staphylococcus spp., Streptococcus pyogenes, Streptococcus pneumoniae, Staphylococcus haemolyticus, Enterobacter spp., Escherichia coli, Salmonella spp., Klebsiella spp., Serratia spp., Enterococcus spp., Proteus spp., Pseudomonas aeruginosa, Haemophilus influenzae, and Neisseria gonorrhoeae (Rahila et al., 2011). different concentrations of Levofloxacin (ranging from 20 μ g/ μ l to 0.01 μ g/ μ l) were used to test the susceptibility of isolated bacterial organisms. Staphylococcus epidermidis showed susceptibility at a very low concentration of 0.31 µg/µl, while resistance was observed below this level. These results are consistent with studies reporting the effectiveness of Levofloxacin in treating bacterial endophthalmitis caused by S. epidermidis, S. aureus, and P. aeruginosa (Ferrer et al., 2008; Yildirim et al., 2002). Corynebacterium pyogenes showed susceptibility at a very low concentration of 0.15 μ g/ μ l, with resistance observed below this level. These findings align with studies demonstrating the inhibitory effect of Levofloxacin on Corynebacterium pyogenes in external ophthalmic infections (Yoshiko et al., 2012; Najma et al., 2013).

CONCLUSION

Staphylococcus epidermidis was observed most prevalent bacterial organism in camel wound as compared to Corynebacterium pyogenes. All the three treatment i.e. pure Aloe vera, its ethanol extract and Levofloxacin inhibited the growth of isolated bacterial organisms at certain levels. Pure Aloe vera inhibited the growth of both Staphylococcus epidermidis and Corynebacterium pyogenes at same concentration, i.e. $5\mu g/\mu l$. Ethanol extract of Aloe vera inhibited the growth of Staphylococcus epidermidis and Corynebacterium pyogenes at 20 $\mu g/\mu l$, $10\mu g/\mu l$, $5\mu g/\mu l$ and $2.5\mu g/\mu l$, $1.25\mu g/\mu g/\mu l$ respectively. Levofloxacin showed its minimum inhibitory concentration against Staphylococcus epidermidis and Corynebacterium pyogenes at 0.31 $\mu g/\mu l$ and 0.15 $\mu g/\mu l$ respectively. Corynebacterium pyogenes was noticed to be more susceptible to Levofloxacin and ethanol extract of Aloe vera than Staphylococcus epidermidis.

ACKNOWLEDGEMENTS

The research work was accomplished by utilizing budget of Department of Veterinary Pharmacology, Sindh Agriculture University Tandojam, Pakistan.

STATEMENT OF CONFLICT OF INTEREST

The mentioned authors have declared no conflict of interest.

CONTRIBUTION

MMF, RB, SUB conceived and designed the experiments. MMF, ZL, FK, AN, II performed the experiments. AK, MS, MA, SA analyzed the data. AK revised the manuscript. AK, wrote the manuscript.

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