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Antibacterial Efficacy of Pure Aloe Vera, Methanol Extract and Gentamicin Against Pathogenic Bacteria

Rizwan Ali Keerio[1], Shamsuddin Bughio[1], Rehana Buriro[1], Muhammad Bilawal[1*], Muneer Ahmed Jamali[2], Mansoor Tariq[3], Imdadullah Jamali[3], Nawab Ali Jamali[4], Fahadullah Jamali[5]

[1]Department of Veterinary Pharmacology, Sindh Agriculture University, Tandojam, Pakistan
[2]Department of Animal Products and Technology, Sindh Agriculture University, Tandojam, Pakistan
[3]Department of Veterinary Pathology, Sindh Agriculture University, Tandojam, Pakistan
[4]Department of Animal Reproduction, Sindh Agriculture University, Tandojam, Pakistan
[5]Department of Molecular Biology and Genetics, Shaheed Benazir Bhutto University, Shaheed Benazir Abad, Pakistan

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***Corresponding Author:**
Muhammad Bilawal Arain
Department of Veterinary Pharmacology, Sindh Agriculture University, Tandojam, Pakistan
dr_bilalarain@yahoo.com

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ABSTRACT

The Aloe vera plant has been utilized for thousands of years for traditional medicinal reasons, including the prevention of bacterial development. **Objectives:** To investigate the antibacterial effectiveness of pure Aloe vera and its methanol extract, comparing their efficacy to that of the commonly used antibiotic gentamicin. **Methods:** 50 broiler meat samples were collected from the butcher markets of Tandojam. Isolation and identification of the microorganisms, the minimum inhibitory concentration (MIC) was done on 96 well plates. For this purpose, similar concentrations of pure Aloe vera, its methanol extract and gentamicin were to evaluate their minimum inhibitory concentration against *Staphylococcus aureus* and *Escherichia coli*. The minimum inhibitory concentration was tested based on the transparency and turbidity of the medium. **Results:** Out of 50 meat samples identified 19 (38%) were found positive for *Staphylococcus aureus* and 24 (48%) were positive for *Escherichia coli*. The mean susceptibility value of *Staphylococcus aureus* was noticed at 10, and 20µg/µl for pure Aloe vera, 20, 10, 5µg/µl for methanol extract and 20, 10, 5, 2.5, 1.25µg/µl for gentamicin. While the mean concentration at which *Escherichia coli* growth inhabited was 20 µg/µl and 20, 10 µg/µl, and 20, 10, 5, 2.5, 1.25, 0.625, 0.312 µg/µl for pure Aloe vera, its methanol extract and gentamicin, respectively. **Conclusions:** It was concluded that all treatments i.e., pure Aloe vera, its methanol extract, and gentamicin inhibited the growth of isolated bacterial organisms, but the methanol extract stopped the growth of isolated organisms at lowered concentration in comparison to pure aloe vera.

INTRODUCTION

Poultry meat is believed to be the most common vehicle for the transmission of foodborne diseases, followed by red meat. S. *aureus* is a bacterium that causes several infections in animals including mastitis, arthritis, and urinary tract infections. Food poisoning caused by *Staphylococcus aureus* is the third most common cause of food-borne disease worldwide [1]. It is the pathogen's ability to produce enterotoxins that makes it deadly. This kind of food poisoning is most frequently associated with fresh and prepared meals, namely meat products [2]. *Escherichia coli (E. Coli)* has also important dissemination and association as a foodborne pathogen. This pathogen can cause intestinal and extra-intestinal infections. It has been reported that human foodborne illnesses are caused by the consumption of meat and meat products contaminated by *E. coli* [3]. On the other hand, *E. coli* is an indicator of fecal contamination in poultry meats. Some strains of *E. coli* are highly pathogenic in humans as well as in animals [4]. The Aloe vera plant has been utilized for thousands of years for traditional medicinal reasons, including the prevention of bacterial development [5]. It has been stated that *Aloe vera* gel has antibacterial action

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over gram-negative and gram-positive bacteria and that it has been shown to effectively kill or substantially decrease the growth of *S. aureus* and *E. coli* bacterias [6]. It has been shown that there are variations in the antibacterial properties of different Aloe vera extracts [7]. As previously shown, the methanol extract of *Aloe vera* has strong antimicrobial action, as evidenced by its ability to limit the maximal growth of S. aureus and *E. coli* when compared to alternative solvents such as ethanol and distilled water [8]. It has been stated that the methanolic, ethanolic, and acetone extract showed antimicrobial activity over E. coli and Bacillus subtilis. The methanolic extract of Aloe vera presented pronounced activity against S. aureus than other extracts [9]. Gentamicin is a broad-spectrum antibiotic that has been used to kill gram-positive and gram-negative bacteria strains with significant colonies [10]. It can be applied topically to treat local infections such as infectious impetigo, seborrheic dermatitis, and superficial ocular infections. It has been used to treat endocarditis, meningitis, and systemic biliary tract disorders. Gentamicin, however, exacerbates serious toxicity issues, particularly ototoxicity, nephrotoxicity, and neuromuscular blockade, when administered parenterally at high concentrations over a long period [11]. The above studies indicated that the Aloe vera possessed antibacterial effects to treat various food-borne bacterial infections. To the best of our knowledge, now, some researchers have detected the antimicrobial effects of methanol extract with Aloe vera. Therefore, considering the importance of food-borne diseases in humans, this study was intended to evaluate the antibacterial efficacy of pure Aloe vera its methanol extract and compared it with gentamicin which is a commonly used antibiotic to treat food-borne diseases caused by Staphylococcus aureus and Escherichia coli isolated from broiler meat.

This study aims to investigate the antibacterial effectiveness of pure Aloe vera and its methanol extract, comparing their efficacy to that of the commonly used antibiotic gentamicin.

METHODS

Fifty samples of fresh broiler meat were obtained in an aseptic method. For primary culture, the materials were cultivated on nutrient agar. The isolates were purified by sub-culturing on blood and MacConkey agar and then incubated at 37°C for 24 hours. On the blood agar medium, sub-culturing was used to carry out additional purification. Based on morphological, biochemical, and cultural characteristics, organisms have been identified. For observing the staining reaction, gram staining was used. Similar concentration of pure Aloe vera, its Methanol extract and gentamicin were used i.e., 20 µg/µl, 10µg/µl, 5 µg/µl, 2.5 µg/µl, 1.25 µg/µl, 0.625 µg/µl, 0.312 µg/µl, 0.156 µg/µl, 0.78 µg/µl, and 0.039 µg/µl, to evaluate their MIC against *Staphylococcus aureus* and *Escherichia coli.* For detection of *Escherichia coli* broiler meat samples were subculture on MacConkey agar for the identification of the bacteria. Following that, Petri dishes were examined for dry, doughnut-shaped, dark pink-in-colour colonies with the whole border encircled by a dark pink region of precipitated bile salts, which were found to be present. Further validation was obtained via the use of Gram staining and biochemical reactions with isolates. Samples of broiler meat were subculture on blood agar to detect *Staphylococcus aureus,* which was found in the meat. Following that, colonies of yellow-golden colour were seen in Petri dishes, and additional confirmation was achieved via the use of gram staining and the biochemical response of isolates. Bacterial organisms were further confirmed by biochemical tests, which include the following. Oxidase test and Coagulase test followed by [12]. The Aloe vera plant leaves were obtained from the Tandojam plant nursery, which is located nearby. The plant leaves were cleaned with 70 percent alcohol before being processed. With the aid of a sterile knife, the leaves were incised, and the gel was removed from the leaves. Further, the gel was mixed to ensure homogeneity and then filtered through a muslin cloth before being autoclaved at 121°C at 15 pounds of pressure for 15 minutes to sterilize it. Then, as a stock solution, a sterilized filter was used (100 percent concentration).Fresh leaf gel will be oven-dried at 80°C for 48 hours to prepare methanol extracts. Afterwards, a pestle and mortar will be used to grind the dried gel into a powder, which will then be blended into a fine powder. Twenty grams of this powder will be steeped in 200 milliliters of methanol. After passing the mixture through Whatman filter paper number 1, the filtrate will be evaporated until it is completely dry. After drying, the extract will be further ground into a powder and mixed with distilled water. Gentamicin Stock solution was prepared by adding 10mg of Gentamicin powder in 10ml distilled water then dissolved thoroughly. This solution was autoclaved at 121oC for 15 minutes at 15lb pressure. Subsequently, the solution was kept under refrigeration at -40oC until further analysis. The antibiotic susceptibility test was carried out using *Aloe vera,* its methanol extract, and Gentamicin as the test substances. It was decided to use the micro broth dilution method on the Muller-Hinton (MH) medium for the antibiotic susceptibility test. For the MIC test, a dilution of 1:1000 was produced. To do this, 6 ml of Muller Hinton (MH) medium was mixed with 6 µl of bacterial culture in Tryptic Soy Broth (TSB). The minimal inhibitory concentration of Aloe vera, its methanol extract, and Gentamicin against *Staphylococcus aureus* and *Escherichia coli* was determined using 96-well plates. 100µl of concentration was added to all wells, then 80 µl of cultured was added to the first and second wells, with the first well remaining as

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the control of the micro-titer and 20 µl of *Aloe vera* being added to the second well, then 100µl of *Aloe vera* concentration was taken from the second well and added to the third well of the micro-titer, and so on respectively. The isolates were treated with Gentamicin at a concentration of 10mg/10ml (stock solution) to eradicate them. Following that, the methanol extract and Gentamicin were subjected to the same MIC testing method. The MIC plates were incubated at 37°C for a whole night. It was therefore necessary to record the turbidity break points in each well to determine the minimal inhibitory activity of Aloe vera, its methanol extract, and Gentamicin [13]. The turbidity/cloudy appearance in cultured wells was used to determine the breakpoints when bacterial growth was stopped.These breakpoints were referred to as the minimal inhibitory concentrations (MIC) for pure Aloe vera, its methanol extract, and Gentamicin. The collected data were tabulated and analyzed by using one-way ANOVA through the statistical 8.1 version.

RESULTS

A total of 50 broiler meat samples were collected and examined. Out of 50 milk samples, 19 (38%) were found positive for *Staphylococcus aureus*, 24 (48%) were found positive for *Escherichia coli*, and mixed colonies were found 7 (14%) positive in meat samples. The different concentrations of pure Aloe vera were used to check the susceptibility of isolated organisms. The results indicated the susceptibility of *Staphylococcus aureus* at 20 and 10µg/µl, while lower concentrations of pure Aloe vera were found resistant. Whereas *Escherichia coli* growth stopped at 20, µg/µl while concentrations lowered than this, the organism showed resistance. Statistical analysis showed a ($p<0.05$) difference in MIC between both isolated organisms against pure Aloe vera (Figure 1).

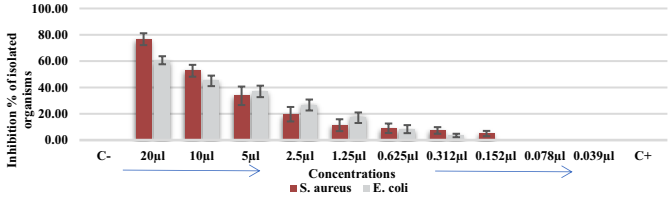

Figure 1: Growth Inhibition of *Staphylococcus Aureus* and Escherichia coli Towards Concentrations of Pure Aloe Vera (20 to 0.039µg/µl)

The various concentrations of methanol extract of Aloe vera were used. The susceptibility of *Staphylococcus aureus* at 20, 10, and 5µg/µl, while lower concentrations of methanol extract of Aloe vera were found resistant. Whereas *Escherichia coli* growth halted at 20, 10 µg/µl, while concentrations lowered than this, the organism showed resistance. Statistical analysis showed ($p<0.05$) a difference in MIC between both isolated organisms against methanol extract of Aloe vera (Figure 2).

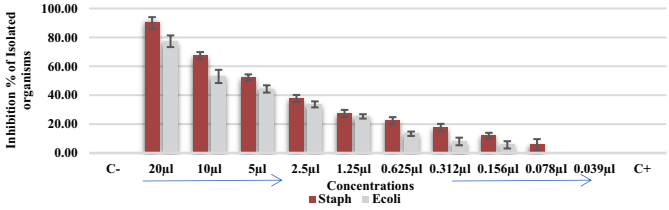

Figure 2: Growth Inhibition of *Staphylococcus Aureus* and *Escherichia Coli* Towards Concentrations of Methanol extract of Aloe vera (20 to 0.039µg/µl)

Different concentrations of gentamicin were used. The *Staphylococcus aureus* obtained susceptibility at 20, 10, 5, 2.5, 1.25 µg/µl, while lower concentrations of gentamicin were found resistant. Whereas Escherichia coli growth was hampered at 20, 10, 5, 2.5, 1.25, 0.625, and 0.312 µg/µl while concentrations lowered than this, the organism showed resistance. Statistical analysis showed a ($p<0.05$) difference of MIC between both isolated organisms against gentamicin (Figure 3).

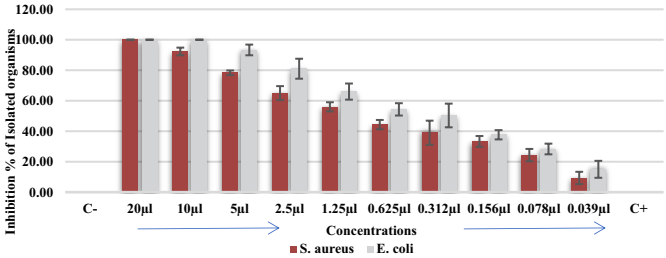

Figure 3: Growth inhibition of *Staphylococcus aureus* and *Escherichia coli* towards concentrations of gentamicin (20 to 0.039µg/µl)

DISCUSSION

The result of the current study is following the previously reported studies that showed bacteriological investigations from 604 samples of raw meat, 601 samples of frozen meat, and 645 samples of ready-to-eat meat, among other sources. S. aureus positive samples were found in all 39 cities, according to both qualitative and quantitative techniques S. aureus-positive samples, as well as *E. coli*-positive samples. The presence of Staphylococcus aureus was identified in 35.0 percent (647/1,850) of the samples. In addition, *E. coli* was found in 48 percent (634/1850) of tested samples. The quantities of the presence of *E. coli* in retail meat were found to be in the high range [14]. The isolated organisms were identified based on their physical characteristics, as well as their culture and staining responses. Furthermore, the biochemical responses of these organisms were used to establish their identity. Our present study agrees with the previous studies in which the author did prevalence studies of microorganisms associated with poultry meat. The most frequent organisms are recorded from poultry meat in a high rate of *Escherichia coli* with 66 (68.7%) and

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Staphylococcus aureus (29.2%) and other frequent mixed colonies were found 46 (47.9%) positive. Further, it was observed that *E. coli* and *Staphylococcus* remained the predominant cause of contaminated poultry meat [15]. It was observed in the present study that the minimum inhibitory concentration of pure Aloe vera at which the growth of *S. aureus* and *E. coli* were inhibited was 20µl and 10µl, respectively. The findings of the current study are also supported by a previous study in which it was reported that *Aloe vera* showed antibacterial efficacy against a wide range of bacteria gram-positive as well as gram-negative. The antibacterial agents of *Aloe vera* were suggested to kill or prevent the growth of *S. aureus, E. coli* [16]. The current study showed agreement with a previous study that *Aloe vera* has antibacterial activity because *Aloe vera* contains some antibacterial substances i.e, aloin, fumaric acid, and anthraquinone [17, 18]. The current study showed agreement with a previous study in which it was found that Methanol extract of *Aloe vera* has great antibacterial activity against both tested microorganisms as compared to other extracts i.e: ether and petroleum with a high zone of inhibition against gram-positive *Staphylococcus aureus,* and moderate zone of inhibition against gram-negative *Escherichia coli*. The Aloe vera gel extracts were shown to have moderate to high antimicrobial effects on both gram-negative and gram-positive bacteria when compared to the other extracts evaluated for antibacterial activity [19]. The recent study showed agreement with the previous findings in which it has been reported that gentamicin is a broad-spectrum aminoglycoside antibiotic which effective in a wide range of gram-positive and gram-negative pathogens by producing bactericidal action [10]. The finding of the present study has been supported by previous studies that Gentamicin is the most frequently used aminoglycoside antibiotic which showed antibacterial action against moderate to severe bacterial infections caused by sensitive agents, including *Escherichia coli* and *Staphylococcus aureus* which produce different moderate to severe bacterial infections like other aminoglycosides. Additionally, the present study is by previous study explained that gentamicin was found effective antibiotic with the MIC standards of 1.56 µg/mL, 6.25 µg/mL, 3.13 µg/mL, 6.25 µg/mL against *S. aureus,* P. aeruginosa, *E. coli* and B. cereus, respectively [20]. Although, this antibiotic is termed as bactericidal. Thus, in the present study, Gentamicin produced a significantly lower minimum inhibitory concentration as compared to pure *Aloe vera* and its methanol extract. Consequently, it is concluded from this study that *Escherichia coli* is more prevalent than *Staphylococcus aureus* in broiler meat samples. The average susceptibility of both organisms is 1.25 µg/µl and 0.312 µg/µl to Gentamicin through MIC. However, the antibacterial activity of Gentamicin is significantly higher than pure Aloe vera and its methanol extract.

CONCLUSIONS

It was concluded that all three treatments i.e., pure *Aloe vera*, its methanol extract, and gentamicin inhibited the growth of isolated bacterial organisms, but the methanol extract halted the growth of isolated organisms at lowered concentration in comparison to pure *Aloe vera. Escherichia coli* is more prevalent than *Staphylococcus aureus* in broiler meat. Growth inhibition of *Staphylococcus aureus* was observed at lower concentrations as compared to *Escherichia coli* following the usage of various treatments. Gentamicin exhibited MIC at a lowered concentration in comparison to other used treatments and *Escherichia coli* remained more susceptible to it.

Authors Contribution

Conceptualization: RAK, SB, RB
Methodology: RAK, MAJ
Formal analysis: MT, IJ, NAJ, FJ
Writing review and editing: SB, RB, MB

All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

All the authors declare no conflict of interest.

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