Aloe vera extract: A novel antimicrobial and antibiofilm against methicillin resistant Staphylococcus aureus strains

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Abstract: Bacterial infectious maladies are the leading cause of death worldwide. Microbial drug resistance boosts the severity of the maladies. In the present study, the antibacterial impact of Aloe vera leaf aqueous extract against six strains of methicillin resistant Staphylococcus aureus (LN872136, LN872137, LN871238, LN871239, LN871240, LN871241) was investigated in vitro. The effect of different concentrations (5-20mg/ml) of the plant extract on bacterial growth was evaluated by estimating the dry weight of bacterial biomass obtained from cultures at 24 and 48 h after exposure to the plant extract. The results revealed that the plant extract at concentrations of 15-20mg/ml, markedly reduced the dry weights of most S. aureus strains after 24 and/or 48 h exposure periods. The effects of the plant extract (20mg/ml) on the inhibition zones and biofilm formation by S. aureus six strains were also investigated. The largest inhibition zone was recorded against S. aureus (LN871241) and confirmed by scanning electron microscope. The plant extract could also block the biofilm formation by most S. aureus strains. In conclusion, the current results may support the use of A. vera extract as antibacterial agent against methicillin resistant S. aureus infections.

Keywords: Aloe vera, Staphylococcus aureus, methicillin resistant, biofilm.

INTRODUCTION

The extensive use of antibacterial drugs for prophylaxis or treatment of human infections has caused the spread of multidrug resistant bacterial strains. These strains are considered a serious threat to human health (Goulda et al., 2012). Currently, most nosocomial and community-acquired infections as well as the thirteen million deaths occurring worldwide are clearly associated to the multidrug resistant bacterial strains (Lushniak, 2014). Methicillin resistant Staphylococcus aureus (MRSA) are antimicrobial resistant bacteria that have been disseminated worldwide, showing resistance to many classes of antibiotics (Ness, 2010). MRSA infection often occurs endogenously through mucocutaneous boundary due to injury, skin ailments, intravenous injection, etc. This can cause wound infection and abscesses, skin and urinary infections. Spread of MRSA into the blood can cause sepsis and serious metastatic infections including pericarditis, myocarditis, pneumonia, meningitis and osteomyelitis (Goulda et al., 2012). The mechanization of antimicrobial resistance to drugs involves low absorption and tendency to biofilm formation (Nikaido, 1989). Elimination of this pathogen is always difficult because of its cells have the ability to bind together and form biofilms. Bacterial biofilm is a viscous gel, consists of organic compounds, including polysaccharides and proteins that can attach on various surfaces (Oral et al., 2010). Many chemical agents have poor ability to eradicate this pathogen because they cannot penetrate into the biofilm and hydrolyze the biofilm matrix (Oral et al., 2010).

The serious impact of multidrug resistant pathogens such as MRSA in human health supports the need of new therapeutic alternative agents than the currently available drugs. Accordingly, medicinal plants are still the main source of bioactive compounds with antimicrobial properties, including potential activity against biofilm formation and can be utilized as alternative therapies for treatment of microbial diseases (Da Silva et al., 2014; Bazargani and Rohloff, 2016).

Aloe vera (Aloe barbadensis Miller, family Liliaceae) is a succulent plant of a therapeutic importance (Huseini et al., 2012). It has been used in a traditional medicine as a remedy for several horridness. The leaves of A. vera contain active constituents, including anthraquinones, sterols, acemannan, vitamins and amino acids (Verma, 2011). The majority of these constituents possess many pharmacological properties, such as immunomodulatory (Budai et al., 2013), antidiabetic, hypolipidemic (Huseini et al., 2012), antioxidant (Kammoun et al., 2011), antiviral (Li et al., 2014) and antimicrobial (Pandey and Mishra, 2010) activities. Different extracts from A. vera leaf gel were reported to exhibit suppressing impact on the growth of several microbial pathogens, including Bacillus subtilius, Helicobacter pylori, Escherichia coli, Salmonella typhi and Staphylococcus epidermidis (Irshad et al., 2011, Cellini et al., 2014). Other authors demonstrated that the methanolic leaf gel extract possess strong growth inhibiting impact on gram positive (S epidermidis and S. aureus) and gram negative bacteria (E coli, P. aeruginosa, K pneumonia, Proteus vulgaris and Proteus mirabilis) (Udghire and Pathade, 2014).

The current study aimed to evaluate the antibacterial and antibiofilm impacts of an aqueous extract of A. vera
leaves against six strains of methicillin resistant \textit{S. aureus} using an \textit{in vitro} assay.

\section*{MATERIALS AND METHODS}

\textbf{Plant}

\textit{A. vera}

The leaves of \textit{A. vera} were collected from the garden of King Abdulaziz University, Jeddah, Saudi Arabia. The plant leaves were identified by a taxonomist in the Department of Biological Science, King Abdulaziz University, Jeddah, Saudi Arabia.

\textbf{Preparation of \textit{A. vera} leaf aqueous extract}

Fresh leaves of \textit{A. vera} were washed with a running tap water to remove all dust particles and rinsed in sterile distilled water. The leaves were then cut longitudinally and the colorless gel was removed without the leaf fibers. 50g of \textit{A. vera} leaves without the gel were dried at room temperature and mixed with distilled water (100ml) in a blender for ten minutes. The extract was filtered utilizing muslin cloth, then centrifuged at 3000 rpm for thirty minutes. The supernatant was collected and then dried by lyophilization (Devi \textit{et al.}, 2012).

\textbf{Bacteria}

Six strains of methicillin- resistant \textit{S. aureus} (MRSA, Gram +ve, resistant to ampicillin, tetracycline, vancomycin, amoxicillin and penicillin) were isolated from clinical cases with corneal infections. Identification and characterization of \textit{S. aureus} were carried in accordance to microscopic and colonial morphology, catalase and coagulase tests (Udo \textit{et al.}, 2006). All \textit{S. aureus} strains were examined for methicillin resistance. The disk diffusion assay was used with a 1 µg oxacillin disk (Oxoid) (NCCLS, 1993). Zone sizes were recorded for 24 hours after incubation at 37°C. Strains with zone sizes ≤ 8 mm were regarded as methicillin resistant (Abu-Shanab \textit{et al.}, 2006). The different \textit{S. aureus} strains were identified by analysis of 16S ribosomal RNA (16S r RNA) genes for bacterial species (Chanama 1999).

\textbf{Bacterial growth culture}

23 g of Mueller Hinton Agar were dissolved in one liter of bidistilled water and sterilized in an autoclave for 15 min, then cooled in water bath at 50°C. 25 ml of agar was poured into sterile glass plates with half-open lid (90 mm diameter Greiner Laborteknik, UK) to avoid condensation and then kept at 4°C. Bacteria various strains were streaked onto nutrient agar petri dishes to get isolated purified colonies. The bacterial plates were then incubated at 37°C for about 17 hours.

\textbf{Antimicrobial assay of \textit{A. vera} leaf extract}

\textit{A-Impact of the plant extract on the biomass of \textit{S. aureus} strains}

The effect of different concentrations of the plant extract on bacteria growth was determined by estimating the dry matter of bacterial biomass obtained from cultures at 24 and 48 h after inoculation. The weight of dry matter content of bacterial biomass is proportional to the number of bacteria. Therefore, the decrease in the weight of the dry matter of bacterial biomass is indicative to the decrease in the bacterial number resulted from inhibition of bacterial growth. For each bacterial strain, four sterile glass tubes of MRS broth growth medium, containing serial dilution of plant extract (5-20mg/ml) were prepared. The solutions were then vortexed, and each sample was inoculated with 500 mm³ (10^6 CFU/ml) of the bacterial suspension. Negative control was represented by the bacterial culture without the addition of the plant extract. The cultures, after 24 and 48 incubation periods, were centrifuged at 10,000 rpm for 15 min. to obtain the cell pellet. The pellets of bacterial different strains were dried at 80°C till a constant weight was obtained, then cooled in desiccators and weighed. All tests were performed in triplicate. The bacterial biomass was expressed as mg dry weight/ml (Samanta \textit{et al.}, 2008).

\textbf{Effect of the plant extract on bacterial inhibition zone}

Six strains of methicillin-resistant \textit{S. aureus} were grown at 37°C in Mueller Hinton Agar for 18 hours. Suspensions of different strains of the tested bacteria were made in a sterile normal saline. The agar–well diffusion method was performed for determination the antibacterial potential of the plant extract (Perez \textit{et al.}, 1990). Suspensions of \textit{S. aureus} six strains, 10^6 colony forming unit/ml (CFU/ml) each, were spread on Mueller Hinton Agar medium. Wells, with diameter of 10 mm, were digged in the agar medium and placed onto the incubated plates. \textit{A. vera} leaf extract was dissolved in a bidistilled water (20mg/ml) and added into the labeled medium plates. Controls of bacterial strains without the plant leaf extract were also involved in the experiment. The plates were then kept at 4°C for 2h to allow maximum diffusion of the extract. The tested dishes were then incubated for 24 hours at 37°C. The sensibility of \textit{S. aureus} different strains to the plant extract was assayed by measuring the diameter of zone of inhibition (mm) produced after incubation. The experiment was carried out in triplicate for each \textit{S. aureus} strain and the average of inhibition zone was calculated (Agarry \textit{et al.}, 2005).

\textbf{Effect of \textit{A. vera} leaf extract on MRSA biofilm formation.}

The impact of the plant extract (20mg/ml) on biofilm formation by different strains of MRSA was assayed according to Sandasi \textit{et al.} (2010). The assay method was carried out in a sterile flat-bottom 96-well polystyrene tissue culture plate (Minitek, USA). \textit{S. aureus} strains were assayed individually for biofilm formation by mixing 40µL suspensions of \textit{S. aureus} strains (10^6 CFU/ml) with 230 µL of tryptone soy broth (TSB) in the wells of tissue culture dish and then incubated at 37°C for 4 hours to permit cell adhesion and formation of biofilm. After the biofilm formation, the plant extract (20mg/ml) was added.
to the wells. The negative control wells were filled with the bacterial biofilm without the plant extract. The culture plate was then incubated at 37°C under immobile state for 48h. The contents of the wells were removed and washed three times with PBS, and fixed by drying for one hour at 37°C. The wells were stained after drying with 0.1% crystal violet stain (100µL) for 15 minutes, and then washed off with running tap water to remove the excess stain. After the plates were dried at room temperature, the dye bound to the attached cells was extracted with 250 µL glacial acetic acid (33%, v/v) per well. The absorbance (Ab) of each well was recorded spectrophotometrically at 570 nm. The percentage of inhibition of bacterial biofilm was determined for each bacterial strain. The assay method was carried out in triplicate (three times) for each S. aureus strain.

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\text{Percentage of inhibition} \% = \frac{\text{Ab (Negative control)} - \text{Ab (Experimental)}}{\text{Ab (Negative control)}} \times 100
\]

**Scanning electron microscopy (SEM)**

SEM was carried out on the most sensitive S. aureus strain (LN871241) to the extract. Cells of S. aureus LN871241 in nutrient broth media were treated with the plant extract (20mg/ml) and incubated at 37±2°C for 24h. Bacterial cells without plant extract were served as controls. The bacterial cells were collected by centrifugation and washed with a sterile phosphate buffered saline (PBS). The bacterial cells were fixed in 2.5% glutaraldehyde and phosphate buffer (0.1M, pH 7.3), at 4°C overnight, and then fixed in osmium tetroxide (1%) in the phosphate buffer for 1 h at room temperature before processing for SEM (XL20-Philips).

**STATISTICAL ANALYSIS**

The results were represented as mean ± standard deviations (SD). Significant differences between values were calculated utilizing One-Way ANOVA, followed by Bonferroni’s test as post ANOVA test. Differences were regarded significant at p ≤ 0.05.

**RESULTS**

**Effect of A. vera extract on the biomass of S. aureus bacterial strains**

The efficacy of various concentrations of A. vera aqueous extract on the biomasses of S. aureus different strains in terms of their dry weights are shown in figs. 1-6 respectively. The results demonstrated that treatment of S. aureus (LN872136) with different concentrations (5-20 mg/ml) of A. vera extract, markedly reduced the weight of the bacterial strain after 24h and 48h versus the control counterpart strain (Zero concentration, fig. 1).

![Fig. 1](image1.png)

**Fig. 1:** Effect of various concentrations of A. vera extract on the dry weight of S. aureus (LN872136). Data are expressed as mean ± SD of triplicate independent experiments. *P≤0.05, **P≤0.01 compared with the control untreated strain (zero concentration) after 24h exposure to the plant extract, aP ≤ 0.05, bP ≤ 0.01 compared with the control untreated strain after 48h exposure to the plant extract.

![Fig. 2](image2.png)

**Fig. 2:** Effect of various concentrations of A. vera extract on the dry weight of S. aureus (LN872137). Data are expressed as mean ± SD of triplicate independent experiments. Non-significant change in the weight of the bacterial strain exposed to different concentrations of the plant extract after 24h or 48h compared with the control counterpart untreated strain (zero concentration).

Fig. 2 demonstrated that different concentrations of A. vera extract showed no significant effect on S. aureus (LN872137) compared with the control untreated strain. The results also demonstrated that the plant extract at 15-20 mg/ml, was effective in decreasing the biomass of S. aureus (LN872138) after 24h exposure, meanwhile the different concentrations of this extract (5-20mg/ml) were more effective in reducing the biomass of this bacterial strain after 48h with respect to the control untreated strain (fig. 3).
The inhibiting impact of *A. vera* extract on the growth of *S. aureus* (LN871239) is illustrated in fig. 4. The result indicated that the extract at 15-20mg/ml significantly reduce the weight of this strain after 24h exposure period, however the extract at 10-20 mg/ml markedly reduce the weight of this strain after 48h exposure period in relation to the control untreated counterpart strain.

**Fig. 3**: Effect of various concentrations of *A. vem* on dry weight of *S. aureus* (LN871238). Data are expressed as mean ± SD of triplicate independent experiments. *P<0.05 compared with the control untreated strain (zero concentration) after 24h exposure to the plant extract, *aP*≤0.05, *bP*≤0.01 compared with control untreated strain after 48h exposure to the extract.

The effect of exposure of *S. aureus* (LN872140) to different concentrations of *A. vera* extract on its dry weight is demonstrated in fig. 5. From the fig. it can be noticed that the plant extract was effective in reducing the biomass of the bacterial strain at a concentration of 20mg/ml after 48 h exposure. The result of fig. 6 showed that the plant extract at the used different concentrations (10-20 mg/ml), was effective in reducing the dry weight of *S. aureus* (LN871241) after 24h or 48h exposure period. The plant extract at 20mg/ml was more effective in reducing the biomass of this strain after 24 and 48 h exposure.

**Impact of A. vera extract on the growth of S. aureus strains in terms of inhibition zone**

The suppressing impact of *A. vera* aqueous extract (20mg/ml) on the growth of *S. aureus* strains in terms of zone of inhibition is illustrated in table 1 and fig. 7. The data revealed that the plant extract could suppress the growth of *S. aureus* different strains. The extract mostly inhibited the growth of *S. aureus* (LN871241) and (LN871238) as they showed the highest inhibition zones (23.33±2.9 and 22.00±0.86 mm respectively) followed by *S. aureus*, (LN872136), (LN871239) and (LN872140). However, the plant extract was less efficient against *S. aureus* (LN872137) as it recorded minimum inhibition zone (10.33±2.08), in relation with the inhibition zones of other strains.

**Fig. 4**: Effect of various concentrations of *A. vera* on dry weight of *S. aureus* (LN871239). Data are expressed as mean ± SD of triplicate independent experiments. 

**Fig. 5**: Effect of various concentrations of *A. vera* on the dry weight of *S. aureus* (LN872140). Data are expressed as mean ± SD of triplicate independent experiments. Non significant changes in weight of the bacterial strain exposed to different concentrations of the plant extract after 24h compared with control counterpart (zero concentration). *P* ≤ 0.05 compared with the control after 48 h exposure to the plant extract.

**Biofilm inhibiting activity of A. vera extract by S. aureus strains**

The biofilm suppressing activity of *A. vera* extract by *S. aureus* strains is depicted in table 2. Result demonstrated that the plant extract (20mg/ml) could inhibit the biofilm formation by *S. aureus* different strains. The plant extract obviously suppressed the biofilm formation by *S. aureus* different strains as they showed highest percentage of inhibition (100%, 95% and 80% respectively) compared with the other strains.
Scanning electron microscope observation (SEM)
The most sensitive *S. aureus* strain (LN871241) to *A. vera* aqueous extract was selected to study the damaging impact of the this plant extract on its surface morphology using SEM. This investigation illustrated that treatment of this bacterial strain with the plant extract caused disorganization in the microstructure of the bacterial cells (fig. 8 B and C) which represented by separation of the cell wall of some bacterial cells or damage of the cell wall with the formation of wrinkles in other cells. A decrease in the diameter of the bacterial cells was also observed compared to the normal untreated bacteria which showed normal morphology (fig. 8 A).

**Fig. 6:** Effect of various concentrations of *A. vera* on the dry weight of *S. aureus* (LN871241). Data are expressed as mean ± SD of triplicate independent experiments. *P*≤0.05, **P**≤0.01 compared with the control untreated strain (zero concentration) after 24h exposure to the plant extract, , *P* ≤ 0.05, **P** ≤ 0.001 compared with the control untreated strain after 48h exposure to the plant extract.

**DISCUSSION**

Antimicrobial resistance is a public health serious problem worldwide that has led to the development of nosocomial and community acquired infections. This presents a therapeutic challenge for controlling these infections. Natural plant extracts with antimicrobial activity may provide a source to develop a new alternative therapy for the treatment of microbial infection related diseases (Scott *et al.*, 2009).

The current study investigated the potential antimicrobial impact of *A. vera* leaf aqueous extract on six strains of methicillin-resistant *S. aureus in vitro*. The suppressing effect of the plant extract on the growth of the bacterial strains was investigated in terms of the dry weight of bacterial biomass and the inhibition zone. The result showed that the plant extract at 10-20mg/ml could significantly inhibit the growth of most bacterial strains after 24 and / or 48 h as indicated by the reduction in their dry weights compared with the control untreated counterpart strains. *S. aureus* (LN871241), (LN871238) and (LN872136) were the most sensitive strains to the plant extract at concentrations of 15-20mg/ml. The decrease in the dry weight of the bacterial biomass by the used plant extract may indicate the potential inhibitory effect of this extract on the growth of the tested bacterial strains. The antibacterial impact of *A. vera* extract on the growth of *S. aureus* strains in term of inhibition zone was carried out at a concentration of 20mg/ ml as it was the most effective concentration in reducing the biomasses of *S. aureus* strains. The result showed that the plant extract markedly suppressed the growth of different strains of *S. aureus*. *S. aureus* (LN871241) and (LN871238) were the most affected strains by the plant extract as they recorded the highest inhibition zones (23.33±2.9 and 21.00±1.00 mm respectively) followed by *S. aureus* (LN872136), (LN871239) and (LN872140).

**Fig. 7:** Effect of *A. vera* leaf extract on the growth of *S. aureus* showing the inhibition zones of the different strains, (A) inhibition zone of *S. aureus* (LN872136); (B) inhibition zone of *S. aureus* (LN872137); (C) inhibition zone of *S. aureus* (LN871238); (D) inhibition zone of (LN871239); (E) inhibition zone of *S. aureus* (LN872140); (F) inhibition zone of *S. aureus* (LN871241).

However, the plant extract was less effective against *S. aureus* (LN872137) as it recorded minimum inhibition zone) compared with the inhibition zones of other strains. Baur (1996) stated that the pathogen is resistant if the inhibition zone is less than 8.00 mm and sensitive if it is greater than 11.00 mm. According to this author, most of
the *S. aureus* strains presented in the current study were considered sensitive to *A. vera* extract. The useful antimicrobial effect of *A. vera* extract is similar to previous studies. Former authors reported that *Aloe* extracts and leaf gel extract possess antibacterial properties against gram negative and gram positive bacteria, including *S. aureus* (Habeeb et al., 2007; Pandey and Mishra, 2010; Pandey et al., 2010; Udgire and Pathade, 2014). In addition, in vivo study revealed that extract from *A. vera* peel has a potential antimicrobial action (Kwon et al., 2011). A report by an in vivo study showed that *A. vera* peel extract has immunomodulatory effect and could stimulate the production of specific antibodies such as IgG and IgA which have a key role in the degeneration of bacteria (Kwon et al., 2011). IgA plays a fundamental role in mucosal immunity and provide a protective role against microbial infection in the intestinal lumen (Johal et al., 2004), meanwhile, IgG can protect against microbial toxins and surface proteins (Pechine et al., 2005). The antimicrobial impact of *A. vera* extract presented in the current study may attribute to the active phytochemical compounds of this extract. The protein extracted from the gel of *A. vera* leaves was reported to have a potential fungicidal activity against *Candida parapsilosis, Candida krusei,* and *Candida albicans* (Pandey and Mishra, 2010).

*A. vera* leaves have an active compound, namely anthraquinones with antimicrobial and immunomodulatory properties and could attenuate bacterial growth (Pandey, 2010). This compound has the ability to inhibit the synthesis of bacterial protein by blocking the ribosomal A site (where the aminoaacylated tRNA enters). Polysaccharides of *A. vera* leaves stimulate the phagocytic activities of leukocytes to destroy bacteria (Pugh et al., 2001). Also phyto-chemical investigation of aqueous extract of *A. vera* showed the presence of tannins and flavonoids (Gangadharan et al., 2014). These phyto-constituents are known to have potential antimicrobial activities. Tannins can bind to proline-rich proteins, forming irreversible complexes, leading to the suppression of cellular protein synthesis (Hagerman and Butler, 1981). Flavonoids have been shown to have strong antibacterial against *S. sobrinus,* *A. vera* and *Enterococcus faecalis* strains (Taleb-Contini et al., 2003). In addition, it has been reported that *A. vera* contains a hydroxylated phenol (pyrocatechol), known to have toxic effect on microbes (Cowen, 1999). Another investigation illustrated that the inner gel of *A. vera* shows antibacterial potential action against both sensitive and resistant *Helicobacter pylori* strains (Cellini et al., 2014). The antibacterial ability of these plant compounds against antimicrobial resistant strains may support their beneficial to be developed into effective antimicrobial therapy against multidrug resistant bacteria.

On studying the inhibitory effect of the plant extract on biofilm formation by *S. aureus* different strains, the results showed that the extract has different effects on the development of biofilm by bacterial strains. The plant extract was successful in inhibiting the development of biofilm by *S. aureus* (LN871241), (LN871238) and (LN872136) as the plant extract recorded the highest percentage of biofilm inhibition by these strains. The potential impact of the plant extract against biofilm formation by these bacterial strains may ascribe to the ability of the extract to penetrate and degrade the bacterial biofilm. The less inhibitory effects of the plant extract on biofilm formation by other stains, including (LN871239), (LN872137) and (LN872140), may be attributed to that the bacterial cells of these strains in a biofilm are less sensitive to plant extract. These results are coped with those found previously by other plant extracts. Bazargani and Rohloff, (2016) have reported that the plant extracts of peppermint (*Mentha × piperita* L.), coriander (*Coriandrum sativum* L.), and anise (*Pimpinella anisum* L.) have a potential activity against biofilm formed by *S. aureus* and could inhibit bacteria cell attachment. Biofilm inhibitory effect of *A. vera* extracts against microbial biofilm formation was also documented (Rehman et al., 2016).

**Fig. 8:** Scanning electron micrographs of *S. aureus* (LN872141) bacterial cells treated with *A. vera* leaf extract at 37°C for 24 h, (A) showing normal shape of *S. aureus* (LN872141). (B) and (C) micrograph of the bacterial strain exposed to *A. vera* extract showing abnormal morphology, including rough of the cell wall due to wrinkles (B), separation of cell wall and a decrease in the size of the bacterial cell diameter (C).
bacterial cells by destroying their membranes. This result is supported by some authors declared that the cell wall of bacteria is the target for antimicrobial drugs (Phansri et al., 2011)

**CONCLUSION**

The current investigation, illustrated that aqueous extract of A. vera leaves have potential antibacterial effect against methicillin-resistant S. aureus (MRSA) strains and could be use as an alternative remedy to treat MRSA infections

**REFERENCES**


