



Antimicrobial Properties of Aloe Vera Gel Extracts against Bacterial Isolates from Wound of Donkey

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ABSTRACT

Despite the exploration of antibacterial activity of the Aloe Vera in different families of animals. However, very limited information is documented about antibacterial activity of Aloe Vera in equines. The present study was designed to check the antibacterial activity of the Aloe Vera against most pathogens found in the wounds of donkey. For this purpose, two different concentrations 30 µl and 60 µl crude as well as ethanol extract of Aloe Vera were used. During this experiment, total 50 wound samples were collected from donkey wounds. All 50 samples were found contaminated with different bacterial organism, among all those 45, 35, 33, and 25 were recorded positive for *S. aureus*, *E. coli*, *Shigella* and *Salmonella* spp respectively. However, out of 50 sample, 34 (68%) were found to have different bacterial species and 16 (32%) were recorded with pure contamination. At 30 µl of crude Aloe Vera isolated bacterial organisms did not show any susceptibility, whereas at 60 µl, organisms showed quit sensitive reactions. In contrast, ethanol extract of Aloe Vera showed better result as compared to crude Aloe Vera at both concentration i-e 30 µl and 60 µl. Among isolates, *S. aureus* showed high sensitivity (15-22 mm), followed by *E. coli* (14-18 mm), *Shigella* (13-15) and *Salmonella* spp (11-14 mm) both concentrations. The results of this study revealed that ethanol extract of Aloe Vera showed better antibacterial activity against all isolates as compared to pure Aloe Vera extract at both concentrations used, due to its high polarity.

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Authors' Contribution

SA, RB, SB and AHS conceived, designed and performed the experiments. JS, QK and SAF interpreted the data. SA, RB and WAV wrote the manuscript.

Key words

Aloe vera, Antibacterial activity, Donkey, Gel, Wound

INTRODUCTION

Aloe vera (*Aloe barbadensis miller*) is a cactus like xerophytes plant and about 360 species of this plant have been identified so far. It has been named due to its therapeutic effectiveness among all tested species. It is cultivated in warm climatic areas of the world. Aloe vera (AV) has been known as “secret plant” because it contains photochemical, vitamins and nutrients (Maenthaisong *et al.*, 2007). This plant has elongated, pointed and fleshy leaves which consists of two parts, an outer skin (green rind or latex) and an inner pulp which is colorless mucilaginous gel (Helal *et al.*, 2003; Rodriguez *et al.*, 2005).

Aloe vera has been used for various therapeutic purposes worldwide including Japan, Greece, and China (Rajasekaran *et al.*, 2004; Parthipan, 2011). It has broad range of therapeutic effect and is used traditionally to treat variety of veterinary and human ailments (Blumenhal *et al.*, 1998).

It is used orally to combat several problems, including constipation, colitis, irritable bowel syndrome, respiratory tract disorders, cardio vascular disorders, immune system enhancement, inflammation, peptic ulcer, different bacterial and viral infection (Rajasekaran *et al.*, 2004; Chatterjee *et al.*, 2013). Topically, aloe vera has been used for the treatment of burns, sunburns, inflammatory skin disorders and wounds (Belo *et al.*, 2006; Reider *et al.*, 2005; Paulsen *et al.*, 2005). This plant recognized to have therapeutic properties used for an array of ailments including mild fever, wounds and burns, gastrointestinal ailments hyperglycemia, sexual vitality, fertility problems, cancer, immune system modulates, AIDS and different skin diseases (Rudrangshu *et al.*, 2015).

Skin wounds in donkeys (*Equus asinus*) can be suitable habitat where microorganisms can grow especially in cases of saddle sores because they accelerate the risk of infection by providing perfect conditions for the propagation of some microorganism. The classic signs of equine skin wounds include pain, erythema, edema, heat and purulent discharge. In advanced stages, other signs might be observed, which include serous exudates, delayed

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healing, discoloration, foul odor and friable granulation tissue at the base of the wound and on disruption of these wounds, there is increased pain (Wells *et al.*, 1988; Devrajani *et al.*, 2000; Bowler *et al.*, 2001).

Several studies indicated that the aloe vera has a notable effect on wound healing. To the best of our knowledge, so far very little work has been conducted on *Staphylococcus aureus*, *Escherichia coli*, *Shigella* and *Salmonella* spp. isolated from donkey wounds. Therefore, considering the importance of the donkey as a domesticated member of the horse family, *Equidae*, which is mainly used for transportation in developing countries like Pakistan, this study has been designed to check the antibacterial effect of aloe vera against *Escherichia coli*, *Staphylococcus aureus*, *Shigella* and *Salmonella* spp. with the aim to provide the cheap source of medication.

MATERIALS AND METHODS

Collection of donkey wound samples

A total of 50 wound samples were collected from veterinary clinics and nomadic animals in the vicinity of Tando jam and taken to the Department of Veterinary Pharmacology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tando Jam. All samples were collected under aseptic condition with the help of Tran'sweb tube.

Isolation and identification of pathogens

For detection of *Staphylococcus aureus* wound samples were sub cultured on the nutrient agar. Plates were observed for such as yellow golden colonies further confirmation was done by Gram's staining and biochemical reactions of the isolates.

For the detection of *E. coli*, wound samples were sub cultured on the MacConkey agar which is typical for *E. coli* growth. Plates were observed for bacterial growth which included pink colonies on MacConkey's agar and further confirmation was done by Gram's staining and biochemical test of the isolates.

For the detection of *Salmonella/Shigella* spp., wound samples were sub cultured on the *Salmonella/Shigella* agar (SS Agar), which is specific for *Salmonella* and *Shigella* spp. Growth plates were observed for typical colonies such as for *Shigella*, as transparent or translucent colorless colonies. *Shigella* spp were observed whereas colonies of *Salmonella* spp may appear with or without black centers and further confirmation was done by Gram's staining and biochemical test of the isolates.

Extraction of gel from aloe vera leaves

The aloe vera plant leaves were collected from the

local plant nursery, Hyderabad, washed with distilled water and was subjected to surface sterilization with 70% alcohol. The leaves were incised and gel was separated with the help of sterile knife. Further, the parenchymatous covering of the leaves was blended to make it homogenous and filtered with muslin cloth and sterilized in autoclave for 15 min (Agary *et al.*, 2005). The filtered gel was used as stock solution (100% concentration).

Ethanol extraction method

For ethanol extraction, fresh aloe vera gel was dried in the oven at 80 °C for 48 h and then powdered. Ten grams of this powder was dissolved in 100 ml of ethanol and left for 24 h. The contents were filtered through Whatman filter paper No.1 and filtrate was evaporated to dryness. This dried extract was further powdered and dissolved in 10 ml of distilled water and sterilized. The resultant solution was kept at 4 °C, and used as stock solution (Chatterjee *et al.*, 2013).

Disc preparation

The 6 mm (diameter) discs were prepared from Whatman No.1 filter paper. The discs were sterilized by autoclaving at 120 °C for 15 min. After sterilization, the moisture containing discs were dried in hot air oven at 50 °C for 1 h (Arunkumar and Muthuselvam, 2009). The prepared discs were kept under aseptic condition for further use.

Crude and ethanol extract of aloe vera gel sensitivity tests

To check the susceptibility of the isolated organisms against crude and ethanol extract of aloe vera at different concentrations i-e 30 µl and 60 µl, aloe vera gel was used to check susceptibility and resistance pattern of above four bacterial isolates.

The surface of Muller-Hinton agar was dried by incubating at 37 °C for 30 min. The isolated colonies were selected and dispensed in barium chloride. The sterile cotton swab was dipped in the bacterial solution and then rolled over the surface of the agar medium and evenly with bacterial suspension and placed in incubator for 30 minutes to get dried. Sterile Whatman NO.1 filter paper discs with absorbed aloe vera extracts, crude as well as ethanol extract at different concentrations i-e 30µl and 60µl, and blank/control (without any aloe vera) discs were placed on Muller Hinton agar plate with the help of sterile forceps and slightly pressed to make it adhere to the surface of the medium. The plates were closed, wrapped in polythene bag, kept inverted (medium up and disc downward) and incubated for 24 h at 37 °C.

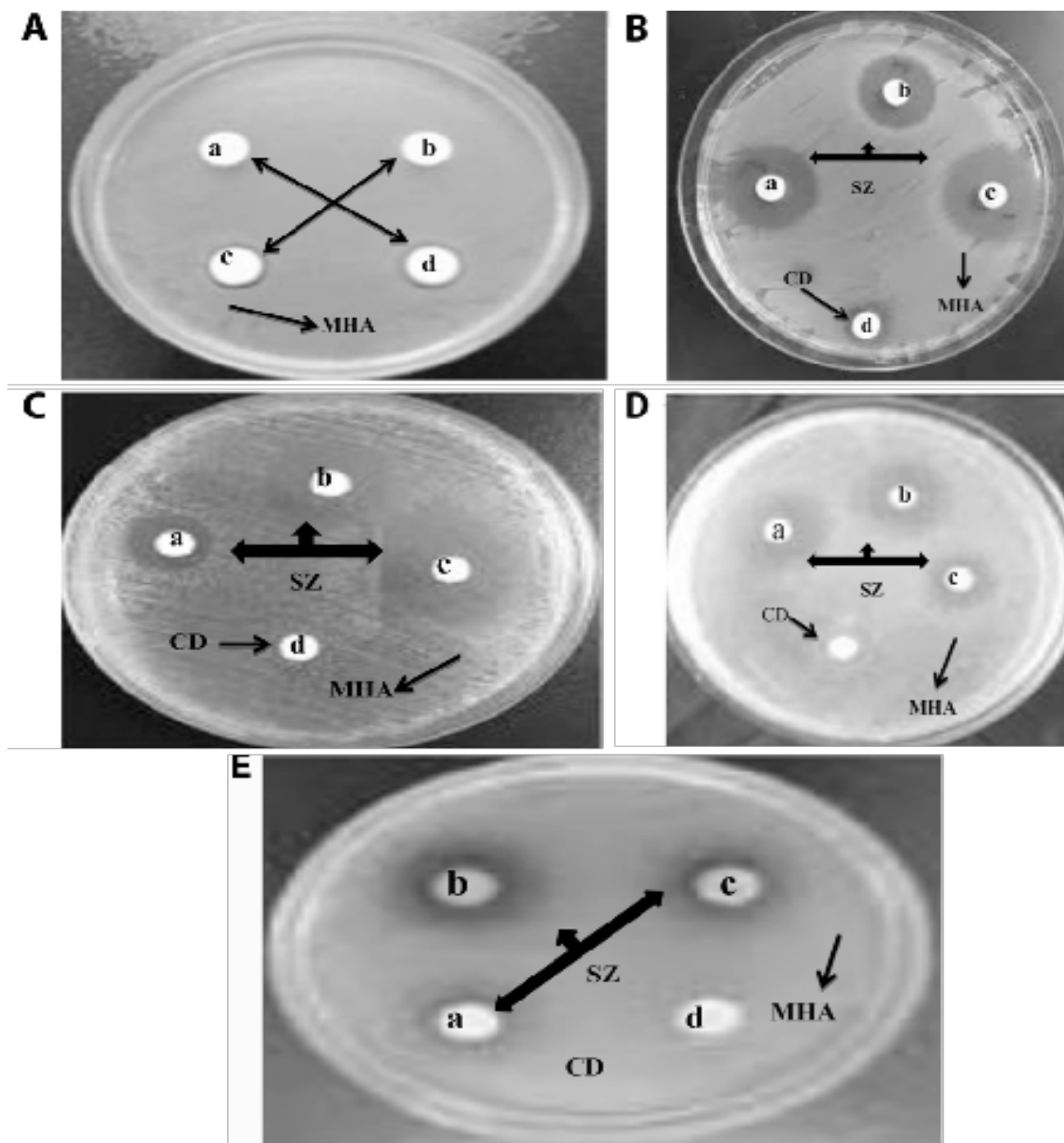


Fig. 1. Effect of crude extract of aloe vera on wounds of donkey; A. shows no sensitivity zones (SZ) produced by crude extract of aloe vera at 30µl on Muller- Hinton agar plate (MHA). B. shows sensitivity zones (SZ) produced by crude extract of aloe vera at 60µl against *Staphylococcus aureus* on Muller- Hinton agar plate a, b, c 60µl containing crude extract of aloe vera, whereas d is control. C. shows sensitivity zones (SZ) produced by 60 µl crude extract of aloe vera against *Escherichia coli* on Muller- Hinton agar plate (MHA) a,b,c, 60 µl containing crude extract of aloe vera whereas d is control. D. shows sensitivity zones (SZ) produced by at 60 µl crude extract of aloe vera against *Salmonella* on Muller- Hinton agar plate (MHA) a,b,c, 60µl containing crude extract of aloe vera whereas d is control. E. shows sensitivity zone (SZ) of inhibition produced by at 60 µl crude extract of aloe vera against *Shigella* on Muller- Hinton agar plate (MHA) a,b,c, 60µl containing crude extract of aloe vera whereas d is control.

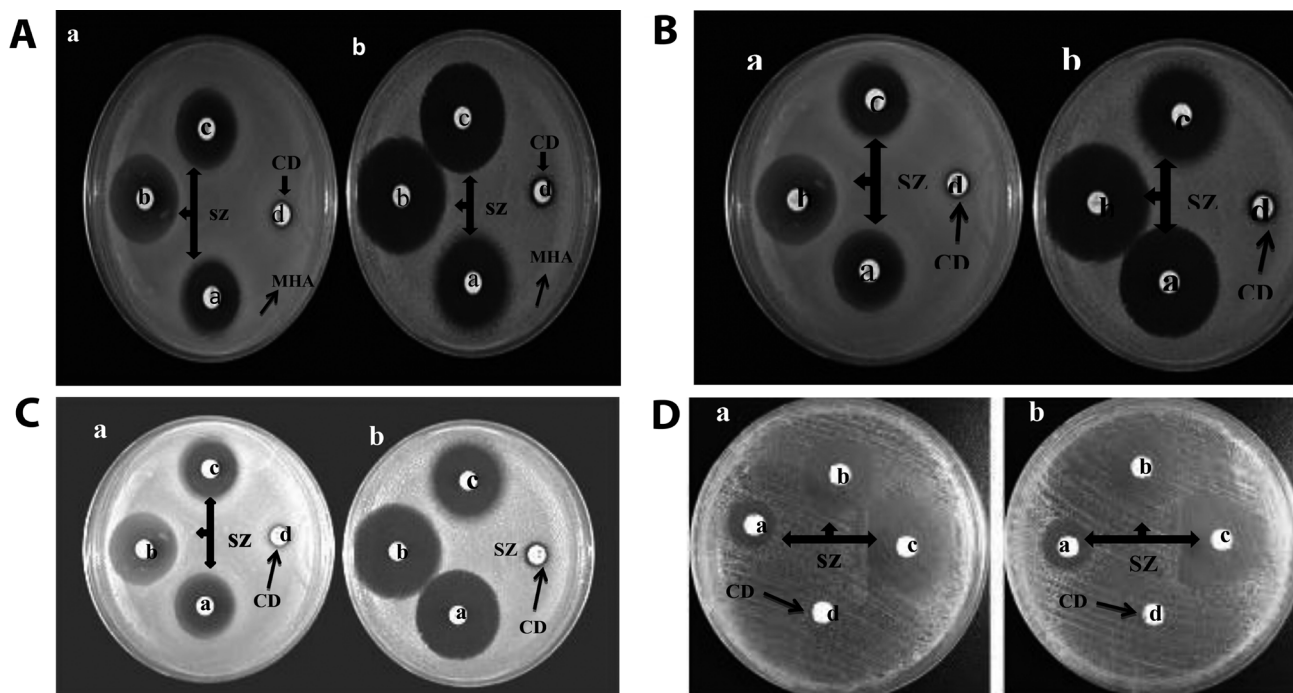


Fig. 2. Effect of ethanol extract of aloe vera on bacterial isolates from wounds of donkey; A. shows sensitivity zones (SZ) produced by ethanol extract of aloe vera at different concentrations i-e (a) 30µl and (b) 60 µl against *Staphylococcus aureus* on Muller-Hinton agar plate (MHA) a,b,c, 30 and 60µl containing ethanol extract of aloe vera whereas d is control. B. shows sensitivity zones (SZ) produced by ethanol extract of aloe vera at different concentrations i-e (a) 30µl and (b) 60 µl against *Escherichia coli* on Muller- Hinton agar plate (MHA) a,b,c 30 & 60µl containing ethanol extract of aloe vera whereas d is control. C. shows sensitivity zones (SZ) produced by ethanol extract of aloe vera at different concentrations i-e (a) 30µl and (b) 60µl against *Salmonella* on Muller- Hinton agar plate (MHA) a,b,c 30 and 60µl containing ethanol extract of aloe vera whereas d is control. D. shows sensitivity zones (SZ) produced by ethanol extract of aloe vera at different concentration i-e (a) 30µl and (b) 60 µl against *Shigella* on Muller- Hinton agar plate (MHA) a,b,c 30 and 60µl containing ethanol extract of aloe vera whereas d is control.

RESULTS AND DISCUSSION

The present study was aimed to compare the antibacterial activity of crude and ethanol extract of aloe vera at different concentrations against some common pathogenic bacterial isolated from donkey wounds.

Prevalence of bacteria isolated from wounds

A total of 50 wound samples were examined and all were recorded positive for different organisms. Out of 50 samples, 45, 35, 33 and 25 were found positive for *Staphylococcus aureus*, *Escherichia coli*, *Shigella* and *Salmonella* Spp, respectively (Table I). All organisms were identified on their morphological, cultural characteristics and staining reactions. Organisms were further confirmed by their biochemical reactions. The incidence recorded 90%, 70%, 66% and 50%, respectively. A similar type of study was done to isolate the bacterial organisms from wound samples of animals by various workers throughout world. However, the comparison of the present figures

can be compared with the results of (Rind and Khan, 2000), who isolated and characterized bacterial species from surgical and non-surgical wounds located on skins and hides of domestic animals. Their results showed, *Staphylococcus aureus* as most prevalent organisms followed by *Escherichia coli* and *Bacillus* spp isolates from buffaloes, cattle, sheep and goat wounds. Another study showed that *Staphylococcus* are the common pathogen isolates from wounds of dogs (Kelly *et al.*, 1993).

The result of the current study is in accordance with the previously reported studies that showed isolated different species of aerobic and anaerobic bacteria from skin wounds of cattle, sheep, goats and dogs and result indicated that among all organisms *Staphylococcus aureus* was a predominant species, whereas *E. coli* was on list after *Staphylococcus*. These studies showed that *S. aureus* was prevalent among all other isolates from other animals. (Kelly *et al.*, 1993; Dewani *et al.*, 2000). Different species of bacteria were isolated from cattle, sheep and goat and besides other animals *Staphylococcus*

aureus, *Streptococcus pyogenes*, *Escherichia coli* and *Micrococcus* were found most prevalent (Khan and Rind, 2001).

Table I. Percentage prevalence of bacterial organisms, and (mixed and individual bacterial species) from donkey wounds.

Bacterial species	Total no. of wound samples	No. of positive wound samples	Percentage
<i>Staphylococcus aureus</i>	50	45	90%
<i>Escherichia coli</i>	50	35	70%
<i>Shigella</i> spp	50	33	66%
<i>Salmonella</i> spp	50	25	50%
Pure bacterial spp	50	16	30%
No of contaminated samples into mixed bacterial spp	34	64	68%

Table II. Zone of inhibition produced by crude ethanol extract of aloe vera extract at different concentrations (30µl and 60 µl).

Bacterial species	30µl	Sensitivity	60µl	Sensitivity
Crude extract				
<i>Staphylococcus aureus</i>	0	--	12mm	+++
<i>Escherichia coli</i>	0	--	10mm	+++
<i>Shigella</i> spp	0	--	9mm	+++
<i>Salmonella</i> spp	0	--	7mm	+++
Ethanol extract				
<i>Staphylococcus aureus</i>	15mm	++++	22mm	++++
<i>Escherichia coli</i>	14mm	++++	18mm	++++
<i>Shigella</i> spp	13mm	++++	15mm	++++
<i>Salmonella</i> spp	11mm	++++	14mm	++++

--, no sensitivity (absence of clear zone); +, weak sensitivity (clear zone with 1-2mm); ++, moderate sensitivity (clear zone with 3-7mm); +++, quite sensitivity (clear zone with 8-11mm); +++++, highly sensitive (clear zone with 12-15mm or above).

The current study observed that *S. aureus* was the most common organism present in wound, as *Staphylococcus* is prevalent in the environment and from where it can get access to the wounds which are source of bacterial propagation. Similarly, the same species were observed the most dominant and common species in the wounds of camel (Devrajani *et al.*, 2000).

Out of 50 wound samples taken from donkey wounds,

16 (32%) were contaminated with pure bacterial species, whereas 34 (68%) were found having contamination with mixed bacterial species (Table II).

It is clear that mixed infection was common in wound sample of donkey. The data of other study showed that out of 46 different animals wound samples 35 were found to be contaminated with mixed organisms, while in 11 samples had single bacterial species. It is concluded that mixed infections are always common in the wound samples (Kamla *et al.*, 2010). In another study in which the bacterial organisms were isolated from human wounds, polymicrobial infection was common and recorded in 59 (27.1%) of the samples (Bessa *et al.*, 2015). It was reported that in wound samples from sheep and goats, more than 80% were with mixed infections, while 20% samples were contaminated with pure infection. It was clear from the results that mixed infections are common in wound samples of animals (Talan *et al.*, 1989).

Susceptibility of bacteria to crude aloe vera extract

Two different concentrations i.e 30 µl and 60 µl of crude aloe vera gel were used to check the susceptibility of the isolated organisms. The results indicated that 30 µl crude extract of aloe vera did not show any result and no clear sensitivity zone was formed around the disc, whereas 60 µl of crude extract of aloe vera produced a clear zone and organisms showed quite sensitive reaction against crude extract (Table II).

Susceptibility of bacteria to ethanol extract of aloe vera

Using ethanol extracts showed the better results as compared to crude aloe vera at both concentrations i.e 30 and 60 µl. *S. aureus* was recorded as most susceptible among all isolates and recorded highly sensitive (15 to 22 mm), followed by *E. coli* (14 to 18 mm), *Shigella* (13 to 15 mm) and *Salmonella* (11 to 14 mm), respectively. Ethanol extract showed greater susceptibility against gram positive as well as gram negative isolates at 60 µl (Table II).

Study was conducted to check the antibacterial activity of ethanol and chloroform extract of aloe vera, ethanol extract of A. Vera which exhibited maximum inhibition against *S. aureus*, *S. pneumonia* and *B. subtilis* (JothiKarumari *et al.*, 2014). A similar kind of investigation was carried out in which ethanol extract was used and it showed the greatest effect on *S. aureus*, *Escherichia coli*, *Klebsiella pneumoniae* and *shigella* compared to pure Aloe extract. Methanol extract exhibited maximum antibacterial activity against *S. aureus* 24 mm, followed by *B. cereus* (Rudrangshu *et al.*, 2015). The result of the current study are in accordance with the previously reported studies showed greater susceptibility against gram positive as well as gram negative isolates

while, significantly least inhibition was with chloroform extract (Cera *et al.*, 1980; Azghani *et al.*, 1995). In another study ethanol extract of aloe vera exhibited greater antimicrobial activity besides methanol, chloroform and Di-chloro Methane extracts (Rudrangshu *et al.*, 2015).

The current study also showed agreement with the previous studies. The result of current study are showed that ethanol extract showed 100% efficacy and susceptibility against gram positive as well as gram negative isolates same as antibiotics. Ethanol extract showed better result as compare to crude extract because ethanol is more polar than water. Apart from that, a lower antimicrobial action against Gram-negative has been recorded as compared to Gram-positive organism because of the presence of additional lipopolysaccharide layer in the Gram-positive bacteria (Matu *et al.*, 2003).

CONCLUSION

It could be concluded from the present study that ethanol extract of aloe vera showed greater efficacy against bacterial isolates as compared to crude aloe vera extract.

Statement of conflict of interest

The authors have declared is no conflict of interests.

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