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Original Article

Antibacterial Effects of *Aloe Vera* Extracts on some Human and Animal Bacterial Pathogens

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Introduction: *Aloe Vera* compounds have inhibitory activity on fungi, bacteria, and viruses. This study examines the antibacterial activity of *A. Vera* purified extracts including gel, boiled skin, boiled gel, and distilled extract against pathogenic bacteria, *Staphylococcus aureus*, methicillin-resistant *S. aureus* (MRSA), *Klebsiella pneumonia* and *Pseudomonas aeruginosa* were elucidated. **Method:** The bacterial strains were collected from veterinary hospital. Freshly collected *A. vera* leaves were used for the juice extraction of gel, skin and distilled extracts. Antibacterial effects of various *A. Vera* extracts were evaluated using broth microdilution method. The crude polysaccharides of boiled skin extract were purified by phenol method; and fractionated by anion exchange chromatography. For each bacterium, minimum inhibitory concentration of various *A. Vera* extracts was determined. The protein expression changes of treated bacteria were detected by SDS-PAGE electrophoresis. **Results:** The distillate extract exhibited more antibacterial effects than other extracts. Out of seven-carbohydrate fractions of the skin extract, the fractions 6 and 7 had antibacterial effects on *S. aureus* and MRSA at 0.089 and 0.134 mg/ml, respectively; also fraction 5 showed antibacterial effects on MRSA at 0.113 mg/ml concentration. The protein profiles of these strains before and after treatment with *A. Vera* showed significant differences at 175, 60, 200 and 70 kDa protein bands of *S. aureus*, MRSA, *P. aeruginosa* and *K. pneumonia*, respectively. **Conclusion:** This finding showed that the distillate extract despite the minimal amount of carbohydrate and protein was more efficient against both Gram-positive and Gram negative bacteria. *J Med Microbiol Infec Dis, 2015, 3 (1-2): 6-10.*

Keywords: *Aloe Vera,* Extracts, Antibacterial.

INTRODUCTION

Antibiotics are the first choice for treating bacterial infection. Long time exposure to antibiotics lead to the development of antibiotic resistance; therfore this need alternative approach for treatments of infectious disease. These require other agents with greater antibacterial effect and lower toxicity. However, despite a push for development of new antibiotics, the rate of approved agents has declined. Many plants contain numerous antibacterial constituents that can be used for the treatment of infectious diseases, especially in the cases of multiple bacterial infections. The effects of herbal medicine are relied on their chemical components. About 80% of world population use herbs to treat disease [1]. Biological or pharmacological test on plants has led to the discovery of a significant number of new natural or semi-synthetic antibiotics [2, 3].

Aloe Vera is a juicy plant species originated in northern Africa; it has frequently been cited as being used in herbal medicine since the beginning of the first century AD [4]. It contains several ingredients such as vitamins, sugars, minerals and enzymes, anthraquinones, phenolic compounds, prostaglandins, saponins, glycoproteins, acemannan, sterols, salicylates, magnesium lactate, amino acids and superoxide dismutases with antioxidant activity [5, 6]. These compounds have inhibitory action on fungi, bacteria, and viruses; in addition *A. Vera* has been used for medicinal purposes and as an element in many beauty products [7]. *A. Vera* gel consists of 99.3% water; the remaining 0.7% is made up of solids with glucose and mannose constituting for a large part. The amino acids and sugars together with enzymes give the special properties as a skin care product [8, 9]. The *A. Vera* gel is extensively used in treatments of gastrointestinal disorders for example peptic ulcer [10].

Staphylococcus aureus, Klebsiella pneumonia, and Pseudomonas aeruginosa are the most important pathogens in human nosocomial infection. *S. aureus* colonizes in the nasal cavity, nasopharynx, skin and mucous membranes of human and animals [11, 12] and causes a variety of significant economic disease in human and animals [13]. The rate of methicillin-resistant *S. aureus* (MRSA) continues to raise in nosocomial infections surveillance system hospitals [14]. MRSA infections of *S. aureus* and coagulase-negative *staphylococci* are serious concerns of the human population [15].

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K. pneumonia is an opportunistic pathogen and is known to cause different infections including urinary tract diseases to pneumonia in human and animals [16]. In recent years, *K. pneumonia* has emerged as an important pathogen in nosocomial infections [17]. *P. aeruginosa* is an opportunistic pathogen that causes infection in animals and human in some condition such as trauma, debilitation and change of normal flora. It expressed the efflux systems which plays a major role in antibiotic resistance [18, 19].

The above mentioned bacterial strains can develop resistance to antibiotics; for this reason, the present study was designed to evaluate the antibacterial activity of various *A. Vera* extracts on these bacteria. The gel, boiled skin, boiled gel and distilled extract of *A. Vera* were purified, and their antibacterial effects against *S. aureus, K. pneumonia,* and *P. aeruginosa* were elucidated. Also, the protein profiles of these bacteria were analyzed before and after the treatments to identify the most potent antibacterial extract of *A. Vera*. Because the skin of *A. Vera* often discarded as waste in food processing industry [20]; this research also aimed to analyze the antibacterial properties of polysaccharide fractions of *A. Vera* skin extract.

MATERIAL AND METHODS

The *A. Vera* leaves were collected from different areas of Khuzestan Province. The bacterial strains of *S. aureus, P. aeruginosa,* and *K. pneumonia* were isolated from animals referred to the veterinary hospital of Shahid Chamran University of Ahvaz, and the MRSA was a standard strain (PTCC:33591). Blood agar and Mueller Hinton Broth were used for propagation and maintenance of bacterial cultures. Broth cultures were incubated under aerobic conditions at 37°C.

Preparation of *A. Vera* extracts. Freshly collected *A. vera* leaves (250 gr) were washed and cut in half. Half of inner tissue was squeezed, and the extract was separated (gel extract); the remaining part was cut in small pieces in 100 mL PBS (phosphate-buffered saline: 0.06 mM sodium phosphate buffer containing 0.15 M NaCl, pH 7.4) and boiled at 50°C for 20 min (boiled gel). The outer parts of leaves (150 gr), homogenized with 100 ml PBS in a blender and boiled at 50°C for 20 min (boiled skin). All extracts were filtered on a Whatman no. 2 paper to remove the insoluble materials and then centrifuged at 6000 rpm for 30 min. The precipitate was discarded and the clear yellow supernatant stored at 4°C to be used. In order to prepare distillate extract, the whole leaves were ground with double distilled water and used for distillation by utilization of common distillation apparatuses. These extraction procedures were repeated three times and the recovered extracts were pooled.

Antibacterial and SDS-PAGE analysis. Antibacterial effects of the various *A. Vera* extracts were evaluated using a broth microdilution method in 96-well microplates. For each bacterium, minimum inhibitory concentration (MIC) of various *A. Vera* extracts was determined. The bacterial strains were cultured on blood agar and incubated overnight at 37°C. One colony was selected from the pure culture of each strain and inoculated at 37°C in Mueller-Hinton Broth and checked until the yield the bacterial suspension reached to 0.5 McFarland standard turbidity. A serial of two-fold dilution (1/2, 1/4, 1/8, 1/32, 1/64 and 1/128) of *A. Vera* extracts was prepared in Mueller-Hinton Broth; the equal volume of each prepared bacteria was added to the wells and incubated at 37°C for 24 h. The lowest concentration of each extract which inhibited visible bacterial growth was taken as the MIC of extract [21]. Also, the bacteria were treated with ½ and ¼ dilution of *A. Vera* distilled extract and their total protein profiles before and after the treatment were detected by SDS-PAGE electrophoresis. We used 12% separating gel and 4% stacking gel, and electrophoresis was performed in running buffer (25 mM Tris base pH 6.8, 192 mM glycine, 1% SDS) at 100 V for 90 min. The polyacrylamide gels were stained for 30 min with Coomassie staining solution followed by destaining with 7% acetic acid solution overnight.

Purification and fractionation of the crude polysaccharides of boiled skin extract. The skin extracts polysaccharides were purified by phenol methods; briefly, the same volume of 90% phenol was added to the extracts and shaked at 68°C for 15 min. The suspensions were cooled and centrifuged at 8500 *g* for 15 min. The supernatants were transferred to other tubes and phenol phases were re-extracted. Carbohydrates were precipitated by addition of sodium acetate at 0.5 M final concentration, and ten volumes of 95% ethanol to the supernatants and samples were stored at -20°*C* overnight. Tubes were then centrifuged at 2000×*g*, 4°C for 10 min and the pellets were suspended in distilled water. Extensive dialysis against double distilled water was performed at 4°C [22].

The crude polysaccharides were fractionated by anion exchange chromatography on a DEAE-C (Sigma, Cat No: D6418) column. In brief, 50 mg of skin polysaccharide was filtered through a 0.45-µm Millipore filter (MilliporeCo., Billerica, MA, USA), and applied onto the column of DEAE-cellulose, previously equilibrated with 10mM Tris–HCl buffer (pH 8.5). After removing the unabsorbed carbohydrates, the carbohydrate fractions were eluted with 0.1 to 2 M NaCl gradually [23]. The total carbohydrate content was determined by the phenol-H2SO4 method using glucose as the standard [24]. The different fractions were concentrated and dialyzed in distilled water. The antimicrobial effects of *A. Vera* boiled skin carbohydrate fractions were evaluated using broth microdilution method in 96-well microplates [21].

RESULTS

Protein and carbohydrate analysis. The highest value of carbohydrate and protein were observed in boiled skin and boiled gel extracts, respectively (as shown in Table 1). The minimal amount for these was seen in distillate extract.

The antimicrobial effects of *A. Vera* extracts. The MICs of the *A. Vera* extracts against *S. aureus*, MRSA, *P. aeruginosa* and *K. pneumonia* were determined (Table 2). Out of these four extracts, distilled extracts exhibit the most potent antibacterial effects. There was no difference in the vulnerability of gram-positive and gram-negative bacteria toward distillate extracts.

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Table 1. The protein and carbohydrate contents (mg/mL) of various *A. Vera* extracts.

	Gel	Boiled gel	Boiled skin	Distilled
Protein	5.71	3.35	2.25	0.06
Carbohydrate	11	19	47	0.7

Table 2. The antimicrobial effects of *A. Vera* extracts. The MICs were observed at ¼ dilutions.

	Gel	Boiled gel	Boiled skin	Distilled
S. aureus	-	-	+	+
MRSA	+	+	+	+
K. pneumonia	-	-	-	+
P. aeruginosa	-	-	-	+

SDS-PAGE analysis. SDS-PAGE analysis of whole-cell protein extracts from the four bacteria produced patterns containing discrete bands with molecular weights ranging from >5 to <200 kDa. The protein profiles of these strains before and after treatment with *A. Vera* were compared. Significant differences were seen at 175, 60, 200 and 70 kDa protein bands of *S. aureus*, MRSA, *P. aeruginosa* and *K. pneumonia*, respectively (Figure 1).

Purification and Fractionation of crude polysaccharides. The phenol method showed to be more useful for carbohydrate purification of *A. Vera* skin extract. Seven carbohydrate fractions were purified by DE-C column (Table 3). The fractions eluted with 0.1 M NaCl have the highest carbohydrate concentration and the one eluted with >2 M NaCl have negligible carbohydrate concentration. Out of seven-carbohydrate fractions of skin extract, fractions 6 and 7 had antibacterial effects on *S. aureus* and MRSA at 0.089 and 0.134 mg/ml, respectively. Moreover, fraction 5 showed bacteriostatic effects on MRSA at 0.113 mg/ml concentration (Table 4)

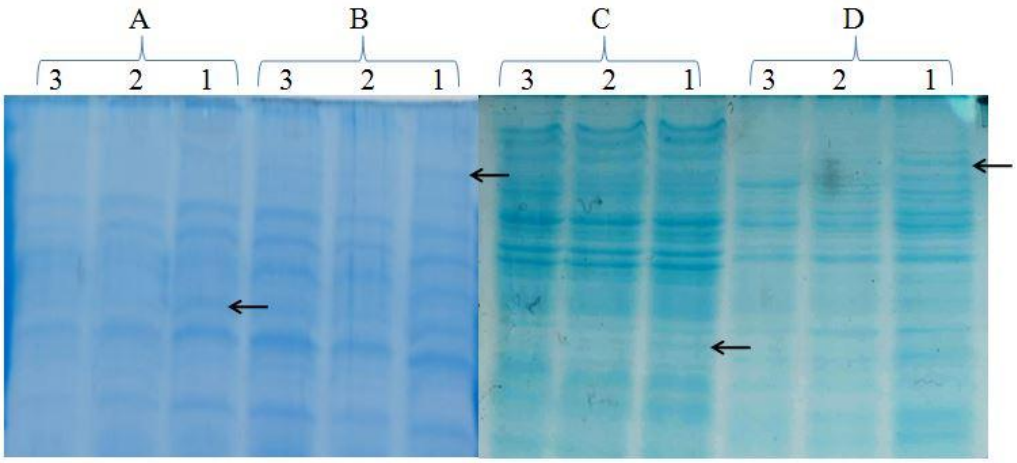


Fig. 1 SDS-PAGE analysis of whole-cell protein of bacterial strains: A) *S. aureus*, B) MRSA, C) *K. pneumonia*, D) *P. aeruginosa*. Lanes 1-3: The first lane of each group was the untreated bacteria, and lanes 2 and 3, treated with ½ and ¼ dilution of *A. Vera* distilled extract, respectively.

Table 3. The carbohydrate concentration of seven isolated fractions (1-7) of boiled skin extract.

Fraction	**1**	**2**	**3**	**4**	**5**	**6**	**7**
Carbohydrate (mg/mL)	**0.16**	**5.8**	**0.88**	**0.2**	**0.452**	**0.356**	**0.536**

Table 4. The antimicrobial effects of *A. Vera* skin boiled carbohydrate fractions.

Fraction	**1**	**2**	**3**	**4**	**5**	**6**	**7**
S. aureus	-	-	-	-	-	+	+
MRSA	-	-	-	-	+	+	+

Fractions 6 and 7 fractions have the bacteriostatic effects on *S. aureus* and MRSA, fraction 5 also has the bacteriostatic effects on MRSA. The MICs were observed at ¼ dilutions.

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DISCUSSION

Previously, various preparations of *A. Vera* including confection, lotion, and juice, have been used as traditional medicine in several communities for curing various diseases [25]. In present study, only *A. Vera* distilled extract was effective against *P. aeruginosa*. However, by using the *A. Vera* gel extracts, Agarry *et al.* [9] and Azghani *et al.* [26] reported a reduction in *P. aeruginosa* infection and inhibition of attaching it to human lung epithelial cells. This disagreement may be due to the difference in the source of *A. Vera* and extracts preparation process, various bacterial isolates or the test conditions. In agreement with the current study, Kaithwas *et al.* [27] showed that the *A. Vera* gel is rich in variety metabolites, such as, anthraquinone, polysaccharides, glycoproteins, glycosides, gamma-linolenic acid and prostaglandins which are effective against gram-positive bacteria in particular against *S. aureus*. Also, previously, antibacterial activity of *A. Vera* essential oil against *S. aureus* and *P. aeruginosa* was reported [28]. In addition, the antibacterial component of *A. Vera* extract was reported to be effective against *S. aureus, Streptococcus pyogenes, Escherichia coli, K. pneumoniae, Salmonella typhi, P. aeruginosa, Helicobacter pylori* and *Propionibacterium acne* [29-31].

In the current research, out of the four *A. Vera* extracts, the distillate extract, despite containing the lowest amount of carbohydrate compared to other extracts, exhibited the most antibacterial effects against four bacterial strains which included both gram positive and gram negative bacteria. This could be due to the activation of some antibacterial elements after distillation.

The gel and boiled skin extracts were only effective against *S. aureus*; such a weak antibacterial effect for aqueous extract was reported by others [32, 33]. Various antibacterial effects by different extraction methods were reported by others; in agreement with the results of the current study, Nejatzadeh-Barandozi [20] suggested the skin extracts as a source of antibacterial agents against *S. aureus* and MRSA. The alcoholic extracts was reported as a stronger antibacterial and antifungal agent than aqueous extract [34] and the methanolic *A. Vera* gel extract had more antibacterial activity against gram-positive bacteria compared to gram-negative bacteria [35]. This may be due to the difference in cell walls of gram positive and negative bacteria. Also, whole leaf component of *A. Vera*, mainly anthraquinones and saponins had antibacterial activities [29, 36].

Cock *et al.* [37] investigated the antimicrobial effects of different methanolic fractions of *A. Vera* inner tissues and showed that five fractions had antimicrobial activities, which first fraction had the highest antibacterial effects; however, in the current study, the three last fractions of the aqueous skin extracts showed antibacterial effects.

In overall, the extraction methods may influence on antibacterial effects of *A. Vera* extracts. The most antibacterial active components of *A. Vera* are volatile or saturated compounds which are mainly obtained in distillate extracts of *A. Vera*. It is noteworthy to suggest the distillate extract as antibacterial agents against the tested bacteria. Although, the most affected protein bands were recognized at SDS-PAGE analysis; this needs an additional research to realize the exact mechanism of antibacterial effects of *A. Vera* extracts.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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