Original Article

Assessment of the Antibacterial Potential of *Aloe vera* as a Source of Antibacterial Agents

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Abstract

Background: Nowadays the use of herbs as an alternative to the chemical drug is considered by researchers. *Aloe vera* belongs to the Asphodelaceae family, a medicinal plant that has been used since ancient times for different pharmaceutical products. This study aimed to evaluate the antibacterial properties of *Aloe vera* grown in Khouzestan, southwest of Iran. **Materials and Methods:**For this purpose, ethanol extract was prepared from aerial parts of *Aloe vera* and its activity was tested against some gram-positive (*Enterococcus faecalis*, *Staphylococcus aureus*, *Bacillus cereus*) and gram-negative (*Pseudomonass aeruginosa*, *Salmonella* Typhi, *Proteus mirabilis*) bacterial species through standard Kirby-Bauer disc diffusion method. Minimum inhibitory concentration and minimum bactericidal concentration were also investigated. **Results:** The *Aloe vera* extract showed antibacterial activity against the majority of bacteria. The highest activity (about 25mm inhibition zone) happened against *P. aeruginosa* but it did not show any inhibitory activity against *S.aureus* and *P. mirabilis*. The MIC was found as 10 mg/ml while MBC ≥ 80. **Conclusion:** Based on the results of this study it can be suggested that *Aloe vera* contains active antibacterial substances that can be used efficiently for bacterial pathogen control and it should be considered as a potent antimicrobial source for finding new antibiotics, especially against resistant species.

Keywords: *Aloe vera*, antibacterial properties, bacterial pathogen, potent antimicrobial source.

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Introduction

Aloe Vera is a medicinal plant used for the management of various infections since ancient times as it has anti-inflammatory, anti-microbial, and immune-boosting properties (1).

Herbal medicine has been the basis of treatment and cure for various infectious diseases and physiological disorders in traditional medicine. Active components of plants play an important role in infection treatments and pathogens control (2).

Aloe vera is a cactus-like plant, although is related to the onion, garlic, and asparagus. It is a stemless plant with triangular and fleshy leaves ranging in color from gray-green to bright green with small white teeth in the margin of its leaves. The leaves are composed of three layers: an inner gel, a yellow sap, and the outer thick layer of 15 to 20 cells called as rind (3).

The parenchymatous cells in the fresh leaves secrete a colorless mucilaginous gel that contains 98% to 99% water and 1-2% active compounds like aloesin, aloin,

aloe-emodin, aloe-mannan, flavonoids, saponin, sterols, amino acids, and vitamins. Free anthraquinones and their derivatives like barbaloin-IO-aloe emodin anthrone, isobarbaloin, and chromones in *Aloe Vera* leaves exert a strong purgative effect and are potent anti-microbial agents (4).

The natural habitat of *Aloe vera* is unclear, as the species has been widely cultivated throughout the world. *Aloe vera* has very effective properties that have given this plant an almost perfect reputation, and it is intended as a wonderful magical herb in medicine V. C (2017). This plant produces antiseptic agents such as salicylic acid, lupeol, cinnamonic acid, sulfur and phenols, all of which are known as growth-inhibiting and/or killing agents for fungi, bacteria, and viruses (5). The gel of *Aloe vera* was used to cure stomachache, gastrointestinal problems, skin disease, constipation, radiation injury, inflammation, healing of wounds, burns, ulcer, and diabetes (6).

Khouzestan has a warm and humid climate and concerning this fact that active constituents of plants can be influenced by ecophysiological conditions, it is necessary to investigate the medicinal properties of plants in different regions.

The purpose of this study was to investigate the antibacterial properties of ethanol extract of cultured *Aloe vera* in Khouzestan, Iran, against some clinical pathogens.

Methods

Extract preparation. The understudy plants were collected from the local market in Khouzestan, Iran, and identified based on taxonomic criteria in the department of biology, Shahid Chamran University. To extract preparation, the leaves of *Aloe vera* were shade dried at room temperature for 72 h and then grind into a fine powder using an electric blender. Using 10 ml of ethanol–distilled water (8:2) v/v, centrifugation (3000 rpm, 15 min), and collecting the supernatant, the ethanolic extract was obtained from one gram of powder. This process was repeated for three times. Finally, the ethanol was removed by evaporation at room temperature (7).

Antibacterial activity assay. Different bacterial species and strains were used for finding the possible antibacterial activity of ethanol extract of *Aloe vera* including *E. faecalis* , *S. aureus, B. cereus, S. Typhi, P. mirabilis* and 5 antibiotic resistance strains of *P.aeruginosa* namely Ps. 11, Ps.7, Ps.4, Ps. 22 and Ps. S.

These bacterial species were originally collected and isolated from clinical specimens of patients and identified based on biochemical identification of the Bergey's manual of systematic bacteriology.

Four concentrations including 50, 100, 200, and 400 mg/ml of extract were prepared in ethanol and their antibacterial activity was evaluated by the disc diffusion method against tested bacteria. The tested bacteria were cultured in Mueller-Hinton broth (Merck, Germany) and incubated at 37°C till 0.5 McFarland turbidity. Then with a sterile cotton swab, a lawn culture was prepared on Mueller- Hinton agar (Merck, Germany) and allowed to remain in contact for 1 min (8, 9). Sterile 6mm filter paper discs were saturated by these solutions (each with 40 µL of solution) and were placed on lawn cultures. Thus, the effective amount of extract in each disc was 2, 4, 8, and 16 mg, respectively. The plates were remained 1h at room temperature for extract diffusion through the agar and then incubated at 37°C for 24 h. The inhibition zone around each disc was measured and recorded (mm) (10).

MIC and MBC determination. MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) of *Aloe vera* ethanolic extract were determined in 2.5-80 mg/mL concentrations in a twofold serial dilution against E. faecalis by macro broth dilution assay method. The tubes were incubated at 37°C for 24 h. The least concentration in the above series with no sign of visible growth was reported as the MIC. A loop full of broth from growth negative tubes in the MIC assay was cultured on Mueller- Hinton agar for determining the MBC.

The plates were incubated at 37°C for 18-24 h. The highest dilution (least concentration) that inhibited colony formation on a solid medium was considered as MBC (11).

Results

The results of antimicrobial activities obtained from the ethanolic extract of *Aloe vera* have been presented

in table 1. As it can be found, the extract was effective against all tested bacteria except *S. aureus*, *P. mirabilis*, and *P.aeruginosa* strain 4. While in other cases the inhibition zone was noticeable. The greatest inhibition zone for E. faecalis was observed at 200 and 400 mg/mL concentrations and the less inhibition zone was found at 50 mg/ml concentration. In general, the highest activity (about 25 mm inhibition zone) was found against P. aeruginosa strain 22 (Table 1).

The results of MIC and MBC were presented in table 2. The MIC was found as 10 mg/ml while MBC ≥ 80.

Table 1: Antibacterial activity of ethanol extract from *Aloe vera.*

Bacteria species	**Ethanol extract(mg/mL)**			
Gram positive	**50**	**100**	**200**	**400**
E.faecalis	13	17	20	20
S. aureus	R	R	R	R
B. cereus	R	R	10	11
Gram-negative				
P.aeruginosastrain4	R	R	R	R
P.aeruginosastrain7	10	11	16	20
P.aeruginosastrain11	8	12	17	20
P.aeruginosastrain 22	R	15	15	25
P. aeruginosastrains	R	R	12	15
S. Typhi	R	R	12	14
P. mirabilis	R	R	R	R

R: Resistant, * Inhibition zone diameter (mm).

Table2: MIC and MBC ($^{mg}/_{mL}$) of *Aloe vera* ethanol extract against *E. faecalis*

Enterococcusfaecalis	***Ethanolic extract (mg/mL)***
MIC	10
MBC	≥ 80

Discussion

Today, one of the main causes of human infection is the antibiotic resistance of bacteria due to the use of antibiotics which causes the emergence and spread of antibiotic-resistant bacterial strains (12). The search for the discovery of healthy and effective antimicrobial agents continues it can be used for both therapeutic and preventive purposes in the case of a broad range of bacterial infections. So pharmaceutical companies are now looking for alternative drugs from other sources, including plants. Because it is known that medicinal plants produce substances with antimicrobial activity (13).

The inhibitory activity of aqueous, ethanol and acetone extracts of the A. vera gel against some human and plant pathogens was measured by the disc diffusion method (14). Ibrahim et al.,(2011) find that the ethanol and acetone extracts have significant inhibition against all tested pathogens. Cock et al. (2008) studied the antimicrobial activity of *Aloe barbadensis leaf* gel components (15). At this test they fractionated the methanol extracts of A. *Pseudomonas aeruginosa* inner leaf gel by RP-HPLC tested these fractions for antimicrobial activity against a range of bacteria and fungi. The result showed that five fractions have antimicrobial activity. Thiruppathi et al (2010) prepared different extracts of *Aloe vera* with various solvents like as hexane, ethyl acetate, petroleum ether, and ethanol and determined their antimicrobial activity against gram-positive bacteria (*Bacillus subtilis*, *Staphylococcus aureus*), and gram-negative bacteria (*Escherichia coli, Klebsiella pneumonia*, *Pseudomonas aeruginosa*). Ethyl acetate (1-9 mm) and ethanol extract (7-12 mm) were identified as having antimicrobial

activity (16).

The result of this study showed that *Alo vera* did not have any inhibitory activity against *S.aureus*, *P.mirabilis*, and *P.aeruginosa* strain4, ie. They were resistant, maybe, because of cell wall permeability or due to other structural and/or metabolic factors (17). Our results confirm those previously reported by Nejat Zadeh et al. (2013) that observed minimum growth suppression against *S. aureus* (18). The antimicrobial effect of *Aloe vera* on Bacillus cereus was not notable. Similar results were also observed by cock et al. The highest inhibition zone of *P.aeruginosa* strain11, 7, and 22 were found at 400 mg/mL concentration. This result does not coincide with studies made by Philip et al. (19). Among the factors that may affect the antibacterial effects of a plant extract Extraction method and type of solvent are important factors. Extracts obtained from a plant by different methods and solvents can have different antibacterial effects on a particular bacterium (20).

This difference may be due to several possible reasons such as permeability barrier provided by the presence of cell wall with multilayer structure in Gram-negative bacteria or the membrane accumulation mechanisms or presence of enzymes in periplasmic space which can break- down foreign molecules introduced from outside.

The inhibitory effect against P. areuginosa isolates that were among resistant isolates is of great importance that creates hope for finding new antibacterial substances from this plant against this pathogen. Soon Choi and Shin., (2017) showed that *Aloe vera* had a concentration-dependent effect on all of the examined bacteria, particularly on *P.aeruginosa* (21). A study by Goudarzi (2015) showed similar results that, *Aloe vera* gel at various concentrations can be used as an effective antibacterial agent to prevent wound infection caused by P. aeruginosa (22).

Some studies have found that bioactive components of Aloe extract, polyphenol of anthraquinone such as Aloe-emodin, aloin cause such desirable effects of the Aloe. Several phytoconstituents like flavonoids, phenolics, and polyphenols, tannin, terpenoids, sesquiterpenes, etc. are effective antimicrobial substances against a wide range of microorganisms (4). These studies have discovered that the phenolic component of *Aloe vera* has antimicrobial and anti-tumor effects. The phenolic component of *Aloe vera* has been reported to have potent anti-lipid peroxidation inhibitory effects in the livers and brains of mice (21). These findings were in agreement with Abakar et al. (2017) who stated that the gel of *Aloe vera* can promote wound healing due to the presence of some components like anthraquinones and hormones which possess antibacterial, antifungal and antiviral activities (23). Also, Sahu et al. (2013) reported that the bitter yellow latex-containing anthraquinones and glycosides have been reported from the middle layers of leaf (24). On the other hand, investigations for the production of new antibacterial compounds because of the high resistance of pathogenic microorganisms to synthetic antibiotics have attracted more attention.

Aloe vera (A. barbadensis Miller L.) is most biologically active among 400 species. According to the World Health Organization (WHO), aloe is the best source for obtaining a variety of drugs, because this plant can survive in both hot and cold temperatures (25).

The MIC was found as 10 mg/mL while MBC $\geq$ 80 mg/mL. It means that the active substances in the ethanolic extract have bacteriostatic activity rather than bacteriocidal activity and are effective as low as 10 mg/ml concentration. It may be that the active ingredients of this extract interfere with metabolic pathways of the bacterial cell and hence growth inhibition happened.

Another possible mechanism of activity is targeting protein synthesis in the bacterial cytoplasm. So as a result of inhibition of protein synthesis and/or error-prone synthesis of proteins, the bacterial metabolism and cell growth and division will be interrupted and growth inhibition has happened.

We can call this extract as bacteriostatic agents which can inhibit bacterial growth but generally do not kill them.

Conclusion

Our results showed that the ethanol extract of *Aloe vera* aerial part was effective against both gram-

positive and gram-negative bacteria. The extract at 200 and 400 mg showed high antibacterial activity, thus these concentrations had inhibited the growth of 2 out of 3 gram-positive bacteria and 5 out of 7-gram negative bacteria. It is hoped that this study could help to introduce some new and natural origin substances to formulate more potent antimicrobial drugs.

Conflict of Interest

The authors declared no conflict of interest.

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