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Research Article

In Vitro Antibacterial Activity of Ethanolic Extract of Aloe Vera and Silver Nanoparticles on Standard Strains of Some Pathogenic Bacteria

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ABSTRACT

Background and objectives: Today, with the increasing rate of antibiotic resistance, treatment of bacterial infections has become challenging. Therefore, it is essential to find suitable alternative antibacterial compounds. The aim of this study was to investigate in vitro effects of silver nanoparticles and ethanolic extract of Aloe vera alone and combined on standard strains of some pathogenic bacteria.

Methods: After collection and verification of A. vera plants, extraction was performed by the Soxhlet extractor method. Antibacterial effects of ethanolic extract of A. vera and silver nanoparticles on standard strains of Staphylococcus aureus, Bacillus cereus, Escherichia coli and Pseudomonas aeruginosa were investigated by agar well diffusion and tube dilution methods, respectively.

Results: The ethanolic extract of A. vera and silver nanoparticles had antibacterial effects on the tested bacteria in a dose-dependent manner. The ethanolic extract of A. vera was more effective against S. aureus and B. cereus compared to gram-negative bacteria. However, silver nanoparticles were more effective against gram-negative bacteria (P. aeruginosa and E. coli). The effect of combination of ethanolic extract of A. vera and silver nanoparticles was much greater than the effect of either alone. This combination showed the greatest and lowest effect on P. aeruginosa and S. aureus, respectively.

Conclusion: For the first time, this study showed that the combination of ethanolic extract of A. vera and silver nanoparticles is effective against potentially pathogenic bacteria. Given the high rate of antibiotic resistance and side effects of conventional antibiotics, it is recommended to identify active compounds of this plant and evaluate the antimicrobial effects of this combination of fungi and other pathogenic bacteria both in vitro and in vivo.

Keywords: Antibacterial susceptibility; Aloe vera; Nanoparticles; Silver; Plant extract

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Introduction

In recent years, the excessive use of synthetic antibiotics in the treatment of bacterial diseases has led to increased rate of antibiotic resistance (1). The use of herbal medicine has a long history in different societies. Such natural medicines have limited side effects and are compatible with genetic and physiological factors of the body (2,3). Plant extracts and essential oils are important sources of natural medicinal compounds that have antimicrobial, antioxidant, free radical scavenging, and anti-cancer properties, which have attracted the attention of scientists in recent years (4,5). Therefore, many studies have been performed on evaluating the antimicrobial (6), anti-parasitic (7), antiviral (8), cytotoxic (9) and mutagenic (10) effects of plant extracts. Aloe vera belongs to the genus Aloe, order Asparagales and Family Asphodelaceae (11). This plant is suitable for subtropical climates and grows in different countries as well as in southern parts of Iran (such as Bushehr, Hormozgan and Balochestan provinces) (12,13). A. vera leaves can be divided into two main parts: the outer green shell, which contains the vascular bundles, and the inner colorless parenchyma (pulp), which contains the A. vera gel. The leaves of this plant contain more than 75 nutrients, 200 active compounds, 20 minerals, 18 amino acids, and 12 vitamins. It also contains various compounds such as acetylated mannans, anthraquinone C-glycosides, anthrones, and anthraquinones such as emodin and lectins (14). The immunostimulatory, antioxidant, antiviral, anti-fungal, anti-cancer, and anti-bacterial effects of this plant have been reported in various studies (15,16). This medicinal plant is useful for the treatment of arthritis, asthma, chronic fatigue syndrome, indigestion and intestinal disorders, skin diseases, epilepsy, migraine, mild burns, skin injuries, acne, diabetes, high cholesterol, and inflammation of the oral mucosa (17-21).

Today, nanotechnology is considered as a novel approach to fight microbes without increasing drug resistance. Extensive research has been conducted on the application of nanoparticles in prevention and treatment of diseases (22). In this regard, silver nanoparticles have been commonly used due to their antimicrobial properties, low price, and simple preparation (23). This study was performed to investigate the in vitro antimicrobial and synergistic effects of ethanolic extract of A. vera leaves and silver nanoparticles on some standard bacterial strains.

MATERIALS AND METHODS

Collection and preparation of ethanolic extract

In this experimental study, A. vera plant was collected from natural cultivation areas in Marand (northwestern Iran). The plant was identified by botanical experts at the Islamic Azad University of Ahar, Iran. The plant was washed several times with water and then shade-dried. For ease of extraction, the samples were pulverized with an electric mill. Extraction was performed by the Soxhlet method. For this purpose, 60 g of dried plant powder and 300 ml of ethanol as a solvent were placed in a Soxhlet extractor for 8 hours. The solvent was slowly evaporated at temperature of 40 ^{o}C using a rotary evaporator. The obtained concentrated extract was diluted to 20, 30, 50 and 400 mg/ml using 5% dimethyl sulfoxide (DMSO) (Merck, Germany) (24).

Preparation of bacterial strains

Standard strains of Bacillus cereus (AT47C: 1247), Staphylococcus aureus (ATCC: 25923), Pseudomonas aeruginosa (ATCC: 27853), and Escherichia coli (ATCC: 25922) were obtained from the Tabriz University of Medical Sciences. To activate the bacteria, first lyophilized cultures were grown in Tryptic Soy Broth (TSB) (Merck, Germany) at 37 °C twice for 18 consecutive hours. Equal volumes of each bacterial suspension were prepared to form an absorption surface equivalent to 0.5 McFarland standard at 600 nm. Turbidity of

the bacterial suspension was proportional to 1.5×10^8 CFU/ml (25).

Determination of the antibacterial effect of A. vera extract by agar well diffusion Due to the diversity of the studied bacteria, Mueller-Hinton medium (Merck Co., Germany) was used. Bacterial suspensions with turbidity equivalent to 0.5 McFarland standard were prepared and cultured on Mueller-Hinton agar, in three directions using sterile swap. Then, using a sterile Pasteur pipette, wells with a diameter of 6 mm and a distance of 2.5 cm were created on the agar surface of each plate. Then, 60 µl of different concentrations of the ethanolic extract of A. vera were poured into each well. In addition, DMSO and streptomycin were used as the negative and positive controls, respectively. The plates were incubated at 37 °C for 24 hours. Next, the mean diameter of the growth inhibition zone was calculated in millimeters to determine antimicrobial activity of the extracts in comparison with the positive and negative control samples.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC of ethanolic extract of A. vera against the tested bacteria were determined using the macrodilution broth method. To determine the MIC values, 6.25, 12.5, 25, 50, 100 and 200 mg/ml dilutions of the extract were prepared using Mueller Hinton broth. Then, 1 ml of bacterial suspension was added to each dilution. A tube containing only bacteria (without extract) and another containing culture medium (without bacteria) were considered as the positive and negative controls, respectively. The tubes were incubated at 37 °C for 24 hours. Next, the tubes were examined for turbidity due to the growth of inoculated bacteria, and the last dilution in which no turbidity was observed (no growth) was considered as the MIC. All tubes in which no bacterial growth was observed were sampled and MBC was determined by culture on agar plates. The plates were incubated at 37 °C for 24 hours. The lowest concentration of the extract which completely inhibited the growth of bacteria was considered as the MBC (26).

Determination of antimicrobial effect of silver nanoparticles by agar well diffusion method Silver nanoparticles in dimensions of 20 nm were purchased from the Nano Sany Engineers Company (Iran). Serial dilutions of 10, 20, 40 and 80 µg/ml were prepared. The MIC and MBC values of silver nanoparticles were determined using the well diffusion method as explained previously (27).

Evaluation of synergistic properties To investigate the synergistic properties of silver nanoparticles and ethanolic extract of A. vera on the selected pathogenic bacteria, the dilution series mentioned in the previous two tests were mixed and used as a concentration. The test procedure was the same as the previous tests.

Statistical analysis

The experiments were repeated five times and the results were reported as mean ± standard deviation (SD). Differences in the obtained results were assessed using analysis of variance and the Chi-square test. Statistical significance level was set at 0.05.

RESULTS

The ethanolic extract of A. vera had the greatest inhibitory effect on S. aureus and B. cereus. However, the extract had little or no inhibitory effects on the tested gram-negative bacteria (Table 1). Concentrations of 400 mg/ml ethanolic extract showed slight inhibitory effect on E. coli. There were significant differences between the tested bacteria in terms of sensitivity to the A. vera ethanolic extract ($p<0.05$). In other words, S. aureus and P. aeruginosa had the highest and lowest sensitivity to the extract, respectively.

Table 1. Diameter of growth inhibition zone and MBC and MIC values of the ethanolic extract of A. vera against the tested bacteria

Concentration (mg/ml) / Bacterial strain	Growth inhibition zone values (mm)						MIC (mg/ml)	MBC (mg/ml)
	20	30	50	400	Negative control	Positive control		
S. aureus	8.8±0.83	10.8±0.83	12.4±0.54	18.8±0.83	--	17	6.25	12.5
B. cereus	6.4±1.14	9±0.70	11.2±0.83	16.4±0.89	--	18	12.5	25
E. coli	--	--	7.2±0.83	10±0.70	--	14	50	100
P. aeruginosa	--	--	--	7±1.41	--	15	--	--

Silver nanoparticles had greater inhibitory effects on gram-negative bacteria compared to gram-positive bacteria. This inhibitory effect was dose-dependent. As shown in (table 2), silver nanoparticles had the greatest and lowest inhibitory effect on P. aeruginosa and S. aureus, respectively.

Table 2. Diameter of growth inhibition zone and MBC and MIC values of silver nanoparticles against the tested bacteria

Concentration (µg/ml) / Bacterial strain	Growth inhibition zone values (mm)						MIC (mg/ml)	MBC (mg/ml)
	10	20	40	80	Negative control	Positive control		
S. aureus	8.6±0.54	10.2±1.09	12.6±0.54	14.4±1.14	-	18	50	100
B. cereus	9.2±1.09	11.8±0.83	13.2±0.83	15.2±1.09	-	17	50	50
E. coli	10±1.22	12.2±0.44	14.2±0.83	17.2±0.83	-	14	12.5	25
P. aeruginosa	11.4±0.89	13.4±0.54	16±0.70	19.6±0.89	-	15	6.25	12.5

The combination of silver nanoparticles with the ethanolic extract of A. vera caused a significant increase in the diameter of growth inhibition zone, particularly in case of gram-negative bacteria. In addition, this combination significantly reduced the MIC and MBC values against the tested bacteria compared to the nanoparticles and the extract alone ($p<0.05$). The combination had the greatest and least inhibitory effects on *P. aeruginosa* and S. aureus, respectively (Table 3).

Table 3. Diameter of growth inhibition zone and MBC and MIC values of silver nanoparticles combined with the ethanolic extract of A. vera

Concentration / Bacterial strain	Growth inhibition zone diameter of ethanolic extract (A) and silver nanoparticles (B)						MIC (A,B) (mg/ml)	MBC (A,B) (mg/ml)
	20mg/ml(A) +10µg/ml(B)	30mg/ml(A) +20µg/ml(B)	50mg/ml(A) +40µg/ml(B)	400mg/ml(A) +80µg/ml(B)	Negative control	Positive control		
S. aureus	9.2±1.09	10.8±0.83	13.4±10.54	15.8±0.44	-	20	6.25 µg/ml , 6.25 mg/ml	25 µg/ml , 25 mg/ml
B. cereus	10.2±1.09	12±1	14.8±0.83	16.4±0.54	-	19	6.25 µg/ml , 6.25 mg/ml	12.5 µg/ml , 12.5 mg/ml
E. coli	11.8±0.83	12.8±0.44	15.4±0.54	17.8±0.83	-	18	6.25 µg/ml , 6.25 mg/ml	12.5 µg/ml , 12.5 mg/ml
P. eruginosa	13.8±0.83	15.4±0.54	17.2±1.09	20.4±0.89	-	17	3.125 µg/ml , 3.125 mg/ml	6.25 µg/ml , 6.25 mg/ml

DISCUSSION

In Iran, a limited number of studies has investigated the antimicrobial properties of A. vera against pathogenic bacteria. Olaleye and Bello-Michael (2005) reported that A. vera leaf and gel are effective against Pseudomonas and Candida albicans, which is not consistent with the results of our study (28). However, in line with our findings, Abraham et al. reported that A. vera extract exert significant, dose-dependent antimicrobial effects on S. aureus (29). George et al. reported the favorable inhibitory effects of A. vera toothpaste gel on C. albicans, Streptococcus mutans, Lactobacillus acidophilus, Enterococcus faecalis, Prevotella intermedia, and Peptostreptococcus anaerobius (30). Martinez et al. reported that the aqueous extract of A. vera leaves had no antimicrobial activity (31). Goudarzi and colleagues reported that A. vera gel had antibacterial activity against P. aeruginosa isolated from burn infections (32). Sadrnia et al. demonstrated that 17.85 mg/ml of A. vera extract inhibited growth of clinical strains of Staphylococcus epidermidis, S. aureus, Klebsiella pneumoniae, and E. coli (33). The difference in the reported values is due to the different extraction methods and different strains used in the experiments (28). In 2010, Anderl et al. showed that both A. vera gel and leaf inhibit the growth of S. aureus isolates rom wound infection, but A. vera gel had no effect on P. aeruginosa (34).

There are inconsistencies regarding the effectiveness of A. vera extract in different solvents. Irshad et al. showed that A. vera methanolic extract has the highest antibacterial activity compared to ethanolic and aqueous extract (35). Ibrahim et al. (2011) showed that the antibacterial and antifungal activity of Estonian extract was higher than that of aqueous and ethanolic extracts (36). Pandey and Mishra investigated the antibacterial properties of A. vera extract on pathogenic bacteria and reported that the aqueous extract had no inhibitory effect on gram-negative bacteria and had weak inhibitory effect on gram-positive bacteria (37). These inconsistencies may be related to difference in solubility and components of the A. vera, extract, especially in cases of solvents with specific antifungal or antimicrobial activity. In the present study, silver nanoparticles had the greatest effect on P. aeruginosa and the least effect on S. aureus. In 2014, Asadi et al. found that the inhibitory effects of silver nanoparticles on E. coli were greater than those on S. aureus. They also showed that the type of bacteria, contact time, and concentration of silver nanoparticles are effective factors in the antimicrobial properties of silver nanoparticles (38), which is in line with our findings. Cho et al. reported 5 and 10 ppm as the lowest inhibitory concentrations for S. aureus and E. coli, respectively. However, concentrations of 50 ppm for S. aureus and 100 ppm for E. coli were found to be fatal (39). Kim et al. reported that silver nanoparticles with a diameter of 20 nm could inhibit the growth of S. aureus (40). Feng et al. showed that E. coli is inhibited at a lower concentration of silver nanoparticles compared with S. aureus. This may be due to the presence of a thicker peptidoglycan layer in the cell wall of S. aureus (41). In 2007, Pal et al. showed that silver nanoparticles produced by non-biological methods have different antibacterial effects against E. coli (42). Ruparelia et al. examined the antibacterial properties of silver and copper nanoparticles and showed that E. coli was more resistant to both nanoparticles than S. aureus, but this difference was greater in case of silver nanoparticles (43). Differences in the findings of our study and previous studies could be due to nanoparticle concentrations, microbial strains, and nanoparticle production methods. Many sources have shown that the lethal effect of silver nanoparticles is due to their simultaneous action on the wall, their ability to penetrate the cytoplasmic membrane, and their effect on the cellular respiratory chain of RNA and DNA. These structures are the same in gram-positive and gram-negative bacteria.

Therefore, the antibacterial properties of silver nanoparticles are somewhat similar for both groups of bacteria (44).

CONCLUSION

For the first time, this study showed that the combination of ethanolic extract of A. vera and silver nanoparticles is effective against potentially pathogenic bacteria. Given the high rate of antibiotic resistance and side effects of conventional antibiotics, it is recommended to identify active compounds of this plant and evaluate the antimicrobial effects of this combination of fungi and other pathogenic bacteria both in vitro and in vivo.

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Ethics approvals and consent to participate

The study protocol was approved by the Islamic Azad University, Ahar branch, Iran.

Conflict of interest

The authors declare that there is no conflict of interest regarding publication of this article

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