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Effects of the Aqueous Extract of *Aloe vera* on the Morphological and Physiological Properties of *E. coli*

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Abstract

Background: Resistance to antibiotics is a growing worldwide problem. The inappropriate use of antibiotics has rendered some strains of bacteria resistant to antimicrobial drugs, making the treatment of infections much more difficult. However, many herbs and their derivatives are effective against drug resistant microbes.

Objectives: The aim of this research was to evaluate the effects of an *Aloe vera* extract on the morphological and physiological properties of *Escherichia coli*.

Patients and Methods: A clinical isolate of *E. coli* was obtained from a patient harboring a urinary tract infection (UTI), and was identified using biochemical and microbiological methods. Aqueous extracts of *Aloe vera* were prepared, and the minimum inhibitory concentration (MIC) of the extract was determined for *E. coli* via the microdilution method. The bacterium, at a concentration of 1.5×10^8 cells/mL, was exposed to the MIC of the extract. Then, the morphology of the cells was studied using optical microscopy, and the physiological properties were studied using biochemical testing and differential culture media.

Results: The MIC of the *Aloe vera* extract was 2.23 mg/mL, and was able to prevent the growth of *E. coli*. The morphological examination of the bacteria exposed to the extract at the MIC revealed that the cells were shrunken, the concentration was reduced, the number of coccobacilli was increased, and the staining property of this bacteria changed ($P < 0.0001$). In addition, the extract caused a 76% reduction in the bacterial cell number, in comparison to the control ($P < 0.0001$), and a change in the physiological properties and growth of the bacteria.

Conclusions: This study showed that *Aloe vera* has antibacterial effects against *E. coli*, and can result in structural and physiological changes in this bacterium.

Keywords: Morphological Changes, Physiological Changes, *Aloe vera*, *E. coli*

1. Background

Today, many antibiotics have become ineffective due to the widespread expression of antibiotic resistant genes. Moreover, the use of antibiotics has many side effects, such as hypersensitivity reactions, immunosuppression, etc. (1).

The use of medicinal herbs has long been considered in the treatment of infections, and given the intensification of an herb's effects in its extract, and higher impact than in an herb's purified substance, its use is an optimal choice (2). *Aloe vera* (*Barbadensis miller*), from the Liliaceae family, is native to tropical areas, and has a colorless gel in its leaves (3). Clinical studies have determined that there are active medicinal components in this gel, as well as in the green parts of the *Aloe vera* leaves. These bioactive compounds can be used in various treatments, such as in burns, allergic reactions, injuries, and skin diseases, and are effective as anti-diabetic (4), anti-infection (5), anti-bacterial (6), anti-mutation, and anti-cancer medications (7).

Escherichia coli is Gram-negative, motile, facultatively anaerobic, non-sporulating bacteria. It uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate, and carbon dioxide (8). Therefore, the growth of *E. coli* can be driven by aerobic or anaerobic respiration. Some strains possess flagella and are thus motile (9).

2. Objectives

The aim of this study was the evaluation of the effects of the aqueous extract of *Aloe vera* on the cell morphology and physiology of a clinical isolate of *E. coli*.

3. Patients and Methods

3.1. Aloe vera Extraction

In this experimental study, fresh *Aloe vera* plants were acquired from herb farms, and washed several times with tap water. For extraction, the whole leaves were ground using a crusher, and dried over several days in dark conditions, away from sunlight, at a temperature of 24 ± 2°C.

The extraction was accomplished using distillation, with 200 grams of powder in 500 mL of water for one hour, without any change in the temperature. Then, the temperature was gradually increased until the mixture reached the boiling point, and was kept constant at an average of 100°C. This process was continued for 16 hours until, finally, approximately 100 mL of the herbal extract was obtained. Centrifugation was used to purify the extracts.

3.2. Clinical Isolate

The *E. coli* strain was acquired from the purified clinical cultures of the microbial department of tuberculosis and pediatrics infectious diseases research center at the Arak University of Medical Sciences in Iran. The identification of the bacterium was conducted via Gram staining and referential biochemical tests, including growth in urea agar, triple sugar iron (TSI), Voges-Proskauer (VP), methyl red (MR), sulfide indole motility (SIM), and Simmon's citrate media.

The drug susceptibility testing was conducted using the following antibiotics: tetracycline, ceftazidime, amikacin, gentamicin, cephalothin, ampicillin, and ciprofloxacin.

3.3. Disk Diffusion Method

The sensitivity of the bacteria to the *Aloe vera* herbal extract was examined via the Kirby-Bauer method. A concentration of 0.5 was prepared, based on the McFarland scale (1.5×10^8 cfu/mL), and the suspension was spread on Mueller-Hinton agar plates. Blank discs impregnated with 20 μL of the herbal extract, in concentrations of 285.7, 142.85, 71.42, 35.71, 17.85, 8.92, 4.46, and 2.23 mg/mL, were placed on the media at a distance from the plate wall. Then, the plates were placed in an incubator for 24 hours at 37°C, and the zone diameters of the bacterial growth inhibition were measured and reported in millimeters.

3.4. Examining the Morphological and Count Changes

In order to examine the effects of the *Aloe vera* extract on the morphology of the bacteria, a fresh culture of the *E. coli* bacteria was prepared on blood agar media. The 0.5 McFarland scale concentrations of the bacterial suspensions were prepared in Mueller-Hinton broth; then, 4.75 μL of the *Aloe vera* extract and 600 μL of the microbial suspension were poured into a 1.5 mL micro-tube (the final concentration of the extract was 2.23 mg/mL). The suspension was mixed thoroughly and, at intervals of 0.5, 1, 2, 3, 4, 5, and 24 hours after adding the substances, samples from the suspension were placed on slides and stained by alkaline fuchsin. Additionally, a control sample (4.75 μL of normal saline and 600 μL of the 0.5 McFarland microbial suspension) was used for comparison with the bacteria exposed to the extract.

The bacteria exposed to the extract were morphologically examined in terms of any changes in shape by one person, using an Olympus Optical microscope, model CX21. In order to examine the effects of the *Aloe vera* on the number of bacteria, using a modified neo-bar slide, the number of bacteria were counted at intervals of 0, 0.5, 1, 2, 3, 4, 5, and 24 hours after adding the extract. The number of bacteria were also counted in the control slides.

3.5. Examining the Physiological Changes

The bacteria were cultured in differential media consisting of Simmon's citrate, TSI, SIM, and OF (24 hours of incubation at 37°C), to examine the effects of the *Aloe vera* extract on the physiology of the bacteria, at intervals of 0.5, 1, 2, 5 and 24 hours after adding the extract. The results of these cultures were evaluated.

3.6. Statistical Analysis

All of the data in this study were expressed as the means ± standard deviation (SD). The statistical significances of the differences between multiple groups were explored using the analysis of variance (ANOVA) and Kruskal-Wallis test, with GraphPad Prism 6.01 software (GraphPad Software Inc., San Diego, CA, USA). P values of less than 0.05 were considered to be significant.

4. Results

4.1. Results of Clinical Isolate Identification

In the microbiological examinations, it was confirmed that the *E. coli* sample was a Gram-negative, indole positive, MR positive, VP negative, citrate negative, urea negative, motile, acid/acid bacterium. To eliminate the possibility of contamination, at 0.5, 1, 2, 5, and 24 hours intervals, samples from the test and control groups were cultured on Simmon's citrate media, and citrate-negative bacteria were observed at all of the intervals.

4.2. Results of Antibiogram Testing

The results of this study showed that the *E. coli* bacteria were sensitive to tetracycline, ceftazidime, ciprofloxacin, amikacin, gentamicin, and cephalothin, and resistant to ampicillin (tetracycline: 15 mm, ceftazidime: 14 mm, ciprofloxacin: 34 mm, amikacin: 18 mm, gentamicin: 14 mm, cephalothin: 17 mm, and ampicillin: resistant).

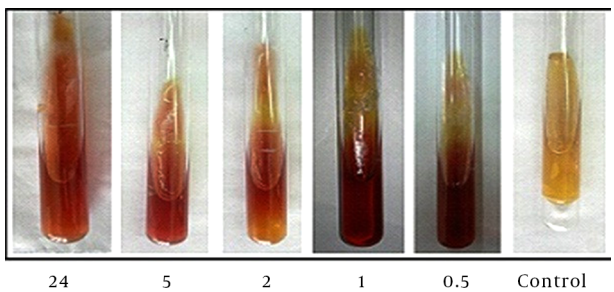
4.3. Results of MIC

Using the micro-plate dilution method, a concentration of 2.23 mg/mL was achieved as a result of the MIC. In addition, the Kirby-Bauer method showed up to an 8.92 mg/mL growth inhibition zone.

4.4. Physiology Results

The results of the biochemical tests showed that the exposed bacteria, when compared to the control bacteria, lost the ability to grow on the EMB and TSI media after 5 hours. The bacteria, after being exposed to the extract for 0.5 hours, transformed from acid/acid to alkaline/alkaline (alk/alk) and lost its ability to produce gas. In addition, after 0.5 hours of being exposed to the extract, the *E. coli* lost the ability to dissolve tryptophan, thus becoming indole-negative, and lost the ability to move in the SIM medium after 2 hours. Finally, the bacteria's ability to produce acid, when exposed to the extract, was lower than the control bacteria, causing the metallic luster of the bacteria to be lost after 2 hours on the EMB medium.

Figure 1. The Results of Culturing the Control Bacteria, the Bacteria Exposed to the *Aloe vera* Extract, and the Control on the TSI Medium

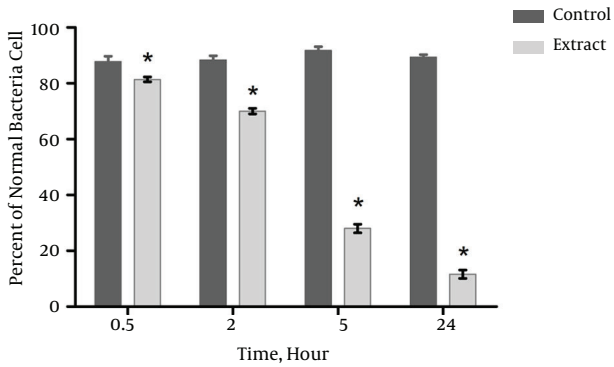


As observed, the extract caused the transformation of the bacteria from acid/acid to alk/alk, and the ability to produce gas was lost after 0.5 hours.

4.5. Morphology and Number

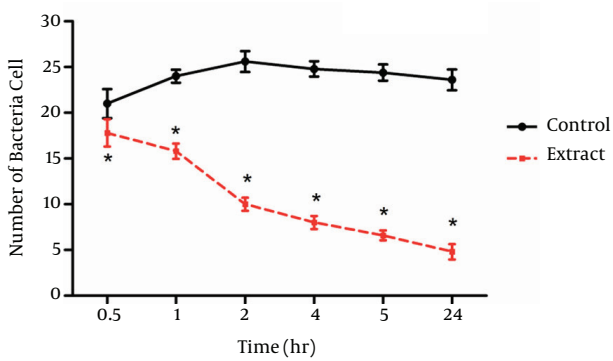
The morphological comparison of the *E. coli* exposed to the *Aloe vera* extract and the control bacteria showed that the extract caused the destruction of the bacteria, reduction in size, reduction in concentration, transformation to coccobacilli, and change in the staining properties of the bacteria. In addition, the comparison of the cell numbers of the *E. coli* exposed to the *Aloe vera* extract and the control bacteria showed that the extract caused the numbers of *E. coli* to be reduced by 76%, when compared with the control bacteria.

Figure 2. The effects of the *Aloe vera* Extract on the Morphology of the *E. coli* Compared to the Control Sample, With the Help of Gram Stained Lams



With regard to the effects of the *Aloe vera* extract on the different time intervals of *E. coli*, the percentage of normal cells was reduced as time passed. This effect was quite evident at 30 minutes, and increased through 24 hours. *indicates a significant difference with the control bacteria ($P < 0.0001$).

Figure 3. Effects of the *Aloe vera* Extract on the Number of *E. coli* Compared With the Control Sample, Using Modified Neo-Bar Lams



At the interval of 30 minutes after adding the extract to the bacterial suspension, a reduction in the number is seen. This reduction gradually increases through 5 hours, when the greatest size effect is observed. The reduction in the number after 24 hours is significantly different when compared to that at 5 hours. *indicates a significant difference with the control bacteria ($P < 0.0001$).

5. Discussion

Given the increase in bacterial resistance to certain antibiotics, efforts to use compounds present in herbs to

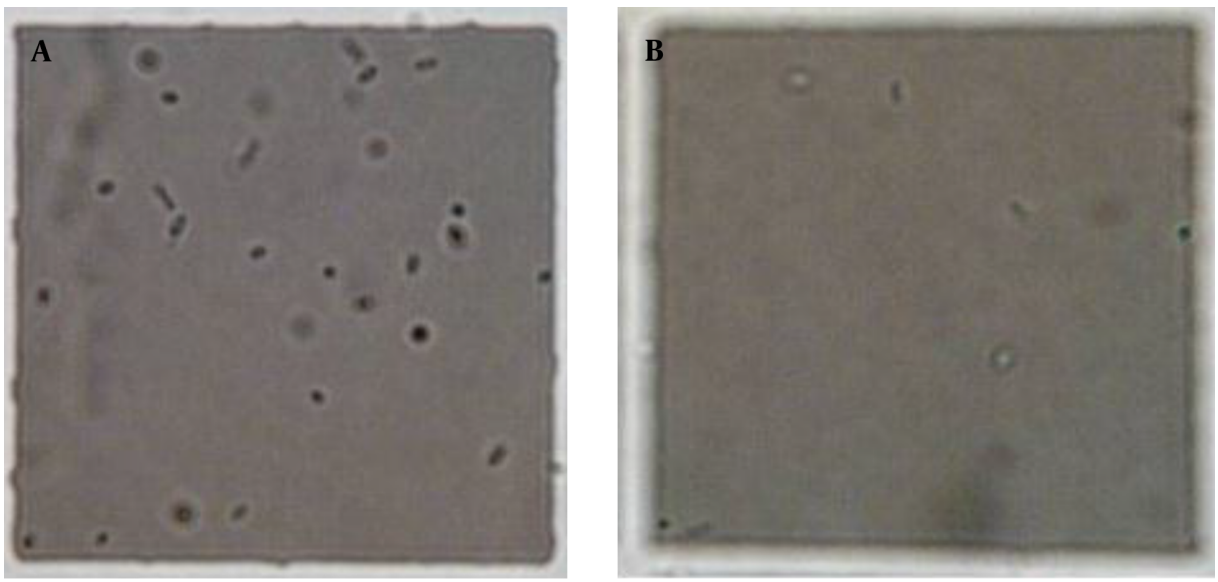
Table 1. Results of Biochemical Tests of the Control Bacteria and the Bacteria Exposed to the Extract (Hours)

	Gas Production	TSI	OF	Citrate	Indole	Motility	Colony Formation on TSI and EMB
Control	+	acid/acid	+	-	+	+	+
0.5	-	alk/alk	-	-	-	+	+
1	-	alk/alk	-	-	-	+	+
2	-	alk/alk	-	-	-	-	+
5	-	alk/alk	-	-	-	-	-
24	-	alk/alk	-	-	-	-	-

Table 2. Results of the Comparison of the Average Cell Number of the Bacteria Exposed to the Extract and the Control Bacteria in Each Small Square of the Neobar Lam

Time, h	Number of Bacterial Cells Exposed to the Extract in the Small Chamber of the Neobar Lam	Number of Control Bacterial Cells in the Small Chamber of the Neobar Lam	P Value
0.5	1.4 ± 18	1.5 ± 21	0.0001 > P
1	0.8 ± 16	0.7 ± 24	0.0001 > P
2	0.7 ± 10	1.1 ± 26	0.0001 > P
4	0.7 ± 8	0.8 ± 25	0.0001 > P
5	0.5 ± 7	0.8± 24	0.0001 > P
24	0.8 ± 5	1.1 ± 24	0.0001 > P

Figure 4. Results of the Examination of the Cell Number of the Bacteria Exposed to the Extract and the Control Bacteria at the 24 hours Interval



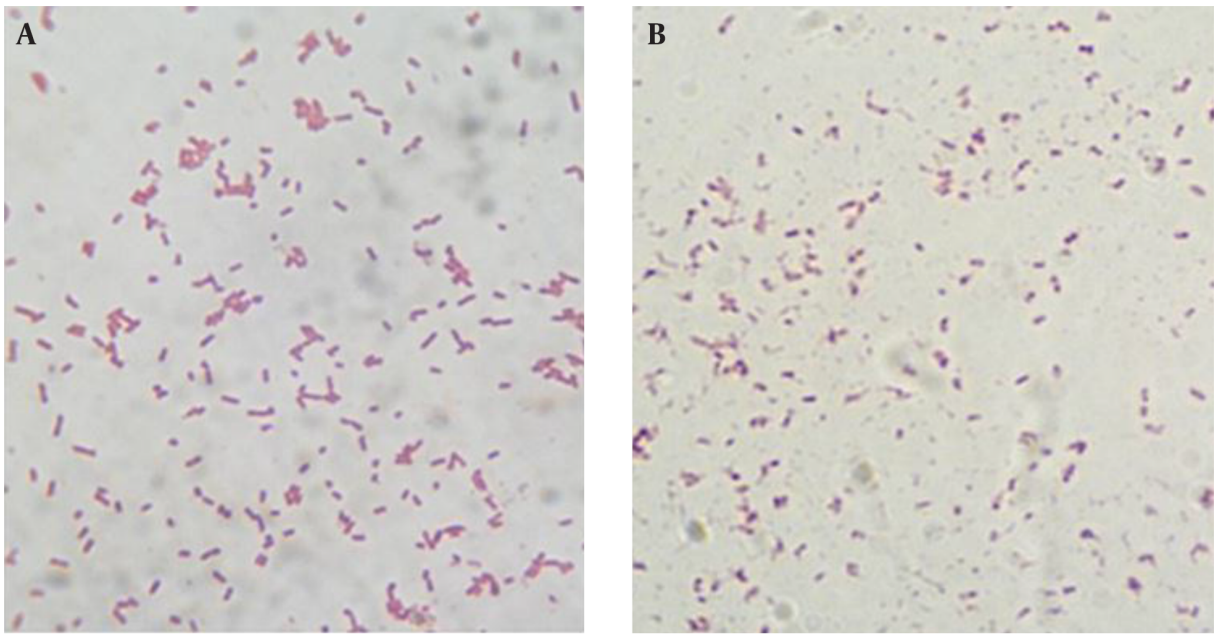
Control

Bacteria Exposed to the Extract

The extract causes a reduction in the average number of bacteria, from 24 ± 1.1 to 5 ± 0.8 (79% reduction).

treat various infections have been doubled. With regard to the study of the anti-microbial effect of medicinal herbs, extensive research has been conducted in our country and many others, mostly on microorganisms. These studies support the anti-microbial effects of the herb under investigation in the present study, based on the results of the

Figure 5. Results of the Examination of the Morphology of the Bacterial Cells Exposed to the Extract and the Control Bacteria at the 24 hours Interval



Control

Bacteria's Cells Exposed to the Extract

The extract caused an increase in the coccobacillus, an increase in the destruction, and a change in the staining properties of the bacteria.

Table 3. Results of the Comparison of the Percentage of Bacterial Cells Exposed to the Extract and the Control Bacteria

Time, h	Percentage of Normal Bacterial Cells Exposed to Extract	Percentage of Normal Control Bacterial Cells	P Value
0.5	81	88	0.0001> P
2	70	88	0.0001> P
5	28	92	0.0001> P
24	12	89	0.0001> P

growth inhibition zone (10).

This study examined the effects of an *Aloe vera* extract on the physiology and morphology of *E. coli.* Based on the MIC obtained, the aqueous extract of *Aloe vera* inhibited the growth of *E. coli*. In this study, the minimum inhibitory concentration of the aqueous extract of *Aloe vera* was calculated at a dilution of 1/128 (2.23 mg/mL). In a study by Shilpakala et al., a dilution of 1/64 in the growth inhibition zone was observed (11). Additionally, a study by Stanley et al. reported the minimum inhibitory concentration of the aqueous extract of *Aloe vera* as 0.25 mg/mL (12). Irshad (13) stated that the methanolic extract of *Aloe vera* had the maximum antibacterial activity; although, in our study, the aqueous extract was the best. In 2012, Athiban (14) reported that *Aloe vera* was indeed effective as a GP decontaminant, which is comparable to the results of our study. In his work, Kaithwas (15) concluded that *Aloe vera* juice was more effective than *aloe vera* gel, which was concordant with the results of our work.

In addition, detectable changes were observed in the shape of the bacteria, such as changes in size, increases in coccobacilli, a reduction in staining, increase in destruction, and reduction in the concentration of the bacteria, when compared to the control bacteria. The results of this research showed that the aqueous extract of *Aloe vera* greatly affects the physiology of the *E. coli*, so that the exposure of the bacteria to the aqueous extract of *Aloe vera* causes the loss of the bacteria's ability to consume tryptophan, glucose, and lactose, as well as the loss of the ability to move and to produce acid and gas. Furthermore, the aqueous extract of *Aloe vera* caused a significant reduction in the number of bacterial cells.

The anti-microbial effects of the *Aloe vera* were promising, showing the potential for producing new medicinal herbs which, in addition to having strong anti-microbial effects, would have less side effects as well.

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Footnotes

Authors' Contribution: Study concept and design, Mahdi Kargaran, Ali Reza Moradabadi, Mohammad Arjomandzadegan; acquisition of data, Mahdi Kargaran, Ali Reza Moradabadi, Mohammad Arjomandzadegan, Hossein Hosseini, Ghasem Habibi, Maryam Tayeboon, Ahmad Akbari; analysis and interpretation of data: Mahdi Kargaran, Mohammad Arjomandzadegan; drafting of the manuscript, Mahdi Kargaran, Mohammad Arjomandzadegan, Ahmad Akbari; critical revision of manuscript for important intellectual content, Mohammad Arjomandzadegan; statistical analysis, Mahdi Kargaran; administrative, technical, and material support, Ghasem Habibi, Maryam Tayeboon, Ahmad Akbari; study supervision, Mohammad Arjomandzadegan.

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