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Evaluation of antioxidant properties and antimicrobial potential of *Aloe vera* extract on a number of Gram-positive and Gram-negative bacteria: an *in vitro* study

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ABSTRACT

Nowadays food products who contain "natural" ingredients are trending among consumers as a result of raising knowledge and awareness about food ingredients and their influence on human's body health. The aim of this study was to investigate the biochemical (total phenol and flavonoid) of Aloe vera aqueous extract as a natural preservative and determination of antioxidant and antibacterial activity of the extract. Total phenol and total flavonoid content of the aqueous extract was measured as 41.61± 0.78 mg GAE/g extract and 783.33 ± 5.13 mg QE/g extract respectively. Free radical scavenging activity determined at different concentrations of Aloe extract; at highest concentration (600 mg/ml) %68.395 inhibitory effect was estimated through DPPH assay and %50.075 through ABTS assay. Aloe extract showed a greater effect on Gram-positive bacteria through disk diffusion agar and well diffusion agar methods. Minimum inhibitory concentrations (MIC) for *Escherichia coli, Shigella dysenteriae, Salmonella typhi, Staphylococcus aureus, Listeria monocytogenes* and *Bacillus cereus* estimated 32, 64, 32, 16, 32 and 16 mg/mL respectively. Based on results in this study, Aloe vera aqueous extract can be a candidate to use as a preservative in food products.

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1- Introduction

So far, many evidences have been obtained about the direct effect of food on human health, and all these evidences have increased the level of consumer's demand for healthy and nutritious foods to be available. Also, the spread of diseases such as cancer, diabetes, and cardiovascular problems caused by diet and improper lifestyle in today's societies; has caused people to worry and reconsider about their consumption limits. Plants have been used by humans for centuries in various fields such as food and medicine. In fact, the presence of compounds derived from the plant's secondary metabolism, such as phenolic, flavonoid, terpenoid, and other biologically useful compounds, as well as high fiber, has made plants not only consumed as food sources, but also one of the oldest ways to prevent or treat various diseases, which are still used today [1 & 2].

In the food industry, plants and their compounds can be used directly or indirectly. One of the ways to increase the shelf life of food is to use artificial preservatives. These preservatives gradually being replaced with natural preservatives or herbal extracts and essences, because of negative view of consumers to their artificiality due to increasing consciousness about food products and its ingredients. Plant extracts are called green liquids, which are extracted from different parts of the plant using different solvents such as water, ethanol, methanol, and ethyl acetate. Antimicrobial and antioxidant properties consider as characteristics of plant extracts due to their composition and nature. It can be stated that the antimicrobial properties of ethanolic extracts are higher than aqueous extracts. Different parts of plants have verity of these compounds, including polyphenols, which have a significant effect on reducing oxidation. In fact, the presence of plant extracts in food products has caused a series of changes in the media, which leading by the food engineers can apply desirable results to the product [3, 4 & 5].

The harsh and unfavorable conditions of plant growth and the accumulation of free radicals have created complex enzymatic and non-enzymatic antioxidant systems in them. The non-enzymatic system with different mechanisms increases the resistance of plant cells against oxidative stress and thus strengthens this feature in the body when consumed by humans. Oxidative stress is responsible for some disorders and diseases such as Alzheimer's disease, cancer, arteriosclerosis and cardiovascular disorders in humans. In fact, the presence of polyphenolic compounds increases the antioxidant capacity and create balance in the human body. It should be noted that these compounds will exert their effect even in low concentrations [6].

Nowadays, the resistance of pathogens to the inhibitory activity or destruction by antibiotics has increased due to their indiscriminate and irregular use. Isolation of bioactive compounds derived from medicinal plants due to their therapeutic nature and many useful compounds, high diversity, cheap and easy access provide a suitable approach to fight against pathogenic microorganisms. The World Health Organization has declared microbial resistance as the most urgent problem that medical community has faced. In order to solve this emerging problem and considering the fact that currently a percentage of the drugs used are of herbal origin; through identifying the antimicrobial compounds of different plants and using them, it is possible to discover and produce new drugs with proper performance against resistant pathogens. As a result, determining the antimicrobial activity of different plants has become a new goal for researchers [7 & 8].

Aloe vera is a green and perennial plant from the *Liliaceae* family that grows mostly in dry and warm regions (Middle East, North Africa, and Southern Mediterranean) which for thousands of years has been used as a medicinal plant for treating wounds, burns, and skin inflammation. This plant has about 98% water and colorless fillet is placed inside its green tulips. Today, the gel obtained from the fillet inside its fleshy leaves is used in the pharmaceutical, cosmetic and food industries. The colorless gel obtained from the inside of the leaves is called *Aloe vera* mucilage. There are more than 75 useful compounds in this gel such as vitamins, enzymes, minerals, anthraquinones, fatty acids and carbohydrates, each of these compounds have important therapeutic properties such as anti-diabetes, anti-cancer and anti-lipid properties. Various researches have proven that the presence of acemannan polysaccharide as the main polysaccharide and anthraquinones in *Aloe vera* is the main factor of its antioxidant and antimicrobial properties, which has led to the use of this gel or the compounds isolated from it as a preservative in various fields and food products. Also, the phenolic

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and flavonoid compounds of this plant have a significant effect in this matter [9 & 10].

Aloe vera gel is known among people as a diet food for weight loss due to its high fiber and water content. Its powdered products, drinks and even gel are used by diabetic patients or people with chronic obesity. Due to its antimicrobial and antioxidant properties, this gel is used in film and edible coatings for bioactive food packaging. The addition of this gel to food products like sauces, dairy products such as cheese and yogurt, as well as ice cream has also been reported [11].

The active compounds reported in the leaves of this plant that are involved in its therapeutic properties include: saponin, aloesin, enzymes, sugars, sterols, chromones, anthraquinones, aloin, aloe emodin, sterols, lignin, salicylic acid, flavonoids, minerals and phenolic compounds. In a study, the antimicrobial activity of ethanolic extract of the leaf of this plant was investigated against bacterial strains such as *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus* and some fungi, which showed good results [12].

Also, the flavonoid content as well as the antioxidant potential of two aqueous and ethanol extracts of *Aloe vera* were investigated and proved [13]. The aim of this research is to determine the amount of total phenol and flavonoids, to evaluate the antioxidant effect and antimicrobial activity of the aqueous extract obtained from *Aloe vera* leaf by disk diffusion method, well diffusion in agar, and to determine the minimum inhibitory concentration and the minimum bactericidal concentration of extract against pathogens such as *Escherichia coli*, *Shigella dysenteriae*, *Salmonella typhi*, *Staphylococcus aureus*, *Listeria monocytogenes* and *Bacillus cereus*.

2- Material and methods

2-1- Extraction method for *Aloe vera* leaves

Green leaves of the plant were washed and their surface were gently cleaned with a brush as the first step to obtaining the extract. Then, to separate the thick yellow liquid present in Aloe vera, the head and base were cut and the leaf was placed vertically in distilled water for 60 minutes. After that, the blades and one side of the green latex were separated with a knife and the colorless sticky *Aloe vera* fillet (mucilage) was extracted. The mucilage were stirred for 3 minutes in a domestic blender so a uniform and homogeneous gel was obtained, and the final extract was prepared by passing this gel through a cotton cloth (such as muslin) to filter the suspended particles and polysaccharides from extract [14].

2-2- Chemical tests

2-2-1- Determining total phenolic content of *Aloe vera* aqueous extract

The folin–ciocalteu assay (colorimetry) was performed and the total amount reported as mg of gallic acid per gram of nanoemulsion. First, a certain concentration of extract was prepared and 0.5 ml of it was added to folin solution (10%). The obtained solution was well stirred and after 6 minutes, 2 ml of sodium carbonate (7.5%) was added to. After 30 minutes of incubating the final solution at room temperature in a dark place, the absorbance of the solution was determined by a spectrophotometer (WPA, England) at a wavelength of 765 nm and measured by placing in the formula obtained from the gallic acid standard curve [15].

2-2-2- Determining total flavonoid content of *Aloe vera* aqueous extract

Total flavonoid content of the Aloe extract was also measured by colorimetry method using a spectrophotometer. Standard solution of quercetin was prepared and standard curve obtained using different concentrations of this solution, the total flavonoid in the extract was measured by placing at the standard curve equation and reported as mg quercetin per gram of nanoemulsion. To perform this test, 75 µl of sodium nitrite solution (5%) was added to the Aloe extract using a sampler and after stirring, incubated for 6 minutes. 150 µl of Aluminum chloride solution (10%) was added to the mixture with a sampler and incubated again for 5 minutes. Finally, by adding 1 ml of sodium hydroxide (1 M), the absorbance of the final solution was immediately measured at the wavelength of 510 nm, and the total amount of flavonoid was reported accordingly [16].

2-2-3- DPPH assay (2,2-Diphenyl-1-picrylhydrazyl)

Evaluating of the percentage of DPPH free radical scavenging of different concentrations of the extract done using spectroscopy, following hojjati *et al.* [17] method with a slight change. First, different concentrations of extract were prepared (60, 80, 100, 200, 400 and 600 mg/ml). Next step, a solution of DPPH powder (methanol as the solvent) was prepared which its absorbance at the wavelength of 517 nm was about 0.7 (control). Different emulsion

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dilutions were mixed with methanol solution in tubes (1:1 v/v) and their absorbance recorded after incubating for 30 minutes in a dark environment. The percentage of free radical scavenging was calculated using equation 1.

$[(A_{control}-A_{sample})/A_{control}] \times 100$ Equation 1

2-2-4- ABTS assay (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid))

In this test, the percentage of free radical scavenging against $ABTS^+$ was calculated and reported by following the Kaparakou *et al*. [18] method with some changes. First, different dilutions of extract (60, 80, 100, 200, 400 and 600 mg/ml) were prepared. Control solution was obtained by mixing certain amount of $ABTS^+$ with distilled water and added to a 2.45 mM solution of potassium persulfate with a ratio of 1:2. Control solution was placed in a dark place for 16 to 24 hours to activate the free radicals on the next day, methanol was added to the solution until its absorbance reached about 0.7 at the wavelength of 734 nm. Finally, different dilutions of the extract were mixed with the control solution at a ratio of 1:1 and their absorption were recorded after 6 minutes. The percentage of radical scavenging related to different concentrations was determined and reported using equation 1.

2-3- Estimating the inhibitory potential of extract against pathogens

Determination of antimicrobial activity of the nanoemulsion was performed against 6 pathogenic bacteria, including *Escherichia coli* ATCC 12435, *Shigella dysenteriae* ATCC 13313, *Salmonella typhi* ATCC 65154 (gram negative bacteria), *Staphylococcus aureus* ATCC 14154, *Listeria monocytogenes* ATCC 19115 and *Bacillus cereus* ATCC 10876 (gram positive bacteria). For this purpose, 4 techniques were used: disc diffusion agar, well diffusion agar, minimum inhibitory concentration and minimum bactericidal concentration.

2-3-1- Preparing foodborne pathogenic bacteria

To perform microbial tests, fresh cultures of pathogenic bacteria were needed. Standard suspensions of the included bacteria were prepared using 0.5 McFarland standard (1.5 x 108 CFU/mL). This standard suspension was prepared using a spectrophotometer at a wavelength of 625 nm at an absorbance between 0.08 and 0.13 [19].

2-3-2- Disc diffusion agar assay

At first, the Aloe extract sterilized by a 0.22 µm syringe filter. Measuring the diameter of inhibition zones, first the blank disc was soaked inside the sterile *Aloe vera* extract for 15 minutes. 100 µl of standard suspensions were transferred on Müller-Hinton Agar using spread plate method. Then, on each petri dish, a disk containing emulsion, a blank disk (negative control) was placed at certain distances from each other and the Petri dish wall. Then the petri dish placed into the refrigerator for 15 minutes and after 24 hours of incubation at 37°C, inhibition zones were measured and reported at millimeter scale [20].

2-3-3- Well diffusion agar

Based on this method, after using spread plate method for bacterial suspensions on the MHA, wells with specific distances from each other and the Petri dish wall were cut on the agar using sterile glass pasteur pipette and the bottoms were closed with semi-solid (milten) agar. Then, 20 µl of the extract was transferred into the well using a sampler. The same amount of sterile distilled water (control) was poured into other well. After the incubation time, the diameters of the inhibition zones around the wells were measured [21].

2-3-4- Minimum inhibitory concentration

The serial dilution method was used to perform this microbial test. First, the initial concentration was prepared mixing Müller-Hinton broth culture medium, extract and 5 ml of dimethylsulfoxide (DMSO). Other sequel concentrations obtained by adding 5 mL of MHB to each one of them. 100 µl of each concentration was taken with a sampler and transferred into the wells of a 96-well plate. Then 20 µl of the prepared suspensions was added to each dilution so that each row contained different concentrations of the extract containing bacteria. A positive and a negative control was considered in each row. The negative control was the mixture without antibacterial agent and the positive control was the mixture without bacteria. After incubation for 24 hours at 37°C, the 96-well plate was removed from the incubator to add color reagent to the houses. A 5% solution of 2,3,5-Triphenyl-tetrazolium chloride (TTC) as color reagent was

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added (20 µl) to the and transferred to the incubator again for 30 minutes. The first well without preserving red color was selected as the minimum concentration that inhibited bacterial growth [22].

2-3-5- Minimum bactericidal concentration

To determine the minimum bactericidal concentration of the nanoemulsion, 100 µl of colorless wells (no bacterial growth) were taken and cultured on the MHA. 24 hours after incubating, Petri dishes were removed and the concentrations that were free of bacterial growth were selected and reported as MBC [23].

2-4- Statistical analysis

The average of data obtained from the experiments with 3 repetitions were analyzed and results reported using analysis of variance (one-way Anova) at a significance level of 0.05 in Duncan's test. Therefore, SPSS (26 version) was used.

3- results and discussion

3-1- Evaluating the compounds and antioxidant potential of Aloe extract

The leaves of edible plants contain large amounts of bioactive compounds such as phenolic and flavonoid compounds, which are referred to as natural antioxidants [24 & 25]. Phenolic and flavonoid compounds (phytochemicals) inhibit free radicals through reaction by different mechanisms. As a result, with the high amount of these bioactive compounds, a higher antioxidant power can be achieved. In the present study, the total phenol content was 41.61 ± 0.78 mg GAE/g extract and total flavonoid content was 783.33 ± 5.13 mg QE/g *Aloe vera* aqueous extract. Kumar *et al.* investigated phytochemicals and antioxidant power of methanolic extracts of *Aloe vera* leaves obtained by different researchers. The total phenol content reported varied from 63.2 to 32.9 mg GAE/g extract. In all studies, the presence of phenolic compounds in the extract was recorded in significant amounts. Also, in this study and previous ones, it was concluded that there is a significant linear relationship between phenolic content and antioxidant power [26 & 27].

Sharma *et al*. compared the total phenolic and flavonoid content of two aqueous and methanolic extracts of *Aloe vera* leaves; based on the reported results of this research, the amount of these two compounds in different extracts did not differ significantly [28]. Bista *et al.* also compared the total phenolic and flavonoid content of methanolic and ethanolic extracts of *Aloe vera* leaves, and the results showed that the amount of these compounds in the methanolic extract was higher and equal to 30.53 mg of gallic acid and 73.26 mg of quercetin per gram of dry weight of the extract. According to several studies, the amount of flavonoid in all extracts of Aloe leaves was higher than the amount of total phenol. The difference in the data obtained in different studies could be due to various reasons such as the the plant growth environment, harvesting time, geographical location, different methods and different solvents used through the extraction, the concentration used in the test and also different standard curves. Nevertheless, based on several studies, the amount of bioactive chemical compounds was usually determined by aqueous extract rather than other extracts with different solvents [29, 30].

The free radical scavenging is the ability to neutralize the free radical by giving them an electron and prevent the reaction and oxidative stress; which causes a decrease at color of the solution through weakening the absorption in the wavelength by combining electron and radical inhibition leading to a stable state [31 and 32]. The results of the investigation of antioxidant activity by two methods of inhibiting DPPH and ABTS free radicals are mentioned in Table 1.

Based on the observed results, the power of *Aloe vera* extract for inhibiting DPPH free radical was more than ABTS free radical. The highest inhibitory percentage of DPPH, corresponding to the concentration of 600 mg/ml was equal to 68.39%, and the lowest inhibitory activity for the concentration of 60 mg/ml of the extract was determined equal to 36.35%. The inhibitory action of these

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concentrations for $ABTS^+$ was 50.07% and 41.64%, respectively.

According to the results mentioned at Table 1, with a gradual increase in the concentration of the extract from 60 to 600 mg/ml, a significant increase in the inhibitory activity (more than 30%) for DPPH was observed; although, with an increase in the concentration with same ratio, the rate of ABTS radical inhibition increased more slowly (less than 10 percent). However, in both methods, the concentration of the extract had a direct relationship with the inhibition percentage and there was a significant difference between them. Manieh *et al*. determined and compared the radical scavenging of aqueous and methanolic extracts of *Aloe vera* in DPPH free radical scavenging. According to a study report, the antioxidant activity of aqueous extract was significantly higher than methanolic extract. DPPH radical inhibition percentage was reported for aqueous extract in the range of 34.8-65.4% and for methanolic extract in the range of 15.4-48.1% [13]. Bista *et al*.'s study showed that the percentage of antioxidant effect of *Aloe vera* extract by DPPH method for methanolic extract was equal to 81.91%, which is higher than the antioxidant power of aqueous extract in this study and contrary to previous studies in which aqueous extract was stronger [29].

The antioxidant effect of *Aloe vera* is due to the bioactive compounds (polyphenols, enzymes, polysaccharides and anthraquinones) present in it. Acemannan is a storage polysaccharide and considered as the primary and important bioactive compound of *Aloe vera*; which plays an important role on inhibition of the ABTS free radical [33]. Caparaco *et al*. [18] investigated and reported the quantitative antioxidant activity of *Aloe vera* gel. Inhibitory activity against DPPH was estimated from 1.64 to 9.21 micromol of Trolox per ml of gel and for ABTS, equivalent to 0.73 to 5.14 micromol of Trolox per ml of gel. Based on the results, the antioxidant activity of ABTS assay was weaker than DPPH, which is in similar to the results of the present study.

Table 1. DPPH and ABTS radical scavenging activity of *Aloe vera* extract

Concentration (mg/mL)	Scavenging effect (%)	
	DPPH	ABTS
60	36.35 ± 0.40^A	41.64 ± 0.13^A
80	45.01 ± 0.30^B	42.61 ± 0.36^B
100	61.18 ± 0.12^C	43.67 ± 0.15^C
200	63.67 ± 0.24^D	44.29 ± 0.21^D
400	64.77 ± 0.55^E	47.95 ± 0.59^E
600	68.39 ± 0.44^F	50.07 ± 0.18^F

The data written in table, indicate "mean ± standard deviation", n=3. The capital English letters in each column show a significant difference at $P< 0.05$ between antioxidant activity of different extract concentrations.

3-2- Investigating the antimicrobial activity of *Aloe vera* aqueous extract

The antimicrobial activity of *Aloe vera* gel has been investigated in several studies so far. This extract has the characteristic of inhibiting the growth or killing of some bacteria, fungi and viruses. In past researches, the antimicrobial potential of the extract on many fungi have evaluated and reached some desirable results [34 & 35]. The disk diffusion method, also known as the Kirby-Bauer method, is one of the most flexible methods for determining the sensitivity of microorganisms to inhibitory agents. Agar well diffusion method is mostly used to evaluate the antimicrobial effect of plants or microbial extracts. This method is similar to disk diffusion, except that instead of using a disk, wells will be prepared in agar [37].

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Based on these two methods, the antimicrobial activity results of the pure extract were reported in Tables 2 and 3. Table 2 shows the diameter of the inhibition zone around the disk containing the extract; the largest diameter of inhibitory zone is related to the Gram-positive bacteria, *Staphylococcus aureus* with a diameter of 15.40 mm and the smallest diameter is related to the Gram-negative bacteria, *Shigella dysenteriae* with a diameter of 9.20 mm. Among gram-positive bacteria, *Bacillus cereus* and *Staphylococcus aureus* significantly showed the highest diameters. following these two bacteria, *Listeria monocytogenes* had the lowest resistance to the extract. Among Gram-negatives, *Salmonella typhi* was identified as the most resistant bacteria to antimicrobial agents.

Table 2. Inhibition zone (mm) of *Aloe vera* extract on bacterial growth using disk diffusion agar method

Inhibition zone (mm)	Pathogenic bacteria					
	E. coli	*S. dysenteriae*	*S. typhi*	*S. aureus*	*L. monocytogenes*	*B. cereus*
Aloe vera	10.10 ± 0.30^{A}	9.20 ± 0.40^{A}	12.00 ± 0.50^{B}	15.40 ± 0.20^{C}	13.30 ± 0.80^{B}	15.00 ± 0.60^{C}

The data written are "mean ± standard deviation", n=3. Similar capital letters show a significant difference at $P< 0.05$ between antimicrobial activity on different pathogens.

Table 3 shows the results related to the antimicrobial properties of the extract on the bacteria used based on the well diffusion method. In this method, the largest diameter of inhibition zone, was related to Gram-positive *Bacillus cereus* bacteria (16.20 mm) and the smallest diameter similar to the disk diffusion method was related to Gram-negative *Shigella dysenteriae* bacteria (10.00 mm). The inhibitory effect on the two pathogens *Staphylococcus aureus* and *Bacillus cereus* was significantly close to each other and more than the others bacteria. In both disk diffusion and well diffusion methods, the antibacterial effect of the extract on gram-positive bacteria was more than gram-negative, but the diameter of the inhibitory zones was slightly larger in the well diffusion method than disk diffusion.

Table 3. Inhibition zone (mm) of *Aloe vera* extract on bacterial growth using well diffusion agar method

Inhibition zone (mm)	Pathogenic bacteria					
	E. coli	*S. dysenteriae*	*S. typhi*	*S. aureus*	*L. monocytogenes*	*B. cereus*
Aloe vera	11.30 ± 0.60^{A}	10.00 ± 0.30^{B}	12.050 ± 0.40^{C}	16.10 ± 0.50^{D}	13.90 ± 0.50^{E}	16.20 ± 0.20^{D}

The data written are "mean ± standard deviation", n=3. Similar capital letters in the data row, show a significant difference at $P< 0.05$ between antimicrobial activity on different pathogens.

Danish *et al.* reported different results compared to our results. According to their report on the antimicrobial effect of ethanolic *Aloe vera* leave extract, this extract showed an inhibition zone with diameter of 18 mm against the gram-negative bacteria *Escherichia coli* at a concentration of 30 µl; Also, the inhibitory zone

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diameters obtained against the gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus*, on which the aqueous extract had the highest effect on them, was equal to 13 and 14 mm, respectively. These results were different from Tables 3 and 4, but according to the data reported in both studies, it can be said that the antimicrobial effect of aqueous and ethanolic extracts of *Aloe vera* leaves is more on gram-positive bacteria. In fact, the antimicrobial effect of *Aloe vera* aqueous extract on the three mentioned bacteria is less than the ethanolic extract of this plant [12].

Bendjedid *et al*. investigated the antibacterial effect of Aloe extract with 5 different solvents at the concentration of 20 mg/ml; the highest antimicrobial effect was related to n-butanol and acetone extracts of *Aloe vera* (respectively with the diameter of the inhibition zones 25.33 and 22 mm compared to *Staphylococcus aureus*). The aqueous extract used in this study showed an inhibition zone (10.33 mm) only against *Escherichia coli* bacteria [20].

In another study that investigated the antimicrobial effect of Aloe aqueous extract on Gram-negative bacteria, the diameter of the inhibition zone against *Escherichia coli* was reported to be 18 mm. In the same study, the MIC and MBC of this bacteria against *Aloe vera* aqueous extract were investigated and reported (0.625 and 1.25 mg/ml), respectively. Antimicrobial results are highly dependent on the used concentration. In some cases, even higher concentrations of the extract can show weaker results than lower concentrations [38].

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were evaluated and reported in Table 4.

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of *Aloe vera* extract

Pathogenic bacteria	MIC (mg/mL)	MBC (mg/mL)
E. coli	32	>512
S. dysenteriae	64	>512
S. typhi	32	512
S. aureus	16	256
L. monocytogenes	32	512
B. cereus	16	256

To determine these characteristics, first, different concentrations of the extract were prepared by the serial dilution method. Consecutive dilutions (512, 256, 128, 64, 32, 16, 8, 4, 2 and 1 mg/ml) were prepared from the extract. According to the obtained results, the minimum concentration of the extract that could prevent the growth of the microorganisms was 16 ml/mg against the gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus*.

The highest concentration of the extract, which had the ability to inhibit bacterial growth, was 64 mg/ml against Gram-negative *Shigella dysenteriae* bacteria. Most to the least antimicrobial effect of the extract against bacteria, in order of the minimum inhibitory concentration, was specified respectively: *Bacillus cereus* and *Staphylococcus aureus* > *Listeria monocytogenes*, *Salmonella typhi* and *Escherichia coli* > *Shigella dysenteriae*. The minimum bactericidal concentration of the extract against bacteria is mentioned in Table 4.

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The MBC of two gram-negative bacteria *Escherichia coli* and *Shigella dysenteriae* was higher than 512 mg/ml, but the gram-negative bacteria *Salmonella typhi* was destroyed at a concentration of 512 mg/ml of the extract. Two gram-positive bacteria, *Staphylococcus aureus* and *Bacillus cereus*, had a lower concentration of MBC extract, which indicates the higher antimicrobial effect of the extract on these two bacteria. And finally, the gram-positive bacteria *Listeria monocytogenes* had MBC at a concentration of 512 of the extract. In general, it can be said that the aqueous extract of *Aloe vera* had a higher antimicrobial effect on Gram-positive bacteria than Gram-negative bacteria. Various antimicrobial agents inhibit or kill bacteria by targeting different parts of the membrane. In fact, the higher resistance of gram-negatives against this extract can be justified by referring to the difference in the membrane structure of gram-positive and negative bacteria [39, 40 and 41].

Arbab *et al.* also investigated the antimicrobial activity of *Aloe vera* gel on bacteria involved in skin infections (*Escherichia coli*, *Staphylococcus aureus*, *Shigella* and *Salmonella*) which the gel showed high activity so that the results on 4 strains were very close to each other. In the same study, the effect of ethanolic extract on pathogens was further determined [42].

The antimicrobial activity of *Aloe vera* gel on two pathogens *Staphylococcus aureus* and *Escherichia coli* was investigated by Mohebbi *et al.* However, this substance was able to inhibit the growth of the mentioned bacteria in lower concentrations (2 and 8 mg/ml, respectively). In this study, *Aloe vera* at the same inhibitory concentration (8 mg/ml) was able to have a lethal effect on the gram-negative bacteria *Escherichia coli* [43].

4- Conclusions

In this study, the total phenol and flavonoid of aqueous extract obtained from *Aloe vera* leaves, were evaluated. Based on the results, subjected extract had a high antioxidant potential and also showed a proper antimicrobial activity against chosen 6 pathogens. A higher free radical scavenging was observed at DPPH assay rather than ABTS assay. Furthermore, antimicrobial effect of the extract reported to be stronger against gram-positive bacteria. At last, it is possible to use the *Aloe vera* extract at food industry as a natural preservative.

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ارزیابی ویژگی‌های آنتی‌اکسیدانی و پتانسیل ضدمیکروبی عصاره آلوئه‌ورا بر تعدادی از باکتری‌های گرم مثبت و گرم منفی: یک مطالعه آزمایشگاهی

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چکیده

در جوامع امروزی مصرف مواد غذایی با ترکیبات و نگهدارنده‌های طبیعی به‌دلیل آگاهی مصرف‌کننده به تأثیر رژیم غذایی بر سلامت و پیشگیری از بیماری‌ها افزایش یافته است. به همین منظور در این پژوهش میزان فنل و فلاونوئید تام، ویژگی‌های آنتی‌اکسیدانی و اثر ضدمیکروبی عصاره آبی به‌دست آمده از برگ گیاه آلوئه‌ورا مورد ارزیابی قرار گرفت. میزان فنل و فلاونوئید کل به ترتیب ٤۱/٦۱ میلی‌گرم گالیک‌اسید در هر گرم عصاره و ۷۸۳/۳۳ میلی‌گرم کوئرستین در هر گرم عصاره اندازه‌گیری شد. بالاترین درصد مهارکنندگی برای رادیکال آزاد DPPH ، ٦۸/۳۹٪ و برای رادیکال آزاد ABTS ، ۵۰/۰۷٪ در بیشترین غلظت (٦۰۰ میلی‌گرم بر میلی‌لیتر) مشخص شد. باتوجه به نتایج به دست آمده، بیشترین اثر ضدمیکروبی عصاره در روش‌های دیسک دیفیوژن آگار و چاهک آگار بر باکتری‌های گرم مثبت بود. کمترین غلظت بازدارنده از رشد برای پاتوژن‌های *اشرشیا کلی*، *شیگلا دیسانتری* ، *سالمونلا تیفی*، *استافیلوکوکوس اورئوس*، *لیستریا مونوسیتوژنز* و *باسیلوس سرئوس* به ترتیب برابر با ۳۲، ٦٤، ۳۲، ۱٦، ۳۲ و ۱٦ میلی‌گرم بر میلی‌لیتر بود. درکل می‌توان گفت عصاره آبی آلوئه‌ورا یک ترکیب آنتی‌اکسیدان و ضدمیکروب مناسب است و می‌توان از آن به‌عنوان یک نگهدارنده طبیعی استفاده کرد.

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