Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

In vitro comparison of antimicrobial effect of probiotic extract from *Lactobacillus casei* with current antibiotics on four strains of pathogenic bacteria

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ARTICLE INFO

Article History:

Received:2024/5/4

Accepted:2024/7/3

Keywords:

Probiotic,

Antibiotic,

Pathogen

DOI: **10.22034/FSCT.21.157.100.**

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ABSTRACT

The purpose of this study was to investigate the activity of probiotic extract achieved from *Lactobacillus casei* against the growth of 4 standard drug-resistant bacterial strains and to compare its antimicrobial effect with some common antibiotics *in vitro*. *L. casei* was cultured in standard MRS medium and under anaerobic conditions. Probiotic dry extract was extracted after separating the mass of living cells by centrifugation and stabilized by lyophilization. The investigation of antimicrobial activity was done using the diffusion-disc method, the results were analyzed using SPSS software with a significance level of $P<0.05$. There was a significant difference between all antimicrobial agents ($P<0.05$). The findings showed that LPE was able to control resistant pathogenic bacteria. The highest inhibitory effect of LPE was evaluated against *Staphylococcus aureus* with a diameter of 26 mm of non-growth halo and on the other hand, the lowest effect was evaluated against *Escherichia coli* with a diameter of 13.3 mm of non-growth halo. Although LPE had the greatest effect compared to antibiotic agents against 3 bacterial strains, it was weaker than gentamicin and streptomycin in the case of *Salmonella typhi*. Despite the significant antibacterial effects of LPE against several strains of gram-negative and gram-positive bacteria, more studies are necessary before its clinical administration and to prove its beneficial role in the treatment of infectious diseases.

[DOI: 10.22034/FSCT.21.157.100]

1- Introduction

Probiotics are living and specific microorganisms that, when consumed in humans or animals, exert beneficial effects on the host's health by modulating the body's microbial flora [1]. These microorganisms are generally sourced from humans and are considered non-pathogenic bacteria, providing a suitable solution for improving and maintaining digestive tract health, reducing antibiotic use, preventing diseases, enhancing the immune system, and eliminating pathogens through competition or the production of antimicrobial compounds, nutrients, and growth factors [2, 3].

Numerous studies have shown that the effects of probiotics are due to the production of active biological compounds, which are considered their metabolites. These metabolites can be found in the supernatant of bacterial cultures and can be dried and accessed in their dry form. They include a range of organic acids, bacteriocins, and polyamine compounds that have demonstrated bacteriostatic and bactericidal effects on gram-positive and negative opportunistic pathogenic microflora in the digestive system of humans and animals.

Due to the therapeutic properties of probiotics and their metabolites, the addition of these substances to dairy products such as milk, cheese, and yogurt has attracted significant interest. Consequently, many pharmaceutical and food products with therapeutic and strengthening purposes are being produced, incorporating probiotics, including compounds containing dried probiotic cells. The use of metabolites as a substitute for living cells is also a new concept currently under investigation [2, 4, 5].

It is crucial to identify the prepared metabolites in order to determine the precise consumption amount in formulations containing biological metabolites. Given the complexity and time-consuming nature of identifying all compounds, chromatographic or enzymatic methods are considered indicators for identifying the organic acids that are present in the metabolites. With the increasing evidence demonstrating the anti-pathogenic effects of probiotics, they are projected to serve as a suitable and effective alternative to antibiotics, aiming to combat the adverse effects and the development of bacterial resistance to antibiotics. Live and dried cells of probiotics are consumed in two forms:

1. As medicinal supplements in the form of powder, syrup, or tablets.
2. In foods enriched with probiotics [6, 7].

Various clinical studies on humans have demonstrated that the consumption of lactic acid-producing bacteria in amounts ranging from 10^9 to 10^{11} per day can reduce the incidence, duration, and severity of gastrointestinal diseases. Probiotics have been found to maintain intestinal integrity and alleviate complications from various digestive diseases such as antibiotic-related diarrhea, inflammatory bowel diseases, children's diarrhea, traveler's diarrhea, lactose intolerance, Helicobacter pylori infection, irritable bowel syndrome, and intestinal diseases caused by Clostridium difficile. Additionally, laboratory and clinical studies indicate that probiotics show promise in preventing or treating urinary-genital infections, high blood fat, allergies, and cancer [8-10].

In addition to their relatively low cost, the use of probiotics in the treatment of various diseases offers numerous benefits, including safety and the multiple mechanisms through which probiotics inhibit pathogens, thereby reducing the likelihood of developing resistance [8, 11].

One of the most commonly used probiotics in the dairy industry is *Lactobacillus*. In 1906, Dr. Ilya Ilyich Mechnikoff, a Nobel Prize winner, attributed the longevity of Balkan people to their consumption of fermented foods rich in lactobacilli and other lactic acid-producing organisms, particularly yogurt. He discovered that the substances in yogurt hinder the activity of pathogens and possess anti-toxic properties [6].

Following Mechnikoff's death in 1916, research in this area shifted to the United States, where it was discovered that bacteria of intestinal origin likely have beneficial effects in the gut. In 1935, highly active strains of *Lactobacillus acidophilus* were identified, yielding significant results in relieving chronic constipation [6, 12].

The term "probiotics" was first coined in 1953. Probiotics are defined as microbial agents that stimulate the growth of other microorganisms. In 1989, Ray Fuller provided a widely-used definition: probiotics are live microbial food supplements that improve microbial balance in the gut and have beneficial effects on the host. Fuller's definition underscores the bioactivity of probiotics and emphasizes their health-promoting effects on the host [6, 13].

In recent decades, various intestinal lactic acid bacteria (LAB) species with proven health benefits have been identified as probiotics, including *Lactobacillus rhamnosus*, *Lactobacillus casei*, and *Lactobacillus johnsonii* [14, 15].

Antibiotics are substances produced by different types of microorganisms that inhibit the growth of other microorganisms. Nowadays, most antibiotics are synthesized through chemical methods. Antibiotics vary in their physical, chemical, and medicinal properties, indicating differences in their antimicrobial spectrum and mechanism of action. It is worth noting that out of the numerous antibiotics produced in nature, only a limited number are non-toxic and therefore suitable for use as medications. Antibiotics exert their inhibitory activity in cells by interfering in cell wall synthesis, membrane function, protein synthesis, nucleic acid metabolism, and enzymatic reactions. Some antibiotics may have multiple target sites or mechanisms of action [16].

The aim of this study is to investigate the antimicrobial properties of a cell extract from the probiotic *L. casei* and compare its effects with common antibiotics against four resistant pathogen strains.

2-Materials and Methods

The standard strain of *L. casei* (PTCC 1608) was obtained from the collection of industrial and pathogenic fungi and bacteria at the Scientific and Industrial Research Center of Iran in lyophilized form. It was cultured in MRS medium (Merck Cat. No. 1.1.0660.0500) agar. The bacterial colony grown on agar was transferred to liquid MRS medium and cultured overnight. Subsequently, 1 m/L of the overnight culture was inoculated into 50 mL of fresh MRS culture medium and placed in a greenhouse at 37 °C with 250×rpm. The optical density of the culture medium was monitored periodically at a wavelength of 600 nm until reaching an absorbance of one. Following purification of the colonies and conducting biochemical tests, sugar fermentation, microscopic studies, and growth at temperatures of 45, 37, and 15 °C, the bacterium *L. casei* was identified and confirmed [18].

Preparation of Total Extract and *L. casei* Probiotic Extract (LPE) from Culture Medium

The purified *L. casei* was cultured in MRS medium under aerobic conditions at 37 °C until reaching a turbidity equivalent to 0.5 McFarland. To obtain the culture supernatant, the bacteria were centrifuged at 4 °C with 3500×rpm for 25 min. Subsequently, the supernatant was lyophilized to yield a dry and stable probiotic extract, known as LPE [18, 19].

Preparation of Pathogenic Bacteria

Standard and pure lyophilized ampoules of the following bacterial strains were obtained from the Iranian Scientific and Industrial Research Center, and after preparation and cultivation in nutrient broth medium to achieve a turbidity of 0.5 McFarland, they were utilized as pathogens [20]:

1. *Staphylococcus aureus* (PTCC 1431)
2. *Pseudomonas aeruginosa* (PTCC 27853)
3. *Salmonella Typhimurium* (PTCC 1639)
4. *Escherichia coli* (PTCC 2019)

Antimicrobial Activity Investigation

The antibacterial activity of *L. casei* was assessed using Muller Hinton Agar medium. The well method was employed to determine the inhibitory level of LAB and evaluate their antagonistic effect on pathogen strains. Each test was performed thrice to minimize errors. In the well method, a suspension of pathogenic bacteria cultured in nutrient broth medium (0.5 McFarland) was swabbed onto Muller Hinton agar. Wells with a diameter of 5 mm were created on the medium using a sterile cylinder, and *L. casei* bacterium supernatant or 200, 400, and 600 µg of LPE were inoculated into each well. A volume of 100 microliters of total extract or bacterial supernatant was added for assessing the inhibitory effects on pathogen growth. The plates were incubated at 37 °C for 24 h, and subsequently, the diameter of the growth inhibition zone created by LAB against each pathogenic strain was measured and recorded using a millimeter ruler [6, 21].

Statistical Analysis

Statistical analysis of the study results was performed using SPSS ver. 16 software. One-way analysis of variance was employed to test mean differences across multiple groups. Tukey's Post Hoc test was utilized to determine the most significant differences and increase the level of statistical significance.

3-Results and discussion

Results of investigating the inhibitory effects of total extract and LPE of *L. casei* on *P. aeruginosa*

Table 1- The diameter of non-growth halo resulting from the effect of total extract and LPE as well as common antibiotics on halo diameter on *Pseudomonas aeruginosa* in millimeters (n=3)

Bacteria	Antibacterial agent	Average	Standard deviation (±SD)
P. aeruginosa	Imipenem	18.66	3.51
	Gentamicin	17.66	0.57
	Meropenem	20	4.58
	Total extract	18.33	1.15
	LPE (200 µg)	25.00	1.00
	LPE (400 µg)	22.33	0.57
	LPE (600 µg)	20	1.52

Results of investigating the inhibitory effects of total extract and LPE of *L. casei* on *E. coli*

[DOI: 10.22034/FSCT.21.157.100]

Table 2- The diameter of non-growth halo resulting from the effect of total extract and LPE as well as common antibiotics on halo diameter on *Escherichia coli* in millimeters (n=3)

Bacteria	Antibacterial agent	Average	Standard deviation (±SD)
	Ciprofloxacin	0	0.00
	Imipenem	6	4
	Trimethoprim	9.33	3.05
E. coli	Total extract	12.66	2.08
	LPE (200 µg)	14.33	1.15
	LPE (400 µg)	16.66	0.57
	LPE (600 µg)	13.33	0.57

Results of investigating the inhibitory effects of total extract and LPE of *L. casei* on *S. Typhimurium*

Table 3- The diameter of non-growth halo resulting from the effect of total extract and LPE as well as common antibiotics on halo diameter on *Salmonella Typhimurium* in millimeters (n=3)

Bacteria	Antibacterial agent	Average	Standard deviation (±SD)
	Streptomycin	19	6
	Gentamicin	22	2
	Trimethoprim	5.66	5.5
S. Typhimurium	Total extract	12.66	2.08
	LPE (200 µg)	13.66	0.57
	LPE (400 µg)	16.33	1.15
	LPE (600 µg)	15.33	0.57

Results of investigating the inhibitory effects of total extract and LPE of *L. casei* on *S. aureus*

Table 4- The diameter of the non-growth halo resulting from the effect of total extract and LPE and also the common antibiotic methicillin on *Staphylococcus aureus* in millimeters (n=3)

Bacteria	Antibacterial agent	Average	Standard deviation (±SD)
	Methicillin	0.66	1.15
	Total extract	13.33	2.88
S. aureus	LPE (200 µg)	16.66	0.57
	LPE (400 µg)	17.66	1.52
	LPE (600 µg)	26	1.00

Comparison of the inhibitory effects of antibacterial agents on *P. aeruginosa*

Considering that the obtained significance is smaller than the standard significance level ($p<0.05$), it can be concluded that there is a significant difference between the antimicrobial power. In order to check more closely and compare the antibiotics two by two, the results of the post-hoc test or in other words the LSD test are given below.

Table 5. The results of comparison of halo diameter for two by two antibiotics on *Pseudomonas aeruginosa*

Bacteria	Antibacterial agent 1	Antibacterial agent 2	Average differences	Standard error	Significant

	Imipenem	Gentamicin	2.00000	1.91899	0.315
		Meropenem	-1.33333	1.91899	0 .499
		Total extract	.33333	1.91899	0.865
		LPE (200 µg)	-6.33333*	1.91899	0.005
		LPE (400 µg)	-3.66667	1.91899	0.077
		LPE (600 µg)	-1.66667	1.91899	0.400
	Gentamicin	Imipenem	-2.00000	1.91899	0.315
		Meropenem	-3.33333	1.91899	0.104
		Total extract	-1.66667	1.91899	0.400
		LPE (200 µg)	-8.33333*	1.91899	0.001
		LPE (400 µg)	-5.66667*	1.91899	0.010
		LPE (600 µg)	-3.66667	1.91899	0.077
	Meropenem	Imipenem	1.33333	1.91899	0.499
		Gentamicin	3.33333	1.91899	0.104
		Total extract	1.66667	1.91899	0.400
		LPE (200 µg)	-5.00000*	1.91899	0.021
		LPE (400 µg)	-2.33333	1.91899	0.244
		LPE (600 µg)	-.33333	1.91899	0.865
P. aeruginosa	Total Extract	Imipenem	-.33333	1.91899	0.865
		Gentamicin	1.66667	1.91899	0.400
		Meropenem	-1.66667	1.91899	0.400
		LPE (200 µg)	-6.66667*	1.91899	0.004
		LPE (400 µg)	-4.00000	1.91899	0.056
		LPE (600 µg)	-2.00000	1.91899	0.315
	LPE (200 µg)	Imipenem	6.33333*	1.91899	0.005
		Gentamicin	8.33333*	1.91899	0.001
		Meropenem	5.00000*	1.91899	0.021
		Total Extract	6.66667*	1.91899	0.004
		LPE (400 µg)	2.66667	1.91899	0.186
		LPE (600 µg)	4.66667*	1.91899	0.029
	LPE (400 µg)	Imipenem	3.66667	1.91899	0.077
		Gentamicin	5.66667*	1.91899	0.010
		Meropenem	2.33333	1.91899	0.244
		Total Extract	4.00000	1.91899	0.056
		LPE (200 µg)	-2.66667	1.91899	0.186
		LPE (600 µg)	2.00000	1.91899	0.315
	LPE (600 µg)	Imipenem	1.66667	1.91899	0.400
		Gentamicin	3.66667	1.91899	0.077
		Meropenem	.33333	1.91899	0.865
		Total Extract	2.00000	1.91899	0.315
		LPE (200 µg)	-4.66667*	1.91899	0.029
		LPE (400 µg)	-2.00000	1.91899	0.315

[DOI: 10.22034/FSCT.21.157.100]

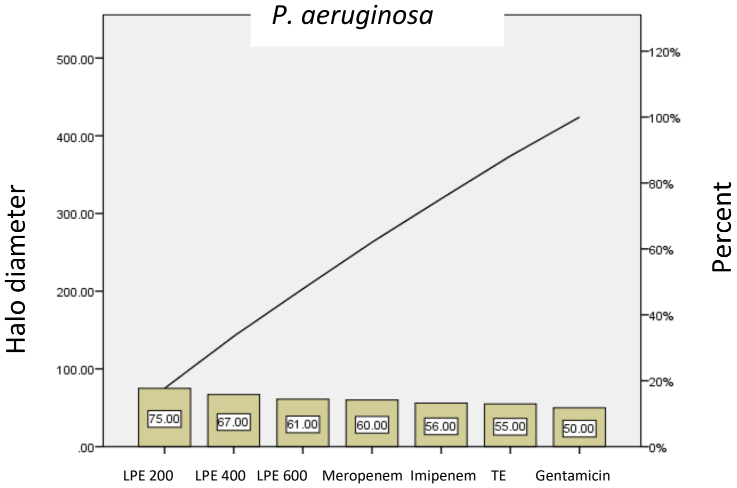

Figure 1-Prato diagram (ranking) to compare the killing power of antibacterial agents, gentamicin, imipenem, meropenem, total extract and LPE in concentrations of 200, 400 and 600 µg of *L. casei* supernatant against *P. aeruginosa*

As seen in Table 5 and Figure 1, the highest antimicrobial power is related to LPE400. The difference between LPE400 and other antimicrobial groups is significant (p<0.05). In other words, it can be said that LPE400 compared to other antimicrobial agents, including gentamicin antibiotics, total probiotic extract, imipenem and meropenem, LPE200 (concentration of 200 µg) from *L. casei* probiotic has the highest inhibitory power against *P. aeruginosa*.

Comparison of the inhibitory effects of antibacterial agents on *S. aureus*

Considering that the obtained significance is smaller than the standard significance level (p<0.05), it can be concluded that there is a significant difference between the inhibitory power of antibacterial agents. In order to check more closely and compare the antibiotics used two by two, the results of the post-hoc test or in other words the LSD test are given below.

Table 6. The results of comparison of halo diameter for two by two antibiotics on *S. aureuS*

Bacteria	Antibacterial agent 1	Antibacterial agent 2	Average differences	Standard error	Significant
S. aureus	Total extract	LPE 200	-3.33333*	1.33333	0.031
		LPE 400	-4.33333*	1.33333	0.009
		LPE 600	-12.66667*	1.33333	0.000
		Methicillin	12.66667*	1.33333	0.000
	LPE 200	Total extract	3.33333*	1.33333	0.031
		LPE 400	-1.00000	1.33333	0.471
		LPE 600	-9.33333*	1.33333	0.000
		Methicillin	16.00000*	1.33333	0.000
	LPE 400	Total extract	4.33333*	1.33333	0.009
		LPE 200	1.00000	1.33333	0.471
		LPE 600	-8.33333*	1.33333	0.000
		Methicillin	17.00000*	1.33333	0.000
	LPE 600	Total extract	12.66667*	1.33333	0.000
		LPE 200	9.33333*	1.33333	0.000
		LPE 400	8.33333*	1.33333	0.000
		Methicillin	25.33333*	1.33333	0.000
	Methicillin	Total extract	-12.66667*	1.33333	0.000
		LPE 200	-16.00000*	1.33333	0.000

	LPE 400	-17.00000*	1.33333	0.000
	LPE 600	-25.33333*	1.33333	0.000

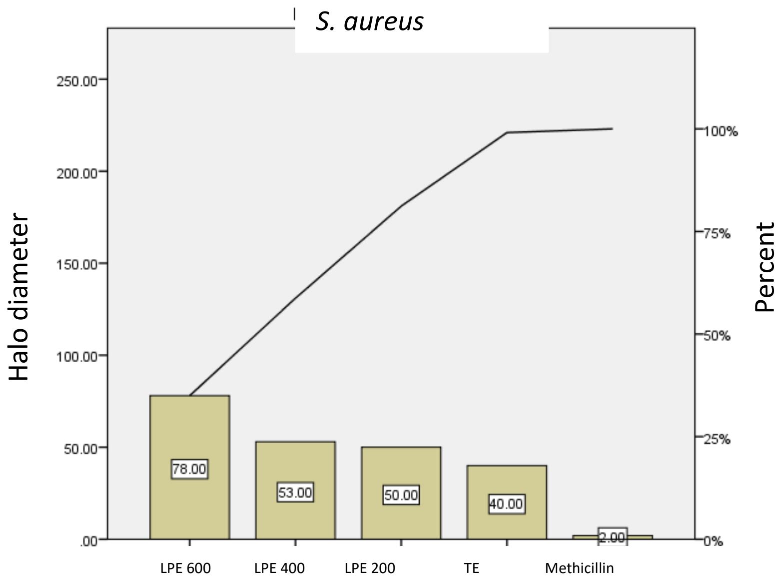

Figure 2- Prato diagram (ranking) to compare the killing power of antibacterial agents, Methicillin, total extract and LPE in concentrations of 200, 400 and 600 µg of *L. casei* supernatant against *S. aureus*

As seen in Table 6 and Figure 2, the highest antimicrobial power is related to LPE600. The difference between LPE600 and other antimicrobial groups is significant ($p<0.05$). In other words, it can be said that compared to other antimicrobial agents, including the antibiotic methicillin, against which *S. aureus* have shown resistance, the probiotic agents obtained from *L. casei*, either in the form of total extract or in the form of LPE, are stronger. It has a higher barrier. Also, the higher the concentration of the active ingredient in LPE, the higher the antimicrobial power.

Comparison of the inhibitory effects of antibacterial agents on the *S. Typhimurium*

Considering that the obtained significance is smaller than the standard significance level ($p<0.05$), it can be concluded that there is a significant difference between the inhibitory power of antimicrobial agents. In order to check more closely and compare the antibiotics used two by two, the results of the post-hoc test or in other words the LSD test are given below.

[DOI: 10.22034/FSCT.21.157.100]

Table 7. The results of comparison of halo diameter for two by two antibiotics on *S. Typhimurium*

Bacteria	Antibacterial agent 1	Antibacterial agent 2	Average differences	Standard error	Significant
S. Typhimurium	Gentamicin	Total extract	9.33333*	2.70214	0.004
		LPE (200 µg)	8.33333*	2.70214	0.008
		LPE (400 µg)	5.66667	2.70214	0.055
		LPE (600 µg)	6.66667*	2.70214	0.027
		Streptomycin	3.00000	2.70214	0.286
		Trimethoprim	16.33333*	2.70214	0.000
	Total Extract	Gentamicin	-9.33333*	2.70214	0.004
		LPE 200	-1.00000	2.70214	0.717
		LPE 400	-3.66667	2.70214	0.196
		LPE 600	-2.66667	2.70214	0.340
		Streptomycin	-6.33333*	2.70214	0.034
		Trimethoprim	7.00000*	2.70214	0.021
	LPE 200	Gentamicin	-8.33333*	2.70214	0.008
		Total Extract	1.00000	2.70214	0.717
		LPE 400	-2.66667	2.70214	0.340
		LPE 600	-1.66667	2.70214	0.547
		Streptomycin	-5.33333	2.70214	0.068
		Trimethoprim	8.00000*	2.70214	0.010
	LPE 400	Gentamicin	-5.66667	2.70214	0.055
		Total Extract	3.66667	2.70214	0.196
		LPE 200	2.66667	2.70214	0.340
		LPE 600	1.00000	2.70214	0.717
		Streptomycin	-2.66667	2.70214	0.340
		Trimethoprim	10.66667*	2.70214	0.001
	LPE 600	Gentamicin	-6.66667*	2.70214	0.027
		Total Extract	2.66667	2.70214	0.340
		LPE 200	1.66667	2.70214	0.547
		LPE 400	-1.00000	2.70214	0.717
		Streptomycin	-3.66667	2.70214	0.196
		Trimethoprim	9.66667*	2.70214	0.003
	Streptomycin	Gentamicin	-3.00000	2.70214	0.286
		Total Extract	6.33333*	2.70214	0.034
		LPE 200	5.33333	2.70214	0.068
		LPE 400	2.66667	2.70214	0.340
		LPE 600	3.66667	2.70214	0.196
		Trimethoprim	13.33333*	2.70214	0.000
	Trimethoprim	Gentamicin	-16.33333*	2.70214	0.000
		Total Extract	-7.00000*	2.70214	0.021
		LPE 200	-8.00000*	2.70214	0.010
		LPE 400	-10.66667*	2.70214	0.001
		LPE 600	-9.66667*	2.70214	0.003
		Streptomycin	-13.33333*	2.70214	0.000

[DOI: 10.22034/FSCT.21.157.100]

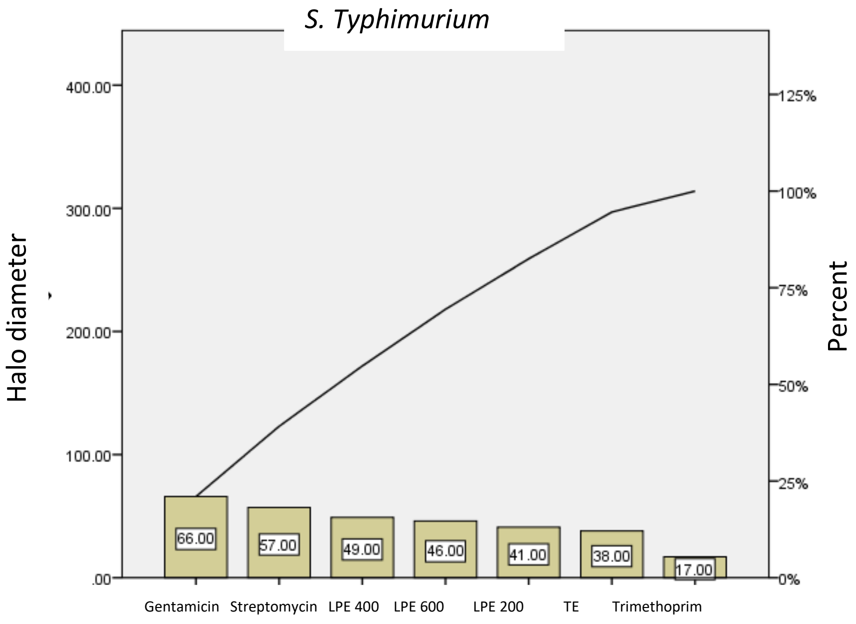

Figure 3-Prato diagram (ranking) to compare the killing power of antibacterial agents, Gentamicin, Streptomycin, Trimethoprim, total extract and LPE in concentrations of 200, 400 and 600 µg of *L. casei* supernatant against *S. Typhimurium*

As seen in Table 7 and Figure 3, the highest antimicrobial power is related to gentamicin and streptomycin ($p<0.05$). The antibiotic trimethoprim had less inhibitory power compared to the total extract and LPE of *L. casei*. It can be said that the antibiotic gentamicin has a better inhibitory power and more suitable performance against *S. typhimurium*.

Comparison of the inhibitory effects of antimicrobial agents on the *E. coli*

Considering that the obtained significance is smaller than the standard significance level ($p<0.05$), it can be concluded that there is a significant difference between the inhibitory power of antimicrobial agents. In order to check more closely and compare the antibiotics used two by two, the results of the post-hoc test or in other words the LSD test are given below.

Table 8. The results of comparison of halo diameter for two by two antibiotics on *E. coli*

Bacteria	Antibacterial agent 1	Antibacterial agent 2	Average differences	Standard error	Significant
E. coli	Imipenem	Total extract	-6.66667*	1.73663	0.002
		LPE (200 µg)	-8.33333*	1.73663	0.000
		LPE (400 µg)	-10.66667*	1.73663	0.000
		LPE (600 µg)	-7.33333*	1.73663	0.001
		Trimethoprim	-3.33333	1.73663	0.076
		Ciprofloxacin	6.00000*	1.73663	0.004
	Total extract	Imipenem	6.66667*	1.73663	0.002
		LPE (200 µg)	-1.66667	1.73663	0.353
		LPE (400 µg)	-4.00000*	1.73663	0.037
		LPE (600 µg)	-.66667	1.73663	0.707
		Trimethoprim	3.33333	1.73663	0.076
		Ciprofloxacin	12.66667*	1.73663	0.000
	LPE 200	Imipenem	8.33333*	1.73663	0.000
		Total extract	1.66667	1.73663	0.353

[DOI: 10.22034/FSCT.21.157.100]

	LPE (400 µg)	-2.33333	1.73663	0.200
	LPE (600 µg)	1.00000	1.73663	0.574
	Trimethoprim	5.00000*	1.73663	0.012
	Ciprofloxacin	14.33333*	1.73663	0.000
LPE 400	Imipenem	10.66667*	1.73663	0.000
	Total extract	4.00000*	1.73663	0.037
	LPE (200 µg)	2.33333	1.73663	0.200
	LPE (600 µg)	3.33333	1.73663	0.076
	Trimethoprim	7.33333*	1.73663	0.001
	Ciprofloxacin	16.66667*	1.73663	0.000
LPE 600	Imipenem	7.33333*	1.73663	0.001
	Total extract	.66667	1.73663	0.707
	LPE (200 µg)	-1.00000	1.73663	0.574
	LPE (400 µg)	-3.33333	1.73663	0.076
	Trimethoprim	4.00000*	1.73663	0.037
	Ciprofloxacin	13.33333*	1.73663	0.000
Trimethoprim	Imipenem	3.33333	1.73663	0.076
	Total extract	-3.33333	1.73663	0.076
	LPE (200 µg)	-5.00000*	1.73663	0.012
	LPE (400 µg)	-7.33333*	1.73663	0.001
	LPE (600 µg)	-4.00000*	1.73663	0.037
	Ciprofloxacin	9.33333*	1.73663	0.000
Ciprofloxacin	Imipenem	-6.00000*	1.73663	0.004
	Total extract	-12.66667*	1.73663	0.000
	LPE (200 µg)	-14.33333*	1.73663	0.000
	LPE (400 µg)	-16.66667*	1.73663	0.000
	LPE (600 µg)	-13.33333*	1.73663	0.000
	Trimethoprim	-9.33333*	1.73663	0.000

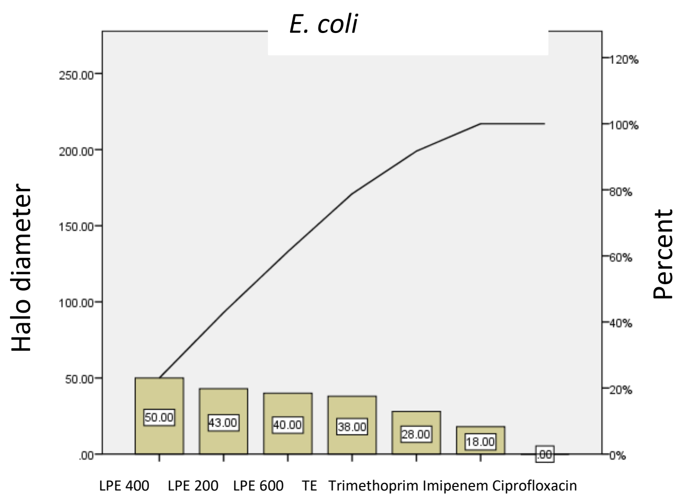

Figure 4-Prato diagram (ranking) to compare the killing power of antibacterial agents, Trimethoprim, Imipenem, Ciprofloxacin, total extract and LPE in concentrations of 200, 400 and 600 µg of *L. casei* supernatant against *E. coli*

As seen in Table 8 and Figure 4, the highest antimicrobial power is related to gentamicin and streptomycin antibiotics (p<0.05). The antibiotic trimethoprim had less inhibitory power compared to the total extract and LPE of *L. casei*. It can be said that the antibiotic gentamicin has a better inhibitory power and a better performance against *E. coli*.

Comparing the inhibitory effects of total probiotic extract on different bacteria

Considering that the obtained significance is smaller than the standard significance level (p<0.05), it can be concluded that there is a significant difference between the inhibitory power of *L. casei* extract against pathogenic agents. In order to check more closely and compare the bacteria used two by two, the results of the post-hoc test or in other words the LSD test are given below.

Table 9-Results of the comparison of the halo diameter of the total extract antibiotic for two pairs of bacteria

Antibacterial	Bacteria 1	Bacteria 2	Average differences	Standard error	Significant
Total extract	*P. aeruginosa*	*S. aureus*	5.00000*	1.74801	0.021
		S. Typhimurium	5.66667*	1.74801	0.012
		E. coli	5.66667*	1.74801	0.012
	S. aureus	*P. aeruginosa*	-5.00000*	1.74801	0.021
		S. Typhimurium	.66667	1.74801	0.713
		E. coli	.66667	1.74801	0.713
	S. Typhimurium	*P. aeruginosa*	-5.66667*	1.74801	0.012
		S. aureus	-.66667	1.74801	0.713
		E. coli	.00000	1.74801	1.000
	E. coli	*P. aeruginosa*	-5.66667*	1.74801	0.012
		S. aureus	-.66667	1.74801	0.713
		S. Typhimurium	.00000	1.74801	1.000

Figure 5- Prato chart (ranking) to compare the inhibitory power of *L. casei* total extract on pathogenic factors

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As can be seen in Table 9 and Figure 5, the sensitivity of *P. aeruginosa* to other pathogenic factors is higher than Lactobacillus casei probiotic total extract (p<0.05).

Comparison of the inhibitory effects of LPE (200 µg) on different bacteria

Considering that the obtained significance is smaller than the standard significance level (p<0.05), it can be concluded that there is a significant difference between the inhibitory power of LPE200 against different pathogenic agents. In order to check more closely and compare the bacteria used two by two, the results of the post-hoc test or in other words the LSD test are given below.

Table 10-Results of comparing the halo diameter of LPE 200 antibiotic for bacteria two by two

Antibacterial	Bacteria 1	Bacteria 2	Average differences	Standard error	Significant
LPE 200	*P. aeruginosa*	*S. aureus*	8.33333*	0.70711	0.000
		S. Typhimurium	11.33333*	0.70711	0.000
		E. coli	10.66667*	0.70711	0.000
	S. aureus	*P. aeruginosa*	-8.33333*	0.70711	0.000
		S. Typhimurium	3.00000*	0.70711	0.003
		E. coli	2.33333*	0.70711	0.011
	S. Typhimurium	*P. aeruginosa*	-11.33333*	0.70711	0.000
		S. aureus	-3.00000*	0.70711	0.003
		E. coli	-.66667	0.70711	0.373
	E. coli	*P. aeruginosa*	-10.66667*	0.70711	0.000
		S. aureus	-2.33333*	0.70711	0.011
		S. Typhimurium	0.66667	0.70711	0.373

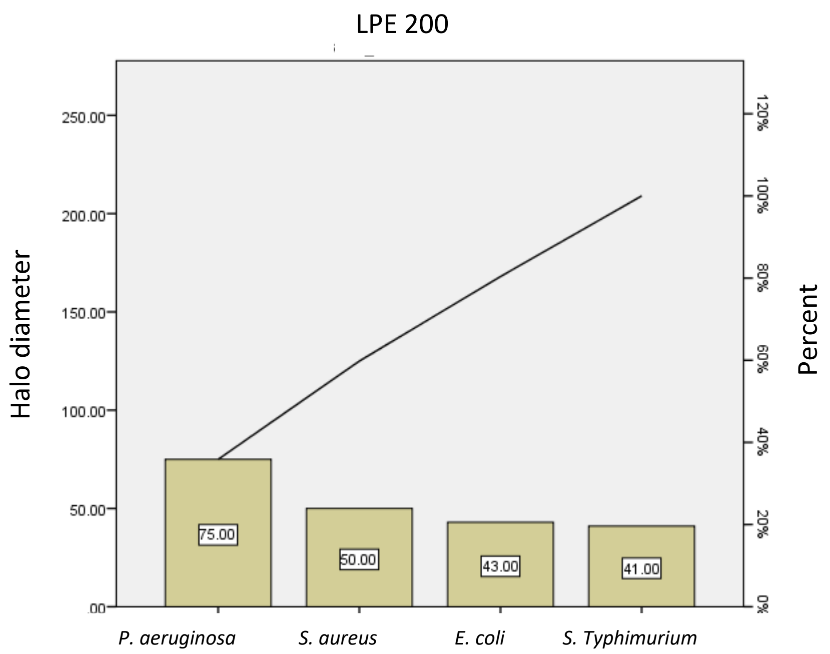

Figure 6 - Prato chart (ranking) to compare the inhibitory power of LPE 200 of *L. casei* on pathogenic agents

As can be seen in Table 10 and Figure 6, the sensitivity of *P. aeruginosa* is higher than other pathogenic agents against *L. casei* LPE200 (P<0.05).

Comparison of the inhibitory effects of LPE (400 µg) on different bacteria

Considering that the obtained significance is smaller than the standard significance level (p<0.05), it can be concluded that there is a

[DOI: 10.22034/FSCT.21.157.100]

significant difference between the inhibitory power of LPE400 against pathogenic agents. In order to check more closely and compare the bacteria used two by two, the results of the post-hoc test or in other words the LSD test are given below.

Table 11-Results of comparing the halo diameter of LPE 400 antibiotic for bacteria two by two

Antibacterial	Bacteria 1	Bacteria 2	Average differences	Standard error	Significant
LPE 400	*P. aeruginosa*	*S. aureus*	4.66667*	0.84984	0.001
		S. Typhimurium	6.00000*	0.84984	0.000
		E. coli	5.66667*	0.84984	0.000
	S. aureus	*P. aeruginosa*	-4.66667*	0.84984	0.001
		S. Typhimurium	1.33333	0.84984	0.155
		E. coli	1.00000	0.84984	0.273
	S. Typhimurium	*P. aeruginosa*	-6.00000*	0.84984	0.000
		S. aureus	-1.33333	0.84984	0.155
		E. coli	-.33333	0.84984	0.705
	E. coli	*P. aeruginosa*	-5.66667*	0.84984	0.000
		S. aureus	-1.00000	00.84984	0.273
		S. Typhimurium	.33333	.84984	0.705

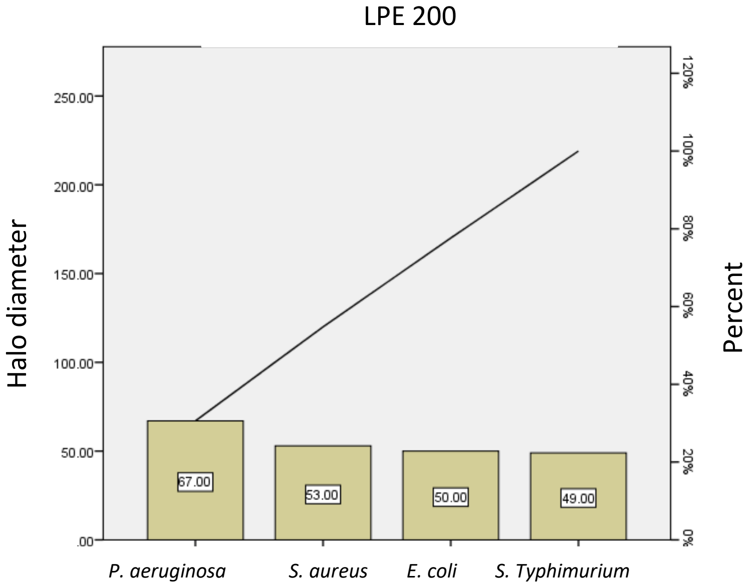

Figure 7- Prato chart (ranking) to compare the inhibitory power of LPE 200 *L. casei* on pathogenic agents

As can be seen in Table 11 and Figure 7, the sensitivity of *P. aeruginosa* is higher than other pathogenic agents against *L. casei* LPE400 (p<0.05).

Comparison of the inhibitory effects of LPE (600 µg) on different bacteria

Considering that the obtained significance is smaller than the standard significance level (p<0.05), it can be concluded that there is a significant difference between the inhibitory power of LPE600 against pathogenic agents. In order to check more closely and compare the bacteria used two by two, the results of the post-hoc test or in other words the LSD test are given below.

[DOI: 10.22034/FSCT.21.157.100]

Table 12-Results of the comparison of the diameter of the non-growth halo obtained from LPE 600 for two pairs of pathogens

Antibacterial	Bacteria 1	Bacteria 2	Average differences	Standard error	Significant
LPE 600	*P. aeruginosa*	*S. aureus*	-5.66667*	0.81650	0.000
		S. Typhimurium	5.00000*	0.81650	0.000
		E. coli	7.00000*	0.81650	0.000
	S. aureus	*P. aeruginosa*	5.66667*	0.81650	0.000
		S. Typhimurium	10.66667*	0.81650	0.000
		E. coli	12.66667*	0.81650	0.000
	S. Typhimurium	*P. aeruginosa*	-5.00000*	0.81650	0.000
		S. aureus	-10.66667*	0.81650	0.000
		E. coli	2.00000*	0.81650	0.040
	E. coli	*P. aeruginosa*	-7.00000*	0.81650	0.000
		S. aureus	-12.66667*	0.81650	0.000
		S. Typhimurium	-2.00000*	0.81650	0.040

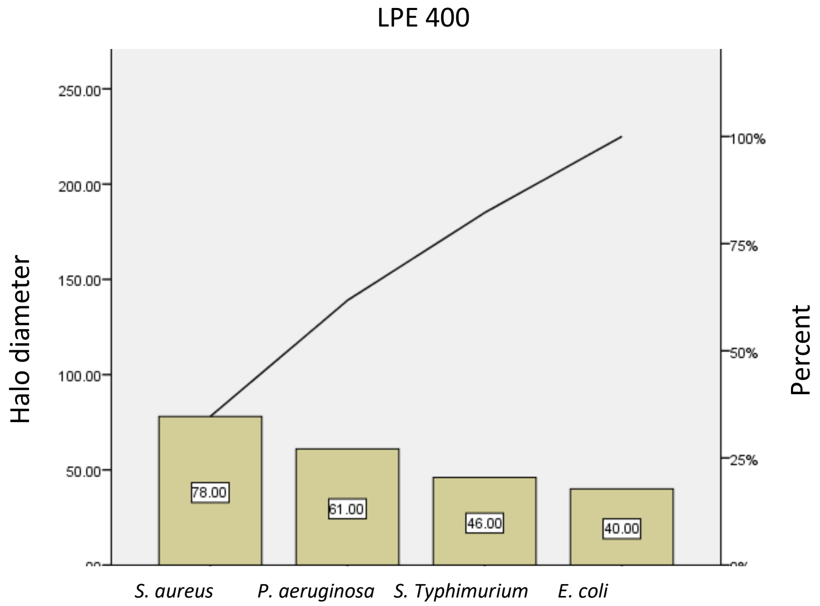

Figure 8 - Prato chart (ranking) to compare the inhibitory power of LPE 600 *L. casei* on pathogenic agents

As indicated in Table 12 and Figure 8, *S. aureus* exhibit higher sensitivity compared to other pathogens against *L. casei* LPE600 (P<0.05).

Probiotic dry extracts or LPE in concentrations of 200, 400, and 600 µg have a stronger inhibitory effect than common antibiotics in treating infections caused by *P. aeruginosa*, with the largest growth inhibition zone observed for LPE200 at an average diameter of 25 mm. Similarly, LPE extract in concentrations of 200, 400, and 600 µg shows a stronger inhibitory effect than common antibiotics in treating infections caused by *S. aureus*, with the largest growth inhibition zone observed for LPE600 at an average diameter of 26 mm. Notably, the total extract of *L. casei*

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exhibits stronger inhibitory abilities compared to antibiotics.

In the case of *S. Typhimurium*, LPE extract at concentrations of 200, 400, and 600 µg shows a stronger inhibitory effect than the antibiotic trimethoprim, although it is weaker than gentamicin and streptomycin antibiotics. The largest non-growth halo diameter for probiotics is observed for LPE400 at an average halo diameter of 16 mm, which is lower than that of gentamicin at 22 mm. For *E. coli* infections,

LPE extract in concentrations of 200, 400, and 600 µg exhibits a stronger inhibitory effect compared to trimethoprim, imipenem, and ciprofloxacin antibiotics. The whole extract also displays more inhibitory power than common antibiotics in treating *E. coli* infections. The results highlight that the highest

inhibitory power against *P. aeruginosa* is associated with LPE at a concentration of 200 µg, while the highest inhibitory power against *S. aureus* is seen with LPE at 600 micrograms. The highest inhibitory power against *S. Typhimurium* is attributed to gentamicin and streptomycin antibiotics, with LPE and total *L. casei* extract displaying stronger inhibitory effects compared to trimethoprim. Moreover,

the highest inhibitory power against *E. coli* is observed with LPE at a concentration of 400 µg, while the total probiotic extract of *L. casei* demonstrates the highest inhibitory power against *P. aeruginosa*.

Reflecting on this material, various studies conducted by research teams, such as the study by Kazemi and colleagues in 2019, have investigated the antimicrobial activity of isolated LAB, particularly probiotic products like Lactobacillus and Bifidobacterium. The study isolated LAB from yogurt and probiotic pill samples, identified them using biochemical methods, and assessed the antimicrobial properties of their cultured supernatant against bacterial pathogens using the disk and well method. Results showed that LAB exhibited good antimicrobial abilities against seven pathogenic bacteria, with Lactobacillus acidophilus displaying the strongest inhibitory effect against Bacillus cereus, showcasing the potential of metabolites produced by LAB in combatting pathogenic bacteria [22].

Saadatzadeh et al. (2013) conducted research on the probiotic extract obtained from *L. casei*, investigating its antimicrobial and antioxidant effects. The group utilized probiotic extract obtained through lyophilization to enhance stability and shelf life, demonstrating a tenfold increase in the antioxidant and antimicrobial potency of this extract, abbreviated as LPE. The study also evaluated the lactic acid content of the probiotic extract as a biological indicator [23].

In 2012, Farah Bakhsh and colleagues isolated probiotic lactobacilli from traditional yogurts in rural areas of Rafsanjan, exploring their antimicrobial effects. Using special culture medium (MRS), selective screening methods, catalase test, and biochemical tests, probiotic lactobacilli were isolated from four samples of local yogurt. The antibacterial effects of these probiotics against common pathogens such as *S. aureus*, *E. coli*, *Streptococcus pyogenes*, and *Proteus vulgaris* were assessed using disk diffusion and well methods. From 40 local yogurt samples, 33 acid-resistant bacilli strains were isolated initially, and eventually, 9 strains exhibiting high resistance to acid and bile salts were identified. These bacteria included *L. casei* (in two locations), *rhamnosus*, *plantarum*, *acidophilus*, *bulgaricus*, *delbrueckii*, *fermentum*, and *brevis*. All probiotic strains demonstrated the ability to combat pathogenic bacteria, with *L. plantarum* displaying the strongest antibacterial effect. Overall, the study suggested the presence of probiotic bacteria with antibacterial activity against pathogenic bacteria in traditionally prepared yogurts, indicating their potential application in industrial dairy product production [24].

In 2015, Kiani and colleagues examined the antagonistic effect of LAB isolated from yogurt against pathogenic bacteria. From 96 strains of LAB isolated from 34 samples of local yogurt, their impact on 7 major digestive pathogens, including *Shigella dysentery*, *Yersinia enterocolitica*, *E. coli*, and *S. Typhimurium*, was evaluated using disk and well diffusion methods on agar with the supernatant solution from the bacterial culture medium. The inhibitory zones around the disk and well were measured, with each test repeated at least three times for accuracy. *L. casei* and *Lactococcus lactis* were found to exhibit the most inhibitory effects in the well method, with a maximum non-growth halo diameter of 18 mm. *Lactobacillus* and *Lactococcus* species showed effective inhibition against intestinal pathogenic bacteria, particularly *Y. enterocolitica*. The study highlighted the inhibitory potential of these LAB against pathogenic strains, especially when derived from local yogurts in the Golestan province [25].

In 2013, Chavoshi Forushani and colleagues explored the antimicrobial effects of *L. casei* cell body and gastric fluid isolated from yogurt against *E. coli* O157:H7, a significant causative agent of diarrhea in developing countries. Given the challenges of drug resistance, disruption of intestinal flora, and verotoxin production induced by certain antibiotics, novel treatment approaches are crucial. The study isolated *L. casei* from yogurt and assessed the impact of the cell body and supernatant derived from its cultivation on the targeted pathogenic bacteria. The stability of the supernatant obtained from the strains' cultivation was demonstrated at temperatures ranging from 56 to 100 °C for 30 and 60 min, as well as against pH levels of 3 to 10. The tube dilution method revealed a minimal concentration required for killing and inhibiting the growth of *Lactobacillus* supernatant at 1.16 and 1.8, respectively. The results suggest the potential utilization of the supernatant as a biological preservative in the food industry, highlighting the antibacterial properties of *L. casei* for treating E. coli-related diseases [26].

In 2004, Okana and colleagues studied the microbial activities and bacteriocin production of two probiotic strains, *L. plantarum* and *L. brevis*, against various pathogens, with the most pronounced inhibitory effect observed on Bacillus cereus (8-10 mm). Additional findings included inhibitory effects on *E. coli* (6-8 mm) and *Y. enterocolitica* (6-7 mm) [27].

4-Conclusion

Based on the research findings, it can be inferred that the dry probiotic extract derived from *L. casei* exhibits significant inhibitory potency against several key pathogenic factors in infections. This probiotic agent shows potential in combating infections induced by prevalent pathogens like *P. aeruginosa*, *S. aureus*, *S. Typhimurium*, and *E. coli*, while also assisting in overcoming microbial resistance to antibiotics. Further investigations are imperative to solidify these conclusions.

5-Acknowledgment

We are very grateful for the sincere cooperation of the drug and food control laboratory of the Ahvaz Faculty of Pharmacy, who were very cooperative in conducting this research.

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[DOI: 10.22034/FSCT.21.157.100]

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مقاله علمی_پژوهشی

مقایسه اثر ضدباکتریایی عصاره پروبیوتیکی حاصل از *لاکتوباسیلوس کازئی* با آنتی بیوتیک‌های رایج علیه چهار سویه پاتوژن باکتریایی بصورت *برون تنی*

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱٤۰۳/۱/۱۷

تاریخ پذیرش: ۱٤۰۳/٤/۱۳

کلمات کلیدی:

پروبیوتیک،

آنتی بیوتیک،

پاتوژن.

DOI:10.22034/FSCT.21.157.100.

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چکیده

هدف از این مطالعه، بررسی فعالیت عصاره پروبیوتیکی استخراج شده از *لاکتوباسیلوس کازئی* علیه رشد ٤ سویه استاندارد باکتریایی مقاوم به دارو و مقایسه اثرضدمیکروبی آن با چند آنتی بیوتیک رایج در شرایط برون تنی بود. *لاکتوباسیلوس کازئی* در محیط استاندارد MRS و شرایط کم هوازی، کشت داده شد. عصاره خشک پروبیوتیکی، پس از جداسازی توده سلول‌های زنده با روش سانتریفوژ استخراج شده و توسط روش لیوفیلیزاسیون، پایدار گردید. بررسی فعالیت ضدمیکروبی با استفاده از روش انتشار-دیسک انجام شد. نتایج با استفاده از نرم افزار SPSS و با سطح معناداری $P< 0.05$ مورد آنالیز قرار گرفت. تفاوت معناداری بین تمام عوامل ضد میکروبی وجود داشت ($P<0.05$). یافته‌ها نشان می‌داد که LPE قادر بود باکتری‌های پاتوژن مقاوم را کنترل نماید. بیشترین اثر مهارکنندگی LPE، علیه باکتری *استافیلوکوکوس اورئوس* با قطر هاله عدم رشد ۲٦ میلیمتر و در مقابل، کمترین اثر علیه باکتری *اشرشیا کلی* با قطر هاله عدم رشد ۱۳/۳ میلیمتر ارزیابی شد. هرچند که LPE، در مقایسه با عوامل آنتی بیوتیک علیه ۳ سویه باکتریایی بیشترین اثر را دارا بود ولی در مورد *سالمونلا تایفی*، از جنتامایسین و استرپتومایسین ضعیف‌تر بود. با وجود اثرات قابل توجه ضدباکتریایی از LPE علیه چند سویه باکتری گرم منفی و گرم مثبت، مطالعات بیشتری قبل از تجویز بالینی آن و اثبات نقش مفید آن در درمان بیماری‌های عفونی ضروری است.

[DOI: 10.22034/FSCT.21.157.100]