Original Article

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The Antimicrobial Effects of Ethanolic and Methanolic Extracts of Rhubarb on *Listeria monocytogenes* and *Yersinia enterocolitica*

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Abstract

Objective(s): The harmful effects of antibiotic misuse have been demonstrated as a cause of the spread of antibiotic-resistant pathogens. Due to the increasing resistance of bacteria to antibiotics, there is a need to use new substances to control bacteria. Recently, there has been an interest in identifying plants with pharmacological and antibiotic effects. Materials and methods: This study was performed to evaluate the antibacterial effects of Iranian rhubarb (*Rheum ribes) in vitro* on *Listeria monocytogenes* and *Yersinia enterocolitica*. For this purpose, ethanolic and methanolic extracts of roots, stems, leaves, and flowers of rhubarb were prepared, and the antibacterial effects of the extracts were evaluated by the broth microdilution method. Results: The results showed the antimicrobial effect of methanolic extracts of different parts of the rhubarb plant is more than its ethanolic extracts and the effect of rhubarb extract on *L. monocytogenes* is more than its effect on *Y. enterocolitica*. The most antimicrobial effect on *L. monocytogenes* belongs to the methanolic extracts of leaves and stems, and the ethanolic extracts of leaves, and stems. Concerning *Y. enterocolitica*, the most antimicrobial properties belong to the methanolic extracts of leaves and ethanolic extracts of stems. Conclusions: Among the ethanolic and methanolic extracts of different parts of rhubarb, the most antimicrobial effect belongs to the methanolic extract of the leaf and the ethanolic extract of the stem. Herbal extracts can be investigated for their beneficial effects in the control of foodborne infectious diseases.

Keywords: *Rheum ribes*, Antibacterial activity, Medicinal plant, Antibacterial agents

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1. Introduction

Foodborne infections are one of the most important illnesses even in developed countries. Foodborne illness is majorly caused by consuming foods or beverages contaminated by various types of pathogens including bacteria, viruses, or parasites. According to a report, only in the United States, there have been 9.4 million cases of foodborne illness, 55,961 hospitalizations, and 1,351 foodborne illness-associated deaths in one year (1).

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Listeria monocytogenes and *Yersinia enterocolitica* are among the bacteria which can cause serious foodborne illnesses. *L. monocytogenes* has also been considered an important pathogen since it causes various infections such as severe fetal infections, stillbirths, and fetal abnormalities, especially in immunocompromised individuals (2). This bacterium that causes listeriosis has been demonstrated to be present in raw milk (2). Treatments for *L. monocytogenes* infection include treatment with parenteral penicillin or ampicillin (2). However, trimethoprim-sulfamethoxazole is used as an alternative treatment in individuals allergic to penicillin (2).

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Y. enterocolitica is the other important pathogen that causes gastrointestinal inflammation and thyroid complications. *Y. enterocolitica* infection causes the disease yersiniosis, which is an animal-borne disease in humans. Yersiniosis is known to be self-limiting obviating the need for any particular treatment. However, in the case of patients with sepsis or severe focal infections, doxycycline in combination with an aminoglycoside is used as the recommended treatment. Moreover, trimethoprim-sulfamethoxazole, fluoroquinolones, ceftriaxone, and chloramphenicol are among other antibiotics with potent and effective clinical activity against *Y. enterocolitica*. In contrast, *Y. enterocolitica* appears to be resistant to penicillin G, ampicillin, and cefalotin (2).

Antibiotics are antimicrobial agents active against bacteria. These agents are majorly used for the prevention and treatment of bacterial infections. The emergence of antibiotic-resistant bacteria is a common phenomenon in which bacteria develop mechanisms and pathways that can help protect them against the activity of antimicrobial agents. Various studies have demonstrated the harmful effects of antibiotic use on the development and spread of antibiotic-resistant human pathogens (3). Increased prescription and distribution of antibiotic drugs as well as their misuse in developing countries are known as the main reasons for the emergence of antibiotic-resistant bacteria. According to estimations, such resistance can result in near a million to several million mortalities per year (4, 5). Therefore, this phenomenon is considered a major public health concern requiring serious actions.

Due to the increased resistance of bacteria to antibiotics, novel antimicrobial agents or substances might be highly required for the control of antibiotic-resistant bacteria. In this regard, herbal antibiotics are considered a suitable option for this aim (6). The antimicrobial activity of herbal plants against foodborne pathogens has always been a subject of investigations with great importance (7). Naturally, plants demonstrate unique activities such as antimicrobial and anti-inflammatory properties due to their particular compounds such as alkaloids, terpenoids, tannins, saponins, glycosides, flavonoids, phenolics, steroids, and essential fatty acids (8). *Rheum ribes*, also known as Rhubarb, is one of the oldest plants in Iran. Naturally, rhubarb grows in mountainous areas in spring and early summer. The stem, petiole, roots, and fruits of the rhubarb plant are among its edible parts. This plant has been extensively used because of its therapeutic properties. These therapeutic properties are also mentioned in traditional medicine books. This plant has also been used for various medicinal purposes including treating constipation, relieving bile and nausea, and treating tuberculosis (9). Researchers have demonstrated the antioxidant activity of the methanol extract of stems and roots of rhubarb (10). Moreover, other studies have evaluated and reported the antibacterial effects of methanolic extracts of *Rheum ribes* on some standard pathogenic bacteria such as *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Pseudomonas aeruginosa* (11). Therefore, in this study, we aimed to evaluate the antibacterial effects and properties of the ethanolic and methanolic extracts of rhubarb on *L. monocytogenes* and *Y. enterocolitica*, two pathogenic bacteria known as the cause of important foodborne infections.

2. Materials and methods

2.1. Materials, bacteria, and sample collection

Ethanol and methanol were both purchased from Merck (catalog number 64-17-5 and 67-56-1, respectively, Merck, Merck KGaA, Darmstadt, Germany). Trypticase Soy Agar (TSA) medium was obtained from Merck (catalog number 22091, Merck, Merck KGaA, Darmstadt, Germany). Sabouraud Dextrose Agar (SDA) was purchased from Neogen (catalog number NCM0008A, Neogen, Lansing, MI 48912 USA). Tryptic Soy Broth (TSB) was purchased from Sigma Aldrich (catalog number 22092, Merck KGaA, Darmstadt, Germany).

L. monocytogenes (ATCC 35152) was obtained from the Food Hygiene and Control Laboratory of Shahrekord University. *Y. enterocolitica* (ATCC 51872) was received from the Biopars Azma equipment group. All bacteria culture was performed under a laminar flow cabinet. The roots, stems, leaves, and flowers of *Rheum ribes* were collected from the Zagros highlands and in Chaharmahal and Bakhtiari region, Iran. Rheum ribes

was collected based on the visual characteristics used by plant scientists to classify this plant (12).

2.2. Preparation of the rhubarb plant extracts

The collected plants were washed several times with distilled water and then the different parts were separated and dried in a cool place away from the direct sunlight. To prepare the plant extract, the powder obtained from each dried part was mixed with 70% ethanol and methanol (a ratio of 1:10 by weight) and added to separate Erlenmeyer flasks. The flasks were kept away from direct light for 72 hours and then placed in a shaker for 120 minutes to mix the content. The content of the flasks was then filtered through a filter paper and the filtered liquid was placed in an oven at 40 ° C to let the water and alcohol evaporate. The dried extract was collected using a spatula and used to prepare the extract solution.

To obtain a 20% extract, the dried extract was mixed with sterile distilled water (with a ratio of 1:5) and then passed through filter paper. Next, the obtained extract was sterilized using sterile 0.22 µm syringe filters, and then kept in the refrigerator (4 °C) for further analysis (13).

To ensure that the extracts were not contaminated with bacteria, 100 µl of each prepared extract was cultured on TSA medium plates. The plates were incubated at 37 °C for 24 hours. Similarly, to ensure that the extracts were not contaminated with fungi and yeast, 100 µl of each extract was cultured on SDA culture plates. The plates were incubated at room temperature for 72 h. After ensuring that the extracts were free of any contamination, we proceeded to the next steps of the experiment.

2.3. Preparation of bacterial samples and bacterial culture

Bacteria were cultured in TSB media and were incubated at 37 °C until adequate morbidity. Next, a dilution of 10^6 colony-forming unit (CFU) bacteria/mL was prepared and used for the following steps of the experiment.

2.4. Micro-dilution

Minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) tests are often used to determine the activity of a given drug on certain species of bacteria. To determine the MIC by the microdilution method, 96-well sterile microplates and TSB medium were used. The first well was prepared without the addition of any extract (as the control) and different concentrations of the extract were added to the following wells of the plate. Next, a concentration of 106 per mL of the mentioned bacteria was inoculated into the wells and the plate was incubated at 37 °C for 24 h. The wells were then examined from the control side and the first well without turbidity was considered as MIC. To determine the MBC of the extract, a sample was taken from each well of the plate. After that, it was cultured on TSA medium and incubated at 37 ° C for 24 h. By examining the plates, the first dilution lower than MIC using which no bacterial growth was observed on the plate was considered as MBC (14).

2.5. Bacterial growth in the presence of concentrations lower than MIC

For this purpose, the first concentration lower than the MIC of the extract in which the bacteria demonstrated growth capability was used to study the growth curve. In detail, in test tubes, 10 ml of TSB medium and a concentration of the extract (which was lower than MIC) were added, and then 10^5 CFU bacteria/mL were inoculated. The tubes were incubated at 37 °C and then cultured on TSA medium 0, 2, 4, 6, 8, 16, and 24 h following incubation at 37 °C. Colonies that appeared on the culture plates were counted after 24 h of incubation.

2.6. Statistical analysis

Each experiment was performed in triplicate. One-way ANOVA was performed for the statistical analysis of the data using Systat SigmaPlot (version 12.3).

3. Resuls

3.1. The MIC and MBC levels of the methanolic extracts

The MIC and MBC results of the methanolic extracts of different parts of rhubarb for *L. monocytogenes* and *Y. enterocolitica* have been presented in Table 1. Accordingly, the methanolic

Table 1. The MIC and MBC levels of methanolic extract of different parts of rhubarb against *Listeria monocytogenes* and *Yersinia enterocolitica*.

Methanolic extract of different parts of the plant	*Yersinia Enterocolitica*		*Listeria monocytogenes*	
	MBC%	MIC%	MBC%	MIC%
Leaves	4.8	3.8	1.2	1.2
Leaves and stems	4.6	4.4	1.2	1.2
Stem	3.6	3.4	1.4	1.2
Roots	1.0	0.8	1.8	1.8
Roots and flowers	1.6	1.4	1.4	1.2
Flowers	4.2	4.0	2.6	2.0

extracts of "leaf" and "leaf and stem mixture" have demonstrated the strongest antimicrobial effects on *L. monocytogenes*. The methanolic extracts of "root and flower" and "stem" had lower antimicrobial effects on *L. monocytogenes*. In terms of antimicrobial effects against *Y. enterocolitica*, the methanolic extracts of "roots" and 'the mixture of roots and flowers" demonstrated the highest antimicrobial properties (Table 1).

3.2. The MIC and MBC levels of the ethanolic extracts

The lowest inhibitory and lethal concentrations of the ethanolic extracts of the different parts of rhubarb on *L. monocytogenes* and *Y. enterocolitica* have been presented in Table 2. In detail, the ethanolic extracts of "leaves", "stems", "leaves and stems", and "roots" showed the greatest to lowest antimicrobial effects on *L. monocytogenes*, respectively. Furthermore, the ethanolic extracts of "stem" and "root" demonstrated the highest antimicrobial effects on *Y. enterocolitica* (Table 2).

3.3. The effect of a lower concentration of MIC on the growth of L. monocytogenes in TSB medium

The growth trend of *L. monocytogenes* in the presence of the plant extract with a concentration lower than MIC is presented in Table 3 and Figure 1. After 24 hours, in the control group, the growth of bacteria reached its maximum level (109 CFU per mL). Also, at 0 and 2 hours following the start of the experiment, there was no statistically significant difference in the growth rate of the bacteria in different experimental groups. At 24 hours, the growth of the bacteria in the treatment groups showed a statistically significant difference in comparison with the control group ($P<0.05$). In detail, the experiment groups with the ethanolic extract of "leaves" and "the mixture of leaves and stems" and the methanolic extracts of "stems" demonstrated statistically significant differences in comparison with the control group ($P < 0.05$).

A comparison between the ethanolic and methanolic extracts of different parts of rhubarb used in this study showed that the methanolic extracts of "leaf" had the highest antimicrobial effects on *L. monocytogenes*. The mixed methanolic extract of "leaf and stem" also showed high antimicrobial effects (even though lower than those of

Table 2. The MIC and MBC levels of ethanolic extracts of different parts of rhubarb for *Listeria monocytogenes* and *Yersinia enterocolitica*.

Ethanolic extract of different parts of the plant	*Yersinia Enterocolitica*		*Listeria monocytogenes*	
	MBC	MIC	MBC	MIC
Leaves	4.5	4.2	1.2	1.2
Leaves and stems	4.4	4.0	1.6	1.2
Stem	1.8	1.6	1.2	1.2
Roots	1.8	1.8	2.4	2.4
Roots and flowers	2.2	2.0	2.4	2.0
Flowers	6.0	6.0	3.8	3.0

Table 3. Effects of ethanolic and methanolic extracts of different parts of rhubarb on the growth of Listeria monocytogenes at a concentration lower than MIC (number of bacteria in log CFU / ml).

Time (h)	Control	Leaf methanolic extract	Stem methanolic extract	Methanolic extract of leaves and stems	Leaf ethanolic extract	Stem ethanolic extract	Ethanolic extract of leaves and stems
0	5.2±1.4Aa	5.2±1.7Aa	5.3±1.3Aa	5.1±1.5Aa	5.2±1.4Aa	5.1±1.2Aa	5.2±1.1Aa
2	5.6±1.2Aa	5±1.2Aa	5.2±1.6Aa	5±1.2Aa	5±1.5Aa	5±1.2Aa	5.1±1.4Aa
4	6.8±1.2Ab	4.6±1.4Cb	6.4±1.2Ab	4.2±1.4Cb	5.7±1.2Ba	6±1.4Ab	5.6±1.2Ba
8	8.5±1.5Ac	3.7±1Cc	6.9±1.4Bb	3.8±1.3Cb	6.3±1.6Bb	6.6±1.5Bb	6±1.5Bb
12	8.8±1.8Ac	2.6±0.4Dd	7.2±1.6Cc	2.9±0.7Dc	6.9±1.4Bb	7.4±1.4Cc	6.8±1.2Bb
24	9.2±1.7Ad	2±0.5Dd	7.6±1.8Bc	2.2±0.5Dc	7.4±1.5Bc	8.1±1.6Cd	7.1±1.6Bc

Small dissimilar letters in each column and uppercase letters in each row indicate a statistically significant difference (P<0.05).

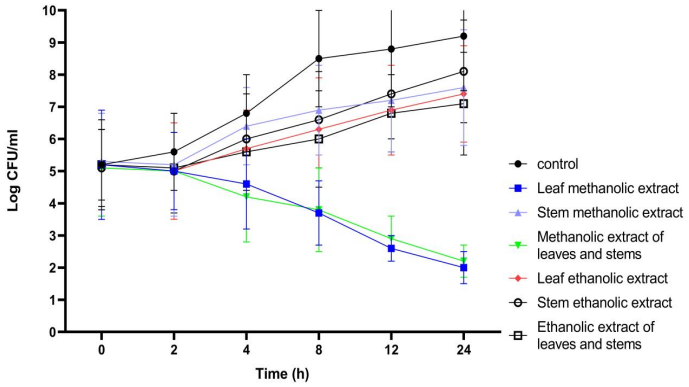

Figure 1. The effects of ethanolic and methanolic extracts of different parts of the rhubarb plant at a concentration lower than MIC on the growth of *Listeria monocytogenes*.

the methanolic extracts of "leaf") in inhibiting the growth of *L. monocytogenes* (Table 3 and Figure 1).

3.4. Effect of a lower dilution of MIC on the growth of Y. enterocolitica in TSB medium

The results of evaluating the effect of the ethanolic and methanolic extracts of rhubarb at a concentration lower than MIC on the growth of *Y. enterocolitica* in TSB medium have been reported in Table 4. From 0 to 24 h, the bacterial growth in the control group increased 1000-fold and reached a CFU of 10^8. On the other hand, in the other treatment groups, the bacterial growth rate was lower than that of the control group. In detail, the bacterial growth reached a CFU of 10^3 per mL in the methanolic extract group which shows a statistically significant difference in comparison with both the control group and the 0-hour time point (P<0.05). However, in the other treatment groups,

Table 4. The effect of ethanolic and methanolic extracts of different parts of rhubarb in a concentration lower than MIC on the growth of *Yersinia enterocolitica* (number of bacteria in log / CFU).

Time (h)	Control	Leaf methanolic extract	Stem methanolic extract	Leaf ethanolic extract	Stem ethanolic extract
0	5.5±1.1Aa	5.5±1.3Aa	5.3±1.5Aa	5.4±1.4Aa	5.5±1.3Aa
2	5.6±1.2Aa	5.2±1.6Aa	5.3±1.3Aa	5.4±1.2Aa	5.1±1.2Aa
4	5.8±1.5Aa	5±1.2Aa	5.5±1.6Aa	5.8±1.6Aa	5.6±1.5Aa
8	6.5±1.4Ab	4.6±1.5Cb	6.2±1.7Ab	7.3±1.3Bb	6±1.7Ab
12	7±1.2Ac	3.8±1.2Cc	6.5±1.4Bb	7.5±1.4Ab	6.2±1.4Bb
24	8.3±1.5Ad	3.2±1.7Cc	6.9±1.5Cb	7.8±1.2Bb	7.5±1.6Bc

Mismatched letters in each column and uppercase letters in each row indicate a statistically significant difference (P<0.05).

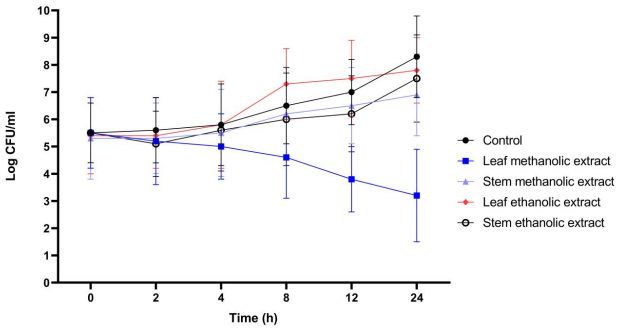

Figure 2. The effect of ethanolic and methanolic extracts of different parts of rhubarb in a concentration lower than MIC on the growth of *Yersinia enterocolitica*.

the bacterial growth presented a statistically significant less growth rate in 24 h in comparison with the control group (Table 4 and Figure 2, P<0.05).

The methanolic extract of "leaf" had the highest level of antimicrobial effects on *Y. enterocolitica*. Moreover, the methanolic extract of "stem" also showed a better result in inhibiting the growth of *L. monocytogenes* with a slight difference after the methanolic extract of the leaf (Table 4 and Figure 2).

4. Discussion

In ancient times, plant extracts and essential oils were used as traditional medicine for the treatment of infectious diseases. After the discovery of antibiotics, the use of herbs was much less considered than before. However, due to the reducing effects of antibiotics, increasing antibiotic resistance, and regulations and restrictions on the use of antibiotics and medicinal residues, the use of plant extracts and essential oils have been reconsidered by researchers. The medicinal properties of components present in different parts of plants have also been a research subject with great importance. For instance, Chipault *et al*. (15) and Chung *et al.* (16) reported that rosemary could be used as a natural antioxidant in food. In 1994, Pandit *et al*. (17) discovered that the antioxidant components of rosemary demonstrate anti-blister activity.

Rhubarb is one of the valuable medicinal plants belonging to the Polygonaceae or Haftbandan family. Various species of this plant grow in different parts of the world including Iran, India, China, and Turkey. Many therapeutic properties have been attributed to different parts of the rhubarb plant such as leaves, barks, stems, and flowers according to scientific sources. One of the most important properties of rhubarb is its antibacterial effects. This study was conducted with the aim of elucidating the antibacterial and medicinal effects of the methanolic and ethanolic extracts of different parts of rhubarb. The findings of this study might pave the way for a new treatment for listeriosis, yersiniosis, and plague. Herein, we can conclude the antimicrobial effects of the methanolic extracts of the different parts of rhubarb is greater than those of the ethanolic extracts. Moreover, the antimicrobial effects of rhubarb extracts against *L. monocytogenes* are greater than its effect against *Y. enterocolitica*.

In the recent years, several studies have been conducted with the aim of evaluating the antibacterial effects of rhubarb. In this regards, Fazlieh Bazaz *et al*. (18) investigated the effects of *Rheum ribes* on several gram-negative pathogens. They first prepared the methanolic extracts from the Iranian rhubarb leaves, roots, and stalks, and then studied the effects of the extracts on *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus*, *Suzodomonas aeruginosa*, and *Neisseria gonorrhoea* by using two methods of disk diffusion and slimming series. Their results showed that rhubarb extract had significant antibacterial activity in both methods. However, stem extracts showed less antibacterial activity than roots and leaves. In addition, the antibacterial effects of the rhubarb extract on *S. aeruginosa* and *Proteus* was more than other pathogens. In 2012, Khan *et al.* (19) examined the antibacterial effects of the Indian Rhubarb on *E. coli* and *Staphylococcus aureus*. The results showed that rhubarb had a 90% antimicrobial ef-

fect against these bacteria. Moreover, Raudsepp *et al*. (20), assessed the antibacterial effects of Siberian rhubarb (*Rheum rhaponticum*). They showed that Siberian rhubarb had a high inhibitory effect on pathogenic bacteria such as *L. monocytogenes*, *E. coli*, and *Campylobacter jejuni*; however, it did not have a high inhibitory effect on probiotics and other beneficial bacteria (19). Another study investigated the antimicrobial effects of the methanolic extract of leaves, stems, and roots of *Rheum ribes* on *E. coli*, *Klebsiella pneumoniae*, *S. aeruginosa*, *Shigella flexneri*, and *S. aureus* using the disk diffusion method. According to the results, the extracts from different parts of *Rheum ribes* demonstrated remarkable antimicrobial effects against the studied bacteria (21). Ultimately, Salehi *et al.* (22) evaluated the antibacterial effect of the aqueous and ethanolic extracts of leaves and stems of Iranian rhubarb on *E. coli* and *S. aureus*. According to the results of this study, the inhibitory effects of rhubarb extracts on *S. aureus* were higher than its antibacterial effects on *E. coli*. Interestingly, the aqueous extracts of rhubarb had no lethal effect (described as MBC) on the studied bacteria.

It has been reported that the antibacterial activity of the rhubarb crude extract is remarkably associated with their anthraquinone content (23, 24). The results of studies on Iranian rhubarb in neighboring countries further support the positive antibacterial effects of the extracts of this plant and are consistent with findings of the present study. For instance, Alan *et al*. (25) studied the antimicrobial effects of Iranian rhubarb grown in Turkey. They evaluated the effects of the rhubarb chloroform, hexane, ethanolic, and methanolic extracts on various pathogenic bacteria. According to the results, the rhubarb leaf extracts had no effect on the studied microorganisms; In contrast, the methanolic and ethanolic extracts of stems and roots demonstrated the highest antibacterial activity. Of note, the most sensitive bacteria were Bacillus subtilis and Enterobacter aerogenes.

L. monocytogenes is known as a great target pathogen for evaluating the antibacterial effects of different types of plant-derived extracts since this bacterium is the cause of serious infections in humans. In 1992, Aurelli *et al*. (26) evaluated the antimicrobial effects of 32 plant-based essential oils on *L. monocytogenes* and found that the essential oils of clove, cinnamon, peppermint, pepper, and thyme demonstrated remarkable antibacterial activity. In 1992, Lund and Nguyen *et al*. (27) reported that components present in carrots have lethal antimicrobial effects on *L. monocytogenes*. These researchers also found that such lethal antimicrobial effects (described as MBC) were dependent on temperature (4-30 ° C), pH (7-5-8), and oxygen availability (inactivity under anaerobic conditions). In 1998, Feo *et al*. (28) studied the antibacterial effects of peppermint essential oils on *Salmonella enteritidis* and *L. monocytogenes* in food models, and reported that the antimicrobial effects of peppermint essential oils are dependent on the extract concentration, nutrient composition, storage temperature, pH, and bacterial species. Moreover, Hao *et al*. (29) stated that cumin, sweet pepper, clove, and thyme plant extracts can affect the growth of *L. monocytogenes* and *Aeromonas hydrophila*. In detail, these extracts can affect the latency phase, growth rate, and maximum growth of the studied microorganisms. On the other hand, these effects depend on the plant species and also bacterial strain. These researchers also stated that *L. monocytogenes* was more sensitive to the essential oils of sweet pepper and cloves.

Researchers (30) reported that plant essential oils contain large amounts of phenolic compounds such as carvacrol (22), gamaterpine (25), pysimone (31), and thymol (32), which are responsible for the antibacterial activity of the essential oils. These researchers also noted that higher amounts of these phenolic compounds in essential oils result in higher antibacterial properties of these essential oils. In 2008, Myrtle *et al*.(33) demonstrated that clove oil of 1% and 2% do not inhibit the growth of *L. monocytogenes*. Moreover, in 2007, Oussalah *et al*. (34) evaluated the antibacterial activity of 28 different plant essential oils on *L. monocytogenes*, *E. coli* (35), *S. aureus*, and *Salmonella typhimurium*. In detail, these 27 essential oils, studied with a concentration of more than 0.4%, reduced the number of *L. monocytogenes* colonies. Notably, the lowest growth inhibition concentration for *L. monocytogenes* was reported to be 0.02>0.8%. Additionally, Celikel *et al*. (36) examined the antimicrobial properties

of plant essential oils (including fragrant leaves, oranges, and thyme) on *S. aureus*, *L. monocytogenes*, *E. coli*, and *Candida albicans* using the agar diffusion method. They reported that these essential oils have both bactericidal and bacteriostatic effects and cause the destruction of genetic material, disruption of energy production as well as structural components of the bacteria, and they also inhibit vital bacterial enzymes. In addition, these researchers reported that thyme had the most antimicrobial effects among the other studied plant parts. Moreover, it was reported that *L. monocytogenes* had the least sensitivity to the plant essential oils among the other bacteria investigated. Finally in 2008, Mashhak *et al*. (37) studied the effects of *Zataria multiflora* on the growth of *L. monocytogenes*, and found that increasing concentrations of *Zataria multiflora* essential oil reduces the number of *L. monocytogenes* bacteria in the logarithmic bacterial growth phase.

Yersinia is the name of a bacterial genus that belongs to the family Enterobacteriaceae. This genus consists of Gram-negative coccobacilli with 11 species (38). Among these species, *Y. enterocolitica*, *Y. pseudotuberculosis*, and *Y. pestis* are the most famous ones since they can cause serious infections in humans (38). *Y. pestis* is the pathogen responsible for the plague. Since studying Yersinia pestis requires high-tech biosafety laboratories, we studied *Y. enterocolitica*. In detail, *Y. pestis* and *Y. enterocolitica* have a high genetic similarity rate and both of these pathogens can cause infectious diseases in humans (39). Therefore, the results obtained in this experiment can be cautiously translated to the pestis-causing bacterium. It is worth mentioning that the aim of this in vitro study was to find a cure for the disease of plague. Therefore, according to the data obtained here, we encourage researchers who do not have the mentioned working condition limitations to expand this research by performing more in-depth experiments on *Y. enterocolitica* and by directly working on *Y. pestis*, and further moving to *in vivo* experiments for more reliable findings. So far, there have been three plague pandemics each causing massive mortality in different eras. Currently, there are numerous cases of plague diagnosed globally each year. Even though vaccines against the plague have been developed, according to the World Health Organization (WHO) recommendation, only individuals with a high risk of infection (including laboratory personnel and health care workers) should be vaccinated. In terms of treatment, some antibiotics are effective for the treatment of plague. These antibiotics include streptomycin, chloramphenicol, tetracycline, gentamicin, and doxycycline. However, antibiotics are not definitive treatments; therefore, the motive for discovering new and effective treatments for plague still exists. To our knowledge, despite the great deal of interest in studying the antibacterial effects of rhubarb; so far, no specific research has been conducted to evaluate the antibacterial effects of the extracts of this plant on *Y. enterocolitica* and *L. monocytogenes*.

5. Conclusion

The results of this study demonstrated the antimicrobial and inhibitory effects of the Iranian rhubarb extracts on *L. monocytogenes* and *Y. enterocolitica*. Due to the high rate of genomic similarity and biological characteristics between *Y. enterocolitica* ptcc1786 (ATCC9610) and *Y. pestis*; ethanolic and methanolic extracts from the different parts of the rhubarb plant may also have antibacterial effects on *Y. pestis*. Therefore, we propose that future investigations can directly study the antibacterial effects of the Iranian rhubarb extracts on *Y. pestis*. In the case of obtaining similar findings consistent with our results, this approach can be considered a basis for the possible drug development process against the plague. It is also suggested that the effects of rhubarb on pathogenic bacteria such as *L. monocytogenes* and *Y. enterocolitica* can be assessed as a preservative in food models and as a drug in pharmaceutical models. It is worth mentioning that whether antibiotics may be replaced with these herbal extracts soon or not is a question that requires further in-depth weighing in both preclinical and clinical stages. Conclusively, our research team aims to chemically characterize these rhubarb extracts, and investigate their effects in animal models of *L. monocytogenes* and *Y. enterocolitica* in the near future.

Ethics Approval

This study was performed in accordance

with the guidelines of Shahrekord University Research Ethics Committee.

Authors' Contributions

Mohammad Ali Fotouhi Lasibi: Data curation, Formal analysis, Investigation, Methodology, Project administration, Validation, Visualization, Writing - original draft, Writing - review & editing, Validation, Supervision. Hamdollah Moshtaghi: Writing - review & editing, Validation, Supervision.

Conflict of Interest

None declared.

References

1. Scallan E, Hoekstra RM, Angulo FJ, Tauxe RV, Widdowson MA, Roy SL, Jones JL, Griffin PM. Foodborne illness acquired in the United States--major pathogens. *Emerg Infect Dis.* 2011 Jan;17(1):7-15.
2. Fleming DW, Cochi SL, MacDonald KL, Brondum J, Hayes PS, Plikaytis BD, Holmes MB, Audurier A, Broome CV, Reingold AL. Pasteurized milk as a vehicle of infection in an outbreak of listeriosis. *N Engl J Med*. 1985 Feb 14;312(7):404-7. doi: 10.1056/NEJM198502143120704.
3. Miller RA, Harbottle H. Antimicrobial Drug Resistance in Fish Pathogens. *Microbiol Spectr*. 2018 Jan;6(1).
4. Dramé O, Leclair D, Parmley EJ, Deckert A, Ouattara B, Daignault D, Ravel A. Antimicrobial Resistance of Campylobacter in Broiler Chicken Along the Food Chain in Canada. *Foodborne Pathog Dis*. 2020 Aug;17(8):512-520.
5. Dadgostar P. Antimicrobial Resistance: Implications and Costs. *Infect Drug Resist.* 2019 Dec 20;12:3903-3910. doi: 10.2147/IDR.S234610. PMID: 31908502; PMCID: PMC6929930.
6. Al-Kobaisi MF. Jawetz, Melnick & Adelberg's Medical Microbiology: 24th Edition. *Sultan Qaboos Univ Med J*. 2007 Dec;7(3):273–5.
7. Jarriyawattanachaikul W, Chaveerach P, Chokesajjawatee N. Antimicrobial Activity of Thai-herbal Plants against Food-borne Pathogens E. Coli, S. Aureus and C. Jejuni. *Agric Agric Sci Procedia*. 2016;11:20-4.
8. Harikrishnan R, Kim DH, Hong SH, Mariappan P, Balasundaram C, Heo MS. Non-specific immune response and disease resistance induced by Siegesbeckia glabrescens against Vibrio parahaemolyticus in Epinephelus bruneus. *Fish Shellfish Immunol.* 2012 Aug;33(2):359-64.
9. Bottone EJ. Yersinia enterocolitica: overview and epidemiologic correlates. *Microbes Infect*. 1999 Apr;1(4):323-33. doi: 10.1016/s1286-4579(99)80028-8. PMID: 10602666.
10. Öztürk M, Aydoğmuş-Öztürk F, Duru ME, Topçu G. Antioxidant activity of stem and root extracts of Rhubarb (Rheum ribes): An edible medicinal plant. *Food Chem.* 2007;103(2):623-30.
11. sayyahi j, Mobaiyen H, Jafari b, Jafari-Sales A. Antibacterial effects of methanolic extracts of Reum ribes L. and Hyssopus officinalis on some standard pathogenic bacteria. *Jorjani Biomed J.* 2019;7(3):34-44.
12. Turkmen O, Crka M, Suat E. Initial evaluation of a new edible wild rhubarb species (Rheum ribes L.) with a modified weighted scaling index method. *Pak J Biol Sci.* 2005;8(5):763-5.
13. Niyyati M, Joneidi Z, KamaliNejad M, Haghighi A, Abadi A, Arab-Mazar Z, et al. In Vitro Activity of Mentha longifolia Leaves and Pimpinella anisum Seeds Against a Clinical Strain of Trichomonas Vaginalis. *Int J Molecul Clin Microbiol*. 2015;5(1):503-9.
14. Parvekar P, Palaskar J, Metgud S, Maria R, Dutta S. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of silver nanoparticles against Staphylococcus aureus. *Biomater. Investig Dent.* 2020;7:105-9. doi: 10.1080/26415275.2020.1796674
15. Chipault JH, Mizuno GR, Hawkins JM, Lundberg WO. The Antioxidant Properties Of Natural Spices. *J Food Sci*. 1952;17(1-6):46-55.
16. Chung KT, Thomasson WR, Wu-Yuan CD. Growth inhibition of selected food-borne bacteria, particularly Listeria monocytogenes, by plant extracts. *J Appl Bacterio*l. 1990 Oct;69(4):498-503.
17. Pandit VA, Shelef LA. Sensitivity of Listeria monocytogenes to rosemary (Rosmarinus officinalis L.). *Food Microbiol.* 1994;11(1):57-63.
18. Fazly Bazzaz B S, Khajehkaramadin M, Shokooheizadeh H R. In Vitro Antibacterial Activity of Rheum ribes Extract Obtained from Various Plant Parts Against Clinical Isolates of Gram-Negative Pathogens. *Innov J Pharm Res.* 2005;4(2):e128230.
.19. Khan SA, Ahmad A, Khan MI, Yusuf M,

Shahid M, Manzoor N, et al. Antimicrobial activity of wool yarn dyed with Rheum emodi L. (Indian Rhubarb). *Dyes Pigm.* 2012;95(2):206-14.
20. Raudsepp P, Anton D, Roasto M, Meremäe K, Pedastsaar P, Mäesaar M, et al. The antioxidative and antimicrobial properties of the blue honeysuckle (Lonicera caerulea L.), Siberian rhubarb (Rheum rhaponticum L.) and some other plants, compared to ascorbic acid and sodium nitrite. *Food Control.* 2013;31(1):129-35.
21. Kazemi Darsanaki R, Parsa Lisar M. Antimicrobial potential of root, stalk and leaves extracts of Rheum Ribes. *J Rep Pharm Sci.* 2014;3(1):10-3.
22. Salehi A, Shariatifar N, Salehi A, Mohammadzadeh A. In-Vitro Antimicrobial Effect of Aqueous and Alcoholic Extracts of Rheum Ribes on Some Food-Borne Pathogens. *Journal of Sabzevar University of Medical Sciences*. 2016;23(3):430-7.
23. Lu C, Wang H, Lv W, Xu P, Zhu J, Xie J, et al. Antibacterial properties of anthraquinones extracted from rhubarb against Aeromonas hydrophila. *Fish Sci.* 2011;77(3):375.
24. Malmir M, Serrano R, Silva O. Anthraquinones as potential antimicrobial agents-A review. Antimicrobial research: Novel bioknowledge and educational programs Formatex. 2017:55-61.
25. Alan Y, Erbil N, Dığrak M. In vivo antimicrobial activity of Rheum ribes ekstracts obtained from various plant parts from Turkey. *J Selcuk Univ Nat Appl Sci*. 2013;1(4):23-9.
26. Aureli P, Costantini A, Zolea S. Antimicrobial Activity of Some Plant Essential Oils Against Listeria monocytogenes 1. *J Food Prot.* 1992 May;55(5):344-348.
27. Nguyen-The C, Lund BM. An investigation of the antibacterial effect of carrot on Listeria monocytogenes. *J Appl Bacteriol.* 1992 Jul;73(1):23-30.
28. Feo VD, Ricciardi AI, Biscardi D, Senatore F. Chemical Composition and Antimicrobial Screening of the Essential Oil of Minthostachys verticillata (Griseb.) Epl. (Lamiaceae). *Journal of Essential Oil Research*. 1998;10(1):61-5.
29. Hao Y, Brackett R, Doyle M. Efficacy of plant extracts in inhibitingAeromonas hydrophilaandListeria monocytogenesin refrigerated, cooked poultry. *Food Microbiol.* 1998;15(4):367-78.
30. Burt S. Essential oils: their antibacterial properties and potential applications in foods--a review. Int J Food Microbiol. 2004;94(3):223-53.
31. Amirmozafari N, Robertson DC. Nutritional requirements for synthesis of heat-stable enterotoxin by Yersinia enterocolitica. *Appl Environ Microbiol*. 1993;59(10):3314-20.
32. Joint FAONWHOSGoFSIAwPfA, World Health O. Food safety issues associated with products from aquaculture : report of a Joint FAO/NACA/WHO study group. Geneva: World Health Organization; 1999.
33. Mytle N, Anderson GL, Doyle MP, Smith MA. Antimicrobial activity of clove (Syzgium aromaticum) oil in inhibiting Listeria monocytogenes on chicken frankfurters. *Food Control*. 2006;17(2):102-7.
34. Oussalah M, Caillet S, Saucier L, Lacroix M. Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: E. coli O157:H7, Salmonella Typhimurium, Staphylococcus aureus and Listeria monocytogenes. *Food Control*. 2007;18(5):414-20.
35. Miller RA, Harbottle H. Antimicrobial Drug Resistance in Fish Pathogens. *Microbiol Spectr*. 2018;6. doi: 10.1128/microbiolspec.ARBA-0017-2017
36. Celikel N, Kavas G. Antimicrobial properties of some essential oils against some pathogenic microorganisms. *Czech J Food Sci.* 2008;26(3):174.
37. Mashak Z, Moradi B, Akhondzadeh basti A, Abasifar A, Gandomi H. Fate of Listeria monocytogenes During the Manufacturing Process of Iranian white Brined Cheese as Affected by Zataria multiflora Boiss. *Essential oil J Med Plant.* 2009;8:114-22.
38. Yersinia enterocolitica and Yersinia pestis. In: Bhunia AK, editor. Foodborne Microbial Pathogens: Mechanisms and Pathogenesis. New York, NY: Springer New York; 2008. p. 227-40.
39. Thomson NR, Howard S, Wren BW, Holden MT, Crossman L, Challis GL, et al. The complete genome sequence and comparative genome analysis of the high pathogenicity Yersinia enterocolitica strain 8081. *PLoS Genet*. 2006 Dec 15;2(12):e206. doi: 10.1371/journal.pgen.0020206.