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In Vitro Study of *Berberis vulgaris*, *Actinidia deliciosa* and *Allium cepa L.* Antibacterial Effects on *Listeria monocytogenes*

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Abstract

Objective: One control method of pathogenic microorganisms is using synthetic chemical preservatives and antibiotics. Because of being generally recognized as safe, antibacterial compounds with organic origin are considered important for health. This study was done in order to investigate the antibacterial effects of methanol extracts of the *Berberis vulgaris* (Barberry), *Actinidia deliciosa* (Kiwi) and *Allium cepa L.* (Onions) on the standard strain (ATCC:19114) of *Listeria monocytogenes (L. monocytogenes)*, as it seems that it is possible to find some important organic and health safe anti-Listerial compounds.

Materials and Methods: After collecting the mentioned plants phytology study was done. Then methanol extracts of named plants were prepared and antibacterial effects of these plants against the mentioned strain of *L. monocytogenes* by the Disk Diffusion, minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) methods were performed. Also Ampicillin (10 µg/disc) was used as the reference antibacterial substance. In order to find the relationship between antibacterial properties of plants extracts independent *t* test, chi-square and analysis of variance (ANOVA) tests were used.

Results: Results showed that the extracts of all the three studied plants had antibacterial effects on *L. monocytogenes*. Average diagonal of growing area in disk diffusion test for barberry, kiwi and onions in order was 12, 15.5 and 11 mm. Also MIC of mentioned plants extracts in order was 125, 62.5, and 125 µg/ml and MBC of named plants was 500, 250 and 500 µg/ ml, respectively.

Conclusion: The results of this work showed that methanol extracts of kiwi had stronger anti-Listerial effect than barberry's and onion's extracts.

Keywords: Actinidia, Anti-bacterial agents, Listeria monocytogenes, Onions

Introduction

Medicinal aromatic herbs have been used traditionaly as a strong source of vegetable, spices, food and natural drugs for many centuries. Recently many researchers have become interested in the chemical extraction, antioxidant and the anti-microorganism properties of these plants (1). *Listeria monocytogenes* (*L. monocytogenes)* is normally found in the environment and gastrointestinal tract of animals. Recent studies have shown that *L. monocytogenes* can be transmitted to humans through consumption of contaminated food and can be found in many types of foods such as raw milk, processed foods, dairy products, meat and related foods e.g. sausage, beef and fresh products like cabbage and also fish and seafoods, egg, fruits and vegetables (2). It is important from the health perspective that food infection caused by this bacteria may lead to complications such as meningitis, septicemia and abortion in pregnant women. However, in cases of outbreak, the mortality rate may be as much as 75% in people prone to infection.

According to the fact that this bacterium can be found in the nature frequently, different foods can be infected. So far many studies have been able to isolate this bacterium from different foods. Since bacteria can easily grow and multiply at refrigerator temperature, preserving the contaminated food at lower temperatures does not eliminate the risk of infection. Mortality rate resulting from this bacterium, very low dose of infection, contamination of most of foods and growth in low temperature have led to efforts to control this bacterium (3).

Recent efforts have been done in order to reduce the economic aspects and health risks of Listeria food-born contamination. One of the control methods for pathogenic micro-organisms is using synthetic chemical preservatives in food. But people are concerned about the use of these chemicals in food since they believe that antimicrobial chemical substances threatens their health so, using natural substances is important for them. Undoubtedly,

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plant extracts and essential oils can be good alternatives. Extracts of plant possess substances that can be used for control of many micro-organisms. The antimicrobial effects against bacteria, yeasts and fungi have been demonstrated.

On the other side antibacterial compounds with organic origins because of being generally recognized as safe (GRAS) are important by having health and nutritional effects (4). Antimicrobial compounds present in foods can extend the shelf life of unprocessed or processed foods by reducing the microbial growth rate or viability. Some of these substances are also known to contribute to the self-defense of plants against infectious organisms (5).

There are more than 1340 plants with defined antimicrobial compounds, and over 30000 components have been isolated from phenol group-containing plant-oil compounds (6). Many of the antimicrobial compounds present in plants can be part of their defense mechanisms against microbial infections (5). Indeed these plant compounds including osides, saponins, tannins, alkaloids, essential oils and organic acids are present as parts of the original plant defense system against microbial infection. Also it has been showed that plants extracts have effect on the gram-positive pathogens bacteria such as *L. monocytogenes*. They can also enhance storage stability by means of active components including phenols, alcohols, aldehydes, ketones, ethers and hydrocarbons, especially in garlic and onions (6).

Due to abundant use of barberry, kiwi and onions in Iran this study was done in order to investigate the antibacterial effects of methanol extracts of the *Berberis vulgaris* (barberry), *Actinidia deliciosa* (kiwi) and *Allium cepa L.*(onions) on the standard strain (ATCC: 19114) of *Listeria monocytogenes (L. monocytogenes)*, as it seems that it is possible to find some important organic and health safe anti-Listerial compounds.

Materials and Methods

Collecting Plants Material

A voucher specimen of plants was identified (Table 1), preserved and deposited at the Herbarium of Food Hygiene Department of Tabriz branch, Islamic Azad University, Tabriz, Iran. Then the plant samples were dried in the shade, powdered and stored at 4°C until in vitro tested (7).

Extract Preparation of Plants for Tests

One gram of plant parts with 100 ml (methanol 80%) were extracted by maceration. Extracts were filtered with Whatman No. 1 filter paper. The filtrates obtained from extracts were evaporated into dry rotary evaporator at 40°C and were stored at 4°C (7).

Preparation of Bacterial Suspension

In this work one of the standard strain of *L. monocytogenes* (ATCC: 19114) obtained from center of scientific-industrial studies of Iran (Karaj, Iran) was used. In considering the fact that preparation of a microbial suspension requires a new culture for every bacterium, therefore about 24 hours before each experiment, a suspension of tested mentioned bacteria was prepared from fresh colonies on blood agar and BHI agar plates (Merck, Germany) after overnight incubation at 37˚C. Then the purified colonies were created in a medium washed with normal saline and the turbidity of bacterial suspension was adjusted to 0.5 McFarland standard. That is mean, microbial suspension used in various testes at present study contained ~1.5×10^8 CFU/ml of the tested bacteria (8).

Preparation of Antibacterial Disks for Antibiogram Test

It should be explained that the disks containing methanol extracts of mentioned plants are prepared from sterile blank disks manufactured in Padtan-Teb company (Tehran, Iran). Thus, the blank disks were placed individually in tubes containing each of the mentioned plants methanol extract's for 30 to 50 minutes and following the complete absorption by disk. Then the disks were placed at 44-45˚C until completely dry and ready for disk. The standard disks of Ampicillin antibiotic (10 mcg/disk) as positive control were prepared by Padtan-Teb company (Tehran, Iran) (9). Antimicrobial effects were investigated in microbial laboratory of Pathobiology Department in Tabriz branch, Islamic Azad University and each test was repeated three times.

Study of Antibacterial Effects of Plant Extracts

Agar Disk Diffusion Test (Antibiogram)

In present study, the antibacterial effects of mentioned plant extracts was tested by using agar disk diffusion method. It is important to note that the sterile blank disks manufactured by Padtan-Teb company (Tehran, Iran) were used in this study. The blank disks were submerged separately in the tubes containing the extracts of these plants and after 30-50 minutes when the extracts were completely absorbed, the disks were heated to approximately 45˚C until completely dried and ready for use. Then 100 µl of mentioned bacterial suspension (prepared in accordance to McFarland 0.5 standard pipe) were cultured on the surface of Mueller-Hinton agar medium. Afterwards, the plates were incubated for 48 hours under a temperature of 37˚C and the antibacterial effect of plants extracts were tested separately. Then the diameter of the inhibition zone around the disks was measured using a scale ruler and recorded with high accuracy. In order to ensure more confidence, each plant extracts tested were

Table 1. Characterization of Onions, Kiwi and Barberry Testing in Present Study

Plants' Name	Scientific Name of Plants'	Used Organ	Collecting Area
Barberry	*Berberis vulgaris*	Fruit	Northwest of Iran, East Azarbayjan, Kaleybar
Kiwi	*Actinidia deliciosa*	Fruit	North of Iran, Gilan, Astara
Onions	*Allium cepa L.*	Bulbs	Northwest of Iran, East Azarbayjan, Ilkhechi

examined repeatedly for 3 times then the mean diameter of inhibition zone of these repetitive measurements was recorded as the final diameter of the inhibition zone of the that plant extracts (9). Also, in all stages of the experiment, the standard antibiotic of Ampicillin (10 mcg/disk) was used as the positive control.

The Minimum Inhibitory Concentration and the Minimum Bactericidal Concentration Tests

By using the method of serial dilution, the minimum inhibitory concentration (MIC) and the minimal bactericidal concentration (MBC) of methanol extracts of each plant were determined. In order to determine the MIC the series of 10 test tubes were used. In this case, 8 test tubes were used for experimenting different solutions of plants extracts while one test tube was used as a positive control and another test tube used for negative control. In a way that from tube number one containing 1 mg/ml of extracts to tube number 8 containing 7.8 mcg/ml of the mentioned solution and all tubes containing 9 ml of the BHI culture medium and also 1 ml the mentioned bacterial suspension. It should be noticed that one test tube containing 9 ml from the mentioned medium plus 1 ml from each of the plant extracts was used as the plants extracts control and another test tube containing 9 ml from same medium plus 1 ml from bacterial suspension of *L. monocytogenes* (ATCC: 19114) was used as bacterial control and we did this separately for each of the extracts. Then, all test tubes mentioned were incubated for 48 hours at 37˚C.

After the incubation period, all test tubes were examined for turbidity as a result of the bacterial growth. It should be noted that this experiment on the extracts of the each mentioned plants were repeated 3 times in a separate manner and the mean concentration desired was calculated. The highest dilution of each plant extract that was inhibited from bacterial growth was considered as the MIC of that plant extract.

It should be noted that this experiment on the extracts of the each mentioned plants were repeated 3 times in a separate manner and the mean concentration desired was calculated.

Also in order to determine the MBC of each extract, sampling was done from all tubes used to determine MIC that had no bacterial growth. For this purpose 1 ml of the mentioned test tubes was mixed in a Petri dish with 20 ml of BHI agar medium (sterile in the laboratory autoclave) at the temperature of 45˚C. After gradual cooling at room temperature and the so called progressive closure agar, all plates were incubated for 48 hours at a temperature of 37˚C; then the cultured plates were controlled for the presence of bacteria.

For this purpose the highest dilution of each plant extracts that was prevented from forming bacterial colony in mentioned culture media was considered as MBC of that extracts (9).

Statistical Analysis

In order to find the relationship between sensitive and resistant properties of plant samples, chi-square test and Independent *t* test were used. Also, in order to determine which samples had significant mean differences, analysis of variance (ANOVA) with equal frequency was used. Also data in the range of $P < .05$ was considered statistically significant (subset for alpha = .05). It is crucial that the statistical methods used in the comparison between MIC and MBC of plant extracts was descriptive statistics.

Results

According to Table 2, each 3 mentioned plant extracts had antibacterial effects on *L. monocytogenes* (ATCC:19114). Also in disk diffusion test, comparison of inhibition zone which is related to kiwi plant extracts with ampicillin (standard antibiotic) as positive control against testing bacterium showed a significant statistical difference (mean ± SD of inhibition zone in disk diffusion test = 9 mm ± 0.1).

Discussion

Edible medicinal and herbal plants and their isolated compounds are known to inhibit the growth of micro-organisms. Also these compounds may be lethal to microbial cells. Several studies showed that the mentioned antimicrobial compounds in plant materials are commonly found in the leaves, flowers, buds, bulbs, seeds, rhizomes and fruits (5). Actually plant-origin antimicrobials compounds are collected by various methods from flowers, buds, seeds, leaves, twigs, bark, herbs, wood, fruits and roots of plants. Various researches have determined that these extracts can be obtained from plants by various methods such as steam, cold, dry and vacuum distillation (6).

The major components with antimicrobial effects found in medicinal plants are phenol compounds, trepans, aliphatic alcohols, aldehydes, ketones, acids, and flavonoides (5). Chemical analysis of a range of these components revealed that the principal constituents of many include carvacrol, thymol, citral, eugenol and their precursors. These compounds are primarily responsible for bactericidal or

Table 2. Results of MIC, MBC and Disk Diffusion Tests of Onions, Kiwi and Barberry Methanol Extracts on *Listeria monocytogenes* (ATCC:19114)

Name of Tested Components	Means ± SD of Inhibition Zones in Disk Diffusion Test (mm)	Results of MIC Test (µg /ml)	Results of MBC Test (µg/ml)
Barberry	12 ± 0. 2	125	500
Kiwi	15.5 ± 0.2	62.25	250
Onions	11 ± 0. 2	125	500

Abbreviations: MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration.

bacteriostatic properties of the plants (5,6).

There are various chemical components present in plant-origin antimicrobials including saponin and flavonoids, thiosulfinates, glucosinolates and saponins. Saponin and flavonoids are found in fruits, vegetables, nuts, seeds, stems and flowers. The antimicrobial activities of saponins and flavonoids in plants and anthraquinones, carbohydrates and alkaloids derived from plants have been proven when extracted from roots, stem bark, leaves and wood (6).

There are increasing reports of nonphenolic compounds of plants, which are effective against both gram-positive and gram-negative groups' bacteria (6). Anti-Listeria effects of herbal extracts and essences have been studied by some researchers. Also many researches have been done, studies which are about anti-bacterial effects of herbal extracts in bacterial cultures (10). Different kind of studies in this field showed that herbal essences of sage, eucalyptus, Indian clove and mint, *Callistemon citrinus*, onions and also cumin have anti-Listerial effects but extracts of some plants like Shirazian thyme, chamomile, rosemary and sumac (*Rhus coriaria*) did not have anti-Listeria effects (11,12).

Various studies have shown that onion extracts have anti-bacterial effects against *Escherichia coli (E. coli), Bacillus subtilis, Salmonella typhi*, *Staphylococcus aureus (S. aureus)*, *Pseudomonas aeruginosa (P. aeruginosa)* and *Candida albicans (C. albicans)* which are related to onion's flavonoids. Moreover onion's raw extracts have strong effect on *P. aeruginosa*, small effect on *C. albicans* and no effect on *S. aureus* and *E. coli*. Whereas, Onion's hot water extracts does not have anti-bacterial effect on any of bacterium (13-19). Also the study of Momeni and Zamanzad in 2010 determined that Onion extracts does not have any remarkable effect on tested *L. monocytogenes* (20). Table 2 of our work also showed that this result is highly similar to the results of our present study. Notably, some plants contain water and methanol extracts and some of them have ether extracts which have high effects on gram- positive bacteria. So it seems that no anti-Listerial effect of onion's extracts in this work is related to kind of effective substances in extracts, techniques of extraction, and kind of solvent even used methods (11,12).

On the other hand, anti-bacterial effects of barberry are reported in multiple studies. Previous studies showed that different kinds of barberry plant have anti-bacterial effects in vivo and in vitro. It is mentioned that certain alkaloid blends are in the structure of wild barberry and responsible for this feature. It is proved that alkaloids intercalate in nucleic acid and cell wall of bacteria and prevent the activity of these two important parts. Also, given that botanical structure of wild barberry has minor differences with other species of this plant therefore; it seems that the mentioned subject can be true about the tested barberry in this study (21). The result of this study somehow showed the anti-Listerial effects of barberry which have similarities with other studies. It seems that these results can justify why in Iranian traditional medicine, different kinds of barberry are mentioned as an anti-bacterial plant. Also some clinical properties of kiwi fruit ingredients such as antibacterial agents have been reported in the literature (22). Previous study has shown the effect of kiwi fruit prevents bacterial proliferation. They suggested that the dramatic antibacterial effect of kiwi fruit induced a significant wound healing in burn ulcers and might be useful in treating chronic ulcers (23). Results of the present work showed that anti-Listerial effect of tested kiwi extracts is more than onions and barberry ($P < .05$). In a similar research by Singh et al in 2003 which was done on anti-Listerial effects of thyme, clove, pimento, rosemary and sage oil extract's in vitro and one form of food (Hot Dog) it appeared that thyme has the most effect of Listeria strains, also clove and pimento showed a moderate anti-bacterial effect; but rosemary and sage did not have any effect (11). On the other hand in the present work, it is revealed that anti-Listerial effects of all 3 extracts are more than ampicillin antibiotic and this result is in line with results of Mahan Nair et al, which showed that Nigella has much more anti-Listerial effect than the antibiotic of gentamycin (24).

Generally, the antimicrobial efficacies of plants are dependent on the chemical structure of their components as well as the concentration (5). Growth level of plants, variety of plants and strain of the tested microorganisms are mentioned (5,25). A plant in different areas can show different combinations, features, and properties. Also in this research, type and technique of extracted can play important role in the laboratory anti-bacterial results of that plant (25).

Conclusion

Results of this research showed that kiwi's methanol extracts in comparison with barberry's and Onions' extracts is a stronger anti-Listerial substance and could be useful. It seems that by studying the organoleptic effects of mentioned extracts in food, it is possible to use them as a preservative substance. Of course many studies are needed to figure out the anti-Listerial effect of these plants, and it is possible by using other techniques of extraction with higher concentration than what we have tested in this research.

Ethical issues

Ethical considerations of this research work was approved by Tabriz Branch, Islamic Azad University.

Conflict of interests

None to be declared.

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