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The Antimicrobial Effects of Medicinal Plants on Pathogenic Food Bacteria

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Abstract

Objectives: This paper aimed to explore the antimicrobial effects *Mentha piperita*, *Solanum nigrum*, *Mentha longifolia*, and *Withania somnifera* on food pathogens.
Methods: Plant extracts were obtained using the rotary system, the minimum inhibitory concentration and diluting method, and examined against bacteria such as *Staphylococcus aureus* ATCC1189, *Shigella dysenteriae* ATCC1188, *Listeria monocytogenes* ATCC1298, *Vibrio cholera* ATCC1611, and *Bacillus cereus* ATCC101.
Results: The results showed that extracts with various inhibitory ppm could inhibit the growth of antigenic bacteria so that the extract of *Solanum nigrum* could strongly inhibit *S. aureus*, *Listeria*, and *Vibrio*; and the extract of *Mentha longifolia* with 6.25 ppm can inhibit *B. cereus*.
Conclusions: The obtained results revealed that the extracts could strongly inhibit the bacteria in foods and that *Solanum nigrum* showed the highest antibacterial effect.

Keywords: Antimicrobial Activities, Food Pathogens, Plant Extracts

1. Background

Consumption of foods containing high amounts of toxic microorganisms is the main cause of human poisoning. In this regard, bacteria are the main bacterial causes. Food researchers and supervisors are concerned about the growth of microbial diseases caused by microorganisms and pathogens in foods.

Food diseases have increased in the recent years despite the amelioration of hygiene. Therefore, it seems necessary to pay more attention to pathogenic microorganisms to prevent their poisoning. Various chemical additives are used to prevent the growth of pathogenic microorganisms. Due to the harmful effects of these chemicals such as causing cancer and poisoning and resistance of pathogens, many consumers are demanding the use of vegetative, animal and microbial conservatives to both increase stability of foods and remove the harmful effects of chemical additives.

Spice essences and vegetables are among natural compounds, which have been widely used in food industries. They not only hinder the growth of microorganisms but also flavor food materials (1, 2).

Herb drugs have been used throughout history for treating diseases. Although most of the current drugs are chemical, yet it has been estimated that about one-third of drugs is vegetative or has been transformed after extraction from plants (3).

Mentha longifolia Ll is a member of the *Lamiaceae* family and a branch of Nepetoideae. It is a multiyear plant. It is used to cure inflammation, spasms and flatulence (4). They also have antimicrobial, antifungal and antioxidant effects (4, 5).

The plant *Withania somnifera* (L.) Dunal, often recognized as "*Ashwagandha*", is famous for its treatment applications in the ayurvedic procedure of traditional medicine. It has been applied as an antibacterial, antioxidant, adaptogen, aphrodisiac, liver tonic and anti-inflammatory agent (6).

Mentha piperita L., a scientifically valuable plant falls under the category of the family Lamiaceae, and is usually famous as peppermint, which is a hybrid of *M. spicata* L. (spearmint) and *Mentha aquatic*

Solanum nigrum commonly known as black night-

shade belongs to the Solanaceae family. It shows medicinal properties like antimicrobial, antioxidant, cytotoxic properties, antiulcerogenic and hepatoprotective activity. It is a potential herbal alternative that act as an anti cancer agent (7).

2. Objectives

The aim of this study was to evaluate the minimum inhibitory concentration (MIC) of commonly used antibiotics and different natural plant extracts against food pathogens.

3. Methods

3.1. Bacterial Strains and Culture Condition

Bacterial strains were obtained from a standard laboratory. The antibacterial activity of the extracts was investigated using *Staphylococcus aureus* ATCC1189, *Shigella dysenteriae* ATCC1188, *Listeria monocytogenes* ATCC1298, *Vibrio cholera* ATCC1611, and *Bacillus cereus* ATCC1015.

3.2. Plant Materials

The plants (*Mentha piperita*, *Solanum nigrum*, *Mentha longifolia*, *Withania somnifera*) were gathered from Zabol, Southeastern Iran, and dried at room temperature. Samples were broken and placed on a glass plate and kept until the extraction process was finished in the laboratory.

3.3. Preparation of Extracts

Twenty grams of the chosen fresh leaf materials were intermingled with 60 mL of 95% ethanol, in a grinding machine for about 10 to 15 minutes for separating the extract phases for one day (shaking occasionally with a shaker). The supernatant was filtered through Whatman No. 1 filter paper. The extracts were aseptically preserved at 5°C for further use.

3.3.1. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Plant Extracts

The broth microdilution method was used to determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5% (v / v). Briefly, serial doubling dilutions of the extract were prepared in a 96-well microtiter plate ranging from 0.3 mg/mL to 100.00 mg/mL. To each well, 10 μL of indicator solution (prepared by dissolving a 10-mg extract in 2 mL of DMSO) and 10 μL of Mueller Hinton Broth were added. Finally, 10 μL of bacterial suspension (10^6 CFU/mL) was added to each well to achieve a concentration of 10^4 CFU/mL. The plates were wrapped loosely with cling film to ensure that the bacteria did not become dehydrated. The plates were prepared in triplicates, and were then placed in an incubator at 37°C for 18 - 24 hours. The color change was then assessed visually. The lowest concentration at which the color change occurred was taken as the MIC value. The average of three values was calculated to determine the MIC and MBC values for the tested extract. The MIC is defined as the lowest concentration of the extract at which the microorganism does not demonstrate visible growth. Growth of the microorganisms was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed.

4. Results

The obtained results revealed that the extracts with various concentrations could inhibit pathogenic bacteria so that the least inhibitory effect was reported for *Mentha longifolia* with 6.25 mg/mL against *B. cereus* while four other bacteria were inhibited by the same extract at a concentration of 12.5 mg/mL. As for *Solanum nigrum*, its least inhibitive effect was at 6.25 mg/mL for *S. aureus*, *Listera* and *Vibrio*, and the highest was at 100 mg/mL for *B. cereus* and *Shigella*. Regarding *Withania somnifera*, the results showed that the lowest and highest inhibitory effects were at 25 and 100 mg/mL, respectively. It inhibited *B. cereus* and *Shigella*. Concerning *Mentha piperita*, it was revealed that *B. cereus* and *Vibrio* were inhibited by this extract (Table 1).

5. Discussion

Based on the results, the extracts could strongly inhibit pathogenic bacteria in at various concentrations, so that *Solanum nigrum* could inhibit *S. aureus*, *Listeria* and *Vibrio*. Furthermore, *Mentha longifolia* at 6.25 mg/mL could inhibit *B. cereus*.

The study of Singh showed that ether and ethyl acetate extracts of *M. piperita* were more effective against *S. aureus* and *K. pneumoniae* when compared to *S. pyogenes* and *E. coli*. A similar trend was observed for ethyl acetate and aqueous extracts (8). The study of Sujana, showed that ethyl acetate leaf extract of *Mentha piperita* demonstrated significant inhibition when compared to chloroform, petroleum ether and hexane. Furthermore, the leaf-extract activity was more on *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus vulgaris* than *Escherichia coli*, *Streptococcus pneumonia* and *Klebsiella pneumonia*. In the current research, we also assessed phytochemical analysis for the presence of different secondary metabolites. The results

Table 1. The Minimum Inhibitory Concentration and Minimum Bactericidal Concentration of Antibacterial Extracts Against Food Pathogens

	W. somnifera, MIC / MBC	*M .piperita*, MIC / MBC	*S. nigrum*, MIC / MBC	*M. longifolia*, MIC / MBC
S. aureus	12.5 / 25	6.25 / 12.5	25 / 50	50 /100
Listeria	12.5 / 12.5	6.25 / 12.5	50 / 100	50 /100
Vibrio	12.5 / 25	6.25 / 12.5	50 / 100	12.5 / 25
B. cereus	6.25 / 12.5	50 / 100	100 / 200	12.5 / 25
Shigella	12.5 / 25	50 / 100	100 / 200	50 / 100

showed the presence of alkaloids, flavonoids, steroids, tannins and phenols (9). Alvandi et al. found that the most important compounds of *Solanum nigrum* are menthol (39.81%), menton (19.55%), neo-menthol (8.835), methyl acetate (8.83%), and sineol (5.81%) (10). The study by Abdolmaleki et al. (2011) revealed that culture of aqueous extract at 500 ppm hindered the growth of *P. drechsleri*, and at 100 ppm hindered the growth of *B. sorokiniana*. Regarding the two other fungi, growth was not hindered even at 2000 ppm (11).

Bokaeian et al. (2014) showed that the highest volume of MIC in the ethanolic extract of *Mentha longifolia* was 250 ppm *Clicila Penomonei* and the least volume was 63ppm (12). Singariya et al. (2012) found that the MIC of the aqueous extract of *Mentha longifolia* was 11.17 + 0.26 and 7.33 + 0.24 against *Proteus merabillis* and *Agrobacterium tumefaciens* (13). Jain and Varshney (2011) realized that the methanolic extract of *Mentha longifolia* formed protective shields of 38, 36, 15, 38, and 32 against *E. coli*, *P. aeruginosa*, *S. aureus*, and *C. albicans* (14). Another Study showed Mentha longifolia with 100 microgram ppm in each disk formed protective shields of 15 + 0.5 against *Aspergillus fumigatus* (15).

5.1. Conclusion

As the attempt for the production of herbal medicines is in progress around the world, the current study will assist the isolation of new products/medicines. Finally, it can be claimed that active chemical compounds prevailing in plant extracts should be used in medication of different bacterial infections and these herbs must be studied more comprehensively to find their potentiality in the medication of infectious diseases.

Footnote

Authors' Contribution: The authors had equal contributions.

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