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# Antibacterial activity of *Mentha longifolia* against *Salmonella typhimurium*

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## ABSTRACT

**Background and aims:** Traditionally, plants have long been applied in the treatment of various diseases throughout the history of human life. The antimicrobial activity of plant extracts in the laboratory is studied on Gram positive and negative strains of human infectious agents. The aim of this study was to evaluate the antibacterial activity of *Mentha longifolia* against important human pathogens.
**Methods:** This was an experimental study and no statistical method was used. The antimicrobial effect of ethanol extracts of *Mentha longifolia were investigated* on *Salmonella typhimurium* were determined using broth microdilution method. The broth microdilution method was used to determine the Minimal Inhibitory Concentration (MIC) and the Minimum Bactericidal Concentration (MBC). All tests were performed in Mueller Hinton broth supplemented with Tween 80 at a final concentration of 0.5%.
**Results:** The results showed that the lowest MIC concentration was 5 mg/ml that 1 strain of Salmonella was inhibited. The highest and the lowest MBC values of extract were 40 and 10 mg/ml, respectively.
**Conclusion:** Since the antibiotic resistant bacteria are increasing in the world, replacing the chemical and synthetic antibiotics with natural products is essential. It can be concluded from the results of this study that using herbal plant instead of antibiotics can resolve some antibiotic resistant problems.

**Keywords:** Antibacterial, Ethanol extract, *Mentha longifolia, Salmonella typhimurium.*

Original article

## INTRODUCTION

Resistance of microorganisms, insufficient chemical agents commonly used in the treatment of infectious diseases, and increased awareness of consumers and the health authorities to the harmful effects of synthetic chemical food preservatives have caused to perform extensive researches to achieve a natural combination with the wide range of biological activities derived from plants and animals.[1,2] Evidence indicates the ancient remains of early human use of plants as medicine in treatment of diseases. With the development of dairy branch of science, the use of chemicals in the production of medicine has enhanced, but soon attracted the attention of researchers to the effects and benefits of these drugs, scientists again have to use herbal preparations in the treatment of diseases.[3] The genus Mentha belongs to the family of Lamiaceae consisting of about 25-30 species. Most of

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them are found in temperate regions of Eurasia, Australia and South Africa. *Mentha longifoliais* has been used as a traditional medicine for stomach ache, anti-asthmatic, anti-spasmodic, digestive and carminative.[4]

The aim of this study was to determine the antibacterial activity of *Mentha longifolia* against *Salmonella typhimurium*.

## METHODS

The leaf of *Mentha longifolia* was purchased from Municipal market in Zahedan, Iran and kept in sterilized screw-cap glass container. Samples were crashed and transferred into glass container and preserved until extraction procedure was performed in the laboratory.

Plants were properly dried and each of 10 g grinded powders was soaked in 60 ml ethanol 95%, separately for one day (shaking occasionally with a shaker). After one day of dissolving process, materials were filtered (Whatman no. 1 filter paper). Then, the filtrates were evaporated using rotary evaporator. At last, 0.97 g of dried extracts was obtained and then stored at 40 °C in air tight screw-cap tube.

All strains were isolated at different times during 2013- 2014 from contaminated birds. Samples were diluted and/or homogenized in Tryptic Soy Broth (TSB) medium, and isolates obtained by *Salmonella* selective enrichment in Rappaport–Vassiliadis (RV) medium after 24 h incubation at 43°C and for antibacterial assay bacterial suspensions with concentration of $1.5\times10^8$ cfu/ml (0.5 McFarland standards) were prepared in nutrient broth. In this method, the bacteria were cultured on a Muller-Hinton agar plate, then ampicillin (10 µg) and penicillin (10 µg) disks were placed on media in 20-30 mm with other disks. The plates were incubated for 18-24 h at 37 °C.

The broth microdilution method was used to determine Minimum Inhibitory Concentration (MIC) of plant extracts 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 ranged from 0.3 mg/ml to 40.00 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 the concentration of $10^4$ CFU/ml. The plates were wrapped loosely with cling film to ensure that the bacteria did not get dehydrated. The plates were prepared in triplicates, and then they were 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 3 values was calculated providing 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 the visible growth. The microorganism growth was indicated by turbidity. The MBC was defined as the lowest concentration of the extract at which the incubated microorganism was completely killed.

## RESULTS

The result of herbal extraction showed that the highest Minimum Inhibitory Concentration (MIC) was 20 mg/ml concentration that 3 strains of them were inhibited by this concentration. The lowest MIC concentration was 5 mg/ml that 1 strain of *Salmonella* was inhibited. The highest and lowest MBC values of extract were 40 and 10 mg/ml respectively (Table 1).

**Table 1**: The minimum inhibitory concentration of extract against *Salmonella*

| Bacterial cod | MIC/MBC for extract plant (mg/ml) | Antibiotic resistant |
|---|---|---|
| 1 | 5.10 | AM-P |
| 2 | 10.20 | AM-P |
| 3 | 10.20 | AM-P |
| 4 | 10.20 | AM-P |
| 5 | 10.20 | AM-P |
| 6 | 10.20 | AM-P |
| 7 | 10.20 | AM-P |
| 8 | 10.20 | AM-P |
| 9 | Any growth | AM-P |
| 10 | 20.40 | AM-P |
| 11 | 20.40 | AM-P |
| 12 | 20.40 | AM-P |

*AM= Ampicillin; P=Penicillin*

# DISCUSSION

*Mentha piperita* L. (Peppermint) is a perennial glabrous and strongly scented herb belonging to the family of Lamiaceae. The plant is aromatic, stimulant and used for allaying nausea, headache and vomiting. Its oil is one of the most popular widely used essential oils in food products, cosmetics, pharmaceuticals, dental preparations, mouthwashes, soaps and alcoholic liquors.

In the study of Stanisavljevic, the essential oil obtained from the herb dried in the natural way has shown the highest antioxidant activity, but this effect was lower than that of the herb dried in the laboratory oven. *Bacillus subtilis*, *M. luteus* and *Enterococcus faecalis* have shown the highest sensitivity on the three samples and the results showed that the inhibitory zone diameter at the concentration of 2% of oregano dried at 45 °C in laboratory against bacteria *M .luteus, M. flavus, S. aureus, S. epidermidis, E. faecalis, B. subtilis, E. coli, K. pneumoniae, P. aeruginosa, C. albicans* ATCC: 10259, *C.albicans* ATCC: 24433 20, 20, 20, 24, 17, 5.18, 5.19, 5.21, 5.13, 5.17 and 19.5 mm and concentration of 4% the obtained results against the studied bacteria were respectively 25, 21, 23, 28.5, 19.5, 17, 21.5, 21, 17, 23, and 25.5 mm.[5] The study results revealed the minimal inhibitory concentration (MIC). In the study of Akroum and colleagues ethanol extract of oregano against bacteria *S. aureus*, *B. cereus* and *B. subtilis* was equal to 0.180, 0.160 and 0.090mg liters while the minimum inhibitory concentration of the methanol extract against bacteria of B. cereus and B. subtilis were 0.250 and 0.170 of *B. cereus* and *B. subtilis* mg liters, Minimum inhibitory concentration of flavonoid Luteolin 7-O-glucoside from oregano against bacteria *S.aureus, B.cereus* and *B.subtilis* was equal to 0.070, 0.090 and 0.050 mg/ml.[6] Research showed that the isolation of an antimicrobial compound from *M. longifolia* leaves validates the use of this plant in the treatment of minor sore throat and minor mouth or throat irritation.[7] Also, the essential oil of *Mentha longifoliassp* has great potential of antimicrobial activity against bacteria and yeasts.[8] The study of Mohkami et al results showed that the minimal inhibitory concentration (MIC) for essential oil of oregano against bacteria *E. coli, L. monocytogenes, B. cereus, B. licheniformis, P. aeruginosa, S. aureus* and *S. typhito* were 20000, 5000, 625, 10000, 25.156, 25.156 and 20,000 mg/ml respectively.[9] Main constituents in *Mentha longifolia* samples collected from various locations such as Tunisia, pulegone (54.41%) were isomenthone (12.02%), 1, 8-cineole (7.41%), borneol (6.85%), and piperitenone oxide (3.19%) (Mkaddem and colleagues), Tajikistan, Cis-piperitone epoxide (7.8-77.6%), piperitenone oxide (1.5-49.1%), carvone (0.0-21.5%), menthone (0.0-16.6%),

thymol (1.5- 4.2%), pulegone (0.3-5.4%), β-thujone (0.2-3.2%), (E)-caryophyllene (0.9-2.5%), myrcene (0.3-2.5%), carvacrol (0.0-2.7%), borneol (0.9-1.8%), and p-cymene (0.2-1.9%),[10] As a major component, Iran, piperitone (44%), limonene (14%), and trans-piperitone (13%).[11] The study of Razavi and colleagues showed that the inhibitory zone diameter/minimum inhibitory concentration of the methanol extract of oregano against bacteria *E. coli, S. aureus, E. faecalis, S. agalactice* and *E carotovora* were 34.512, 40.192, 39.192, 39.192, and 41.128 mm/mg liters.[12] It was previously documented that Methanol, Ethanol and Water extracts of *M. longifolia* presented a good antimicrobial activity.[13,14]

The study of M. Viljoen, by eight samples of *M. longifolia* (representing eight natural populations), two major chemotypes were identified: a menthofuran-rich type (51-62%); and a cis-piperitoneoxide (15-36%) and piperitenone oxide-rich type (15-66%). The constituent analysis showed quantitative variation with higher amounts of oxygen-containing monoterpenes ranging from 57% to 90% whilst the sesquiterpene hydrocarbons ranged from 4% to 17%. The oil from the different geographical areas mostly showed moderate antimicrobial activity against *Staphylococcus aureus, S. epidermidis, Bacillus cereus, Moraxella catarrhalis, Yersinia enterocoliticaand Enterococcus faecalis*. The oils were generally inactive against *Escherichia coli* and *Salmonella typhimurium*. *Candida albicans* and *Cryptococcus neoformans* indicated the highest sensitivities for oil samples from Komukwaneand Prins Albert. These results may partially provide scientific evidence for the extensive use of *Mentha longifolia* in traditional healing.[15]

In the study of Bakht, all extracts from *Mentha longifolia* showed different ranges of antimicrobial activities. Butanol and ethyl acetate fractions showed inhibitory activities against all microbial species. Methanol fraction showed inhibitory effects against all the tested microbial species except *Salmonella typhi.* It was also not controlled by methanol, petroleum ether and dichloromethane extracted samples. The most susceptible gram positive bacteria was *Bacillus atropheus* and *Bacillus subtilis* which were inhibited by all extracts and Staphylococus aureus was the least susceptible among gram positive bacteria. *Klebsiella pneumoniae* was the most susceptible gram negative bacterium and *Salmonella typhi* was highly resistant among the gram negative bacteria. *Erwinia carotovora* and *Agrobacterium tumefaciene* were susceptible to all fractions. All fractions showed antifungal activities against *Candida albicans* except water extracted samples.[16]

In the study of Derwich, the results revealed that essential oil yields and the total oil of *Mentha rotundifolia* were 1.54% and 90.40%. The major component was menthol (40.50%), and other predominant constituents were: menthone (5.0%), menthyl acetate (4.50%), menthofuran (4.20%), oxyde de piperitone (3.80%), linalyl acetate (3.50%), neomenthol (3.20%), piperitone (3.10%), isomenthone (2.50%), 1,8-cineole (2.40%), linalool (2.0%), limonene (1.80%), geraniol (1.70%), myrcene (1.60%), geranyl acetate (1.50%) and trans- Sabinene hydrate (1.40%). Essential oil extracted from *Mentha rotundifolia* showed the highest activity against *Escherichia coli, Staphylococcus aureus, and Staphylococcus intermedius,* with the strongest inhibition zone of 45, 34 and 31mm respectively.[17] In the study of Rodrigus, the essential oil of *M. longifolia* was dominated by the monoterpenes pulegone (52–75%), isomenthone (8–24%), limonene (4–6%), and menthone (1–2%). The antibacterial activity of these EOs was compared to that of the main components

standards. The most effective antibacterial activity was expressed by the EOs against the Gram-negative bacteria, *Escherichia coli* and *Acinetobacter baumanni*, with MIC values of 1 mg/ml. The EOs complex mixtures were more active than the individual aromatic components supporting the hypothesis that the EOs antibacterial activity has a functional synergistic effect in comparison with their different aromatic components. These results show the potential role of *M. cervina* EOs as antibacterial agents and validate the traditional use of this plant.[18] The essential oil of *M. longifolia* has shown interesting antimicrobial activity against *E. coli, S. typhimurium,*[19] L. monocytogenes, *A. flavus, Botrytis cinerea, Fusarium oxysporum, P. aeruginosa, A. niger,*[20] *Trichophyton longifusus, Microsporm canis*[21] *and Mucor ramamnianus*.[22] The most sensitive micromycetes against the extract of this plant were shown to be *cladosporium fulvum, Penicillium ochrochloron and Cladosporium cladosporioides* with a lethal dose of 2.5 µl/ml.[23] The extract of *Mentha longifolia* showed moderate antibacterial activity against gram negative and gram positive bacteria.

## CONCLUSION

Since the antibiotic resistant bacteria were increasing in the world, replacing these chemical with natural product is essential. It can be concluded from results of this study that by using herbal plant instead of commonly used synthetic antibiotics, some antibiotic resistant problems can be resolved.

## CONFLICT OF INTEREST

There is no conflict of interest associated with this study.

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