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Antibacterial and Drug Synergistic Activities of *Mentha longifolia* Essential Oil Against *Shigella flexneri* and *Shigella sonnei*

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Abstract

Background: Microbial infections such as shigellosis are one of the major health challenges in Iran, especially in Khuzestan province in the south west of Iran.

Objective: According to the importance of medicinal plants in the treatment of many infectious diseases, and as a valuable alternative for antibiotics, the aim of this research was to assess the antibacterial and drug synergistic activities of the essential oil from *Mentha longifolia*, a local plant, against *Shigella flexneri* and *Shigella sonnei* as the main causes of shigellosis.

Materials and Methods: The *M. longifolia* essential oil was extracted from the leaves. The antibacterial activities of the essential oil against clinical and standard *S. flexneri* and *S. sonnei* strains were detected by the disk diffusion and micro-broth dilution methods.

Results: The essential oil of *M. longifolia* had the most significant antibacterial activity against the clinical strain of *S. flexneri*. Minimum inhibitory concentration (MIC) of 1024 with a concentration of 0.8 mg/mL of essential oil was detected in both the standard and clinical *S. flexneri* and *S. sonnei* strains. The essential oil of *M. longifolia* showed the highest synergistic effect on gentamicin and ampicillin in the clinical isolates of *S. flexneri*.

Conclusion: The results of this study showed that the essential oil of *M. longifolia* alone or in combination with antimicrobial agents may be useful in the treatment of bacterial infections. In addition, *M. longifolia* may increase the effect of antibiotics and resolve other antibiotic resistance problems.

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Background

Shigella is a gram-negative bacillus of the Enterobacteriaceae family. *Shigella* species are classified into 4 serogroups; *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, and *Shigella boydii*, all of which can cause bacillary shigellosis or dysentery (bloody diarrhea).[1,2] Shigellosis is observed in both developed and developing countries. Shigella is one of the major bacterial agents of illnesses that cause death in children. One of the problems raised in the recent years is the increasing antibiotic resistance of the *Shigella* species to the usual antibiotics throughout the world including Iran.[3]

Due to the indiscriminate use of antibiotics and the increased resistance to these drugs, some antibiotics should be removed from the list of shigellosis-treating drugs. Ampicillin is on top of the list as bacteria show resistance to this antibiotic by 100%. Although drugs such as co-trimoxazole, nalidixic acid, and gentamicin have been considered as effective antibiotics for *Shigella*, at present, a gradual resistance to these drugs has been observed with increasing frequency.[4] With the increasing resistance shown by many bacteria, researchers have made significant efforts for finding new sources of antibiotics and new microbicides. One of the sources found is medicinal plants.[5]

Over the years, natural drugs, especially medicinal plants and the raw materials contained in them have been used in the pharmaceutical industry.[6] Medicinal plants due to their naturalness, low risks, complications, availability, and cheap prices are consumed by people.[5] Due to the antimicrobial activities of medicinal plants, they can be used as alternatives for antimicrobial chemicals.[7]

The mechanisms of essential oils by which they act are set up by their chemical compounds and antimicrobial activity. Although in no cases they show similar mechanisms, the effects of the essential oils on the cell wall structure of bacteria have been confirmed in most studies.[8,9] The toxic effects on the structure and function

of bacterial membrane justify the antimicrobial actions of essential oils of medicinal plants.[10]

Mentha longifolia L. is a medicinal and comestible plant that grows in wet places, such as riversides in the Mediterranean Sea, Europe, Australia, North Africa, and the Middle East, including Khuzestan province in the south west of Iran. This plant is administered orally and used for therapeutic purposes. Several studies have shown that the essential oil of *M. longifolia* has a strong antibacterial activity.[11,12]

Objectives

The incidence rate of the Shigella infection, especially in tropical areas, such as Khuzestan, is quite high. *M. longifolia* is native to this area. Considering these, this study was planned to assess the effect of the *M. longifolia* essential oil on *Shigella flexneri* and *Shigella sonnei,* and also assess its synergistic effect with antibiotics that are commonly used against *Shigella*.

Materials and Methods

Essential Oil Preparation

In this experimental laboratory study, the *M. longifolia* plant was collected from the Goor Kurd area, which is around 15 km from Haftgel city in Khuzestan province, on May 2015. Its herbarium code (A 1516030 AP) was determined. The leaves of *M. longifolia* were washed and shade dried after collecting and finally were milled in order to obtain their powder. Fifty grams of the dried powder of *M. longifolia* was mixed with 1 L of distilled water and essential oil was extracted by the hydrodistillation method using Clevenger machine for 2 hours. The essential oil produced was sterilized by passing through 0.45 nm filters and stored till the time of the test in a sealed glass container in the dark at 4°C.

Shigella Strains

In this study, the effects of the *M. longifolia* essential oil were investigated on clinical and standard *S. flexneri* and *S. sonnei* strains. The standard strains of *S. flexneri* (12022: ATCC) and *S. sonnei* (9290: ATCC) were supplied as a lyophilized powder from Pasture Institute of Iran and microbial collections. The clinical strains of S. *flexneri* and *S. sonnei* were isolated from a pediatric hospital in Ahvaz and were also confirmed by the conventional microbial and serological tests.

Antimicrobial Activity of Essential oil of Mentha longifolia

The weight of the *M. longifolia* essential oil was determined as 830 µg/mL. The antimicrobial activity of *M. longifolia* essential oil was investigated by the disk diffusion method. After the preparation of the bacterial suspension (0.5 McFarland CFU/mL), bacterial strains were subcultured on the Mueller-Hinton agar plate. In the next step, 50 µL of *M. longifolia* essential oil was added to a sterile blank paper disk and 50 µL of dimethyl sulfoxide (DMSO) was added to another blank paper disk and kept for 2 hours until the essential oil and DMSO were completely absorbed by the paper disks. Then the disks were placed on the subcultured bacteria on the Mueller-Hinton agar plate and incubated at 37°C for 18 to 24 hours. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the *M. longifolia* essential oil were detected by micro broth dilution method in a sterile 96-well micro plate.

Antimicrobial Susceptibility Testing

The antimicrobial susceptibility to ciprofloxacin (30 µg), ampicillin (30 µg), gentamicin (10 µg), and trimethoprim-sulfamethoxazole (1.25/23.75 µg) was detected by disk diffusion method, according to the Clinical & Laboratory Standards Institute (CLSI) instructions.[13]

Detection of the Synergistic Effect of Antibiotics and Mentha longifolia Essential Oil

The effect of the combination of *M. longifolia* essential oils and antibiotic disks was investigated by the disk diffusion method. The sub-MIC concentration of essential oils was detected as 1:256 µg/mL. The bacterial suspension in the 96-well plate in 1:256 concentration was subcultured on the Mueller-Hinton agar and then the antibiotics (ampicillin, ciprofloxacin, gentamicin, sulfamethoxazol-trimethoprim) were placed at regular intervals on the media and incubated for 24 hours at 37°C.

Results

Based on our findings, both the standard and clinical isolates of *S. flexneri* and *S. sonnei* were susceptible to the discs containing essential oils of *M. longifolia*. Different inhibition zone diameters around the *M. longifolia* essential oil were observed in the disks; the maximum inhibition zone was detected in the *S. flexneri* by 35 mm and the minimum inhibition was observed around the standard strain of *S. sonnei* by 20 mm.

The MIC value of the essential oils of *M. longifolia* for the standard and clinical strains of *S. flexneri* and *S. sonnei* were determined at the same concentration in 1:1024 equal to 81 mg/mL. The MBC for the standard and clinical strains of *S. flexneri* and *S. Sonnei* were determined at the same concentration in 1:512 equal to 1.28 mg/mL.

The antibiotic susceptibility results are shown in Table 1. The clinical isolates of *S. flexneri* and *S. sonnei* were resistant to ampicillin and no inhibition zone was detected in this antibiotic disk. The highest resistance was detected to ciprofloxacin with an inhibition zone diameter of 40 mm in the clinical isolate of *S. flexneri* (Table 1).

According to the results of the MIC, the sub-MIC concentration of 1:256 was cultured on the Mueller-Hinton agar. A significant difference ($P<0.05$) was detected between the inhibition zone diameters of the antibiotics before and after the use of the disk containing the essential oil of *M. longifolia*. The results indicated a synergistic effect between oregano and the studied

Table 1. Comparison of Activities of Antibiotic and Antibiotic + Essential Oil of *Mentha longifolia* Against Standard and Clinical Isolates of *Shigella flexneri* and *Shigella sonnei*

	Gentamicin A /A+E (mm)	Ciprofloxacin A/A+E (mm)	SXT A/A+E (mm)	Ampicillin A/ A+E (mm)
S. flexneri ATCC	18/22	38/45	33/40	27/36
S. flexneri clinical	25/35	40/46	29/35	0/10
S. sonnei ATCC	20/26	29/37	25/32	25/34
S. sonnei clinical	22/30	31/37	0/17	0/12

SXT: trimethoprim-sulfamethoxazole, A: antibiotic, A+E: antibiotic + essential oil.

antibiotics (Table 1).

Discussion

There are annual reports of the spread of shigellosis, especially in the warm seasons in different parts of Khuzestan province, mainly by *S. flexneri* and *S. sonnei.*[14] One of the complications of shigellosis is the development of spasms and abdominal cramps. The positive effects of *M. longifolia* in traditional medicine have been proven for the treatment of gastrointestinal and liver disorders, ulcerative colitis, vomiting, and loss of appetite.[15-17] The essential oil of *M. longifolia* also has antipyretic, analgesic, and different biological effects.[17-19] The antibacterial activities of *M. longifolia* have been studied in Iran and also in the rest of the world but no research has been conducted on the effect of *M. longifolia* on Shigella. The antimicrobial activity of the *M. longifolia* essential oil against some pathogenic bacteria and its impact on their structural morphology were evaluated by the researcher. The studies on the morphology and cell wall of the bacteria affected by the growth inhibitory concentrations of the *M. longifolia* essential oil have indicated a severe damage that is an important evidence on its antimicrobial activities.[20]

Sağdıç and colleagues studied the antimicrobial activity of the methanolic extract of thyme, sage, cumin and *M. longifolia* against *Escherichia coli* O157 H7 and found that the *M. longifolia* and thyme essential oils showed greater antibacterial activities in comparison with other extracts.[21]

The antimicrobial activities of the *M. longifolia* essential oil have been studied on gram-positive and gram-negative bacteria. Mkaddem et al detected the high activity of *M. longifolia* against *Listeria monocytogenes, Klebsiella pneumonia,* yeasts, and fungi.[22] Some studies have also reported that the natural compounds have widely been used as alternatives for the chemical compounds in foods as protective compounds. Pajohi Alamoti et al examined the antimicrobial activities of the *M. longifolia* essential oils through a dietary model and found that it is appropriate in some foods without any adverse sense, flavor and effects and can therefore be used as protective agent in the food industry.[23]

Conclusion

In conclusion, we may use *M. longifolia* to treat infections caused by *Shigella*. The combination of *M. longifolia* essential oil and antibiotics would increase the antimicrobial activities of the antibiotics and prevent the bacterial resistance to them.

Authors' Contributions

Study concept, design and critical revision of the manuscript: LS and MoM; performance: MaM; drafting of manuscript: MaM.

Ethical Approval

This study was approved by the Medicinal Plant Research Center of the Ahvaz University of Medical Sciences.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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