

# Bioactive Potential of Peppermint Extract Against Some Human Bacterial and Fungal Pathogens

Leila Farzaei,<sup>1</sup> Ali Azizi,<sup>2,\*</sup> Isa Arji,<sup>1</sup> and Razieh Rostami<sup>2</sup>

<sup>1</sup>Department of Medicinal Plants Sciences, Kermanshah ACECR Institute of Higher Education, Kermanshah, IR Iran

<sup>2</sup>Department of Horticultural Sciences, Faculty of Agriculture, Bu-Ali Sina University, Hamadan, IR Iran

\*Corresponding author: Ali Azizi, Department of Horticultural Sciences, Faculty of Agriculture, Bu-Ali Sina University, Hamadan, Iran. Tel: +98-8134424192, Fax: +98-9183072477, E-mail: azizi@basu.ac.ir

Received 2015 May 23; Revised 2015 October 27; Accepted 2015 November 4.

## Abstract

**Background:** Bacterial and fungal infections are an emerging public health concern, especially in the context of nosocomial infections. This study discusses the use of peppermint (*Mentha piperita* L.) methanolic extract against bacterial strains such as *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus* and fungal species such as *Trichophyton mentagrophytes*, *Trichophyton verrucosum*, and *Candida albicans*.

**Objectives:** This research aimed to investigate the inhibitory potential of *M. piperita* methanolic extract against some bacteria and fungi isolated from humans.

**Materials and Methods:** The methanolic extract was prepared by standard procedure, and total phenol, total flavonoid, and total anthocyanin content were determined using a spectrophotometer. Antimicrobial activity against the aforementioned bacteria and fungi strains was evaluated using the disc diffusion method.

**Results:** The contents of total phenol, flavonoid, and anthocyanin as well as antimicrobial activities and antifungal properties were enhanced by the increase of extract concentration. The results showed that the antimicrobial activity of leaf extract was more powerful against *Bacillus subtilis* (1.39 mm) than *Staphylococcus aureus* (1.29 mm), *Escherichia coli* (1.20 mm), and *Pseudomonas aeruginosa* (1.10 mm) based on the growth inhibition zone. The most powerful antifungal activity of peppermint extract was observed against *Trichophyton verrucosum* and *Trichophyton mentagrophytes* (1.49 and 1.48 mm, respectively).

**Conclusions:** Due to phenolic content, methanolic extract of *M. piperita* has moderate antibacterial and antifungal properties against the aforementioned gram positive and gram negative bacteria and fungi strains.

**Keywords:** Antimicrobial Activity, Extract, Peppermint, Phenolic Content

## 1. Background

Infections due to bacterial and fungal species remain a serious therapeutic problem. The widespread use of synthetic drugs has seen antibiotic resistances increase worldwide (1). Because antibiotics in use today may not work tomorrow, the best solution to this serious problem may be the use of substitute drugs (2). One source of new antimicrobial drugs is medicinal plants, which are used in traditional medicine. Since ancient times, medicinal plants have been helpful against many diseases. These plants and their derivatives may be a new approach to cases of resistant bacteria and fungi (3).

*M. piperita* is one of the most important perennial medicinal herbs of the Lamiaceae family. It is cultivated in temperate zones, including America, Asia, and Europe (4). Great attention has been paid to mint species due to their wide range of biological activities. Based on available evidence, the useful effects of *M. piperita* in the control and treatment of irritable bowel syndrome are known. This plant is also used in the treatment of inflammatory bowel diseases and

gall bladder and liver insufficiencies (5). Results of various studies have indicated that the medicinal effects of peppermint might be related to the dominant components, including menthol and menthone, present in its extract and essential oil, which are widely used as flavoring and antioxidants in foods, cosmetics, and hygienic products (6). Menthol is used as a tonic in gastric problems, as a fever reducer, antitussive and antiemetic, and also as a disinfectant in pulmonary inflammations (7). *M. piperita* extract exhibits antimicrobial activity against a range of bacteria, including various strains of *Staphylococcus epidermidis*, *Pseudomonas syringae*, *Bacillus cereus*, and *Escherichia coli* (8) and fungi including *Alternaria alternate*, *Aspergillus niger*, *Penicillium funiculosum*, and *Trichophyton rubrum* (9).

## 2. Objectives

We aimed to assess total phenol, flavonoid, and anthocyanin content and antimicrobial effects of *M. piperita*

methanolic extract against bacteria such as *Staphylococcus*, *Pseudomonas*, *Bacillus*, and *Escherichia coli* and fungi including *Trichophyton* and *Candida*.

### 3. Materials and Methods

Peppermint plants were purchased from Pakan Bazr Company of Isfahan, Iran, in August, 2013.

The leaves were washed under running tap water and then air dried at room temperature for two weeks in the dark. Human pathogenic bacterial and fungal strains gram positive bacteria *Bacillus subtilis* (PTTC 1156, PTTC: Persian Type Culture Collection) and *Staphylococcus aureus* (PTTC 1189), gram negative bacteria *Escherichia coli* (PTCC 1399) and *Pseudomonas aeruginosa* (PTTC 1181), and the fungal species *Trichophyton mentagrophytes*, *Trichophyton verrucosum*, and *Candida albicans* were obtained from the medical diagnostics laboratory at Sina Hospital in Hamadan, Iran.

#### 3.1. Preparation of Leaf Extract

The 2.5 g of dried peppermint leaves were pulverized and soaked in 25 mL of methanol as a solvent and shaken for 48 hours at 1200 r/min at 25°C. Methanolic extract was filtered through Whatman No. 1 filter paper and transferred to the incubator for three hours at 30°C to complete dryness. The resultant extract was collected and stored at 4°C until further tests (10).

#### 3.2. Total Phenol Content

The Folin-Ciocalteu reagent method with slight modifications was used to measure total phenol content (11). Extract of the treated leaves (0.5 mL of 1:10 g/mL) was mixed with Folin-Ciocalteu reagent (1500 µL, 1:10 diluted with distilled water), and after eight minutes, aqueous Na<sub>2</sub>CO<sub>3</sub> (1200 µL, 1 M) was added. Next the mixtures were shaken for 90 minutes in the dark at room temperature. Absorbance was read at 760 nm using a spectrophotometer. The same procedure was performed for all standard gallic acid solutions. Results were expressed as gallic acid equivalents (mg/g dry weight).

#### 3.3. Total Flavonoids Content

Firstly, 2.8 cc distilled water was dropped in an experimental tube. Then 0.1 cc AlCl<sub>3</sub> (10%) was added, and 0.1 cc of acetate potassium (1 mol/L) was added and mixed. Then 0.5 cc diluted methanolic extract was added. After 10 minutes at room temperature, the absorbance of the samples was determined at 430 nm. A standard curve was plotted using quercetin as a standard. Different concentrations of quercetin were prepared in 80% ethanol, and their absorbance was read at 430 nm by spectrophotometer. Findings were reported in mg quercetin/g dry weight by comparison with the quercetin standard (12).

#### 3.4. Total Anthocyanin Content

The 0.05 mg of methanolic extract was diluted to 5 mL using an 80/20 (v/v) mixture of 95% methanol and 37%

HCl. Absorbance was recorded at 532 nm using a spectrophotometer. Concentration of anthocyanin was calculated by the equation:

$$(1) \quad C_{\frac{mg}{L}} = \frac{Abs}{402.3} \times 10000 \times DF$$

Where 402.3 is the absorbance of a solution containing 1% (w/v) of methanolic extract anthocyanins, 10,000 is the conversion factor from g/100 mL to mg/L, and DF is the dilution factor. Results are shown as mg/g dry weight (13).

#### 3.5. Disc Diffusion Method

Screening of antibacterial and antifungal activities was performed by the standard disc diffusion method (14). The previously mentioned bacterial strains were grown on Mueller Hinton Agar (MHA, Merck, 35 g/L) in an incubator at 28°C. The bacterial inoculum suspension was adjusted with distilled water to a concentration of the order of 10<sup>8</sup> CFU/mL. Briefly, 200 µL/mL of each bacterial suspension were added to the surface of sterile petri dishes (8 cm in diameter), which were filled with MHA medium (15 mL/per plate). Sterile discs (6 mm in diameter) were then impregnated with 20 µL of the different concentrations (0.005, 0.01, 0.02, and 0.04 µL/mL) of methanolic extract solution. The paper discs were dried and placed on the surface of the plates. After 48 hours of incubation at 28°C, the diameters of the growth inhibition zones were measured in mm. Gentamicin antibiotic discs were used as positive control, and 20 µL methanol were added to the filter discs as a negative control. The antifungal test was done in the same way as the antibacterial test, with some differences. Fungal strains were cultured on potato dextrose agar (PDA, Merck, 15 g/L) and incubated at 28°C until optimum density was obtained. The fungal suspension was made with distilled water to a concentration of the order of 10<sup>6</sup> spores/mL (15), and the disc diffusion was performed as described. In addition, nystatin antibiotic discs were used as a positive control. Antimicrobial activity was evaluated by measuring the zones of inhibition against the test organisms.

#### 3.6. Statistical Analysis

The experiment was carried out as a completely randomized design. It was triplicated. Results presented are the average of the obtained values. Data was calculated by analysis of general linear model (GLM) using SAS software version 9.2 followed by Duncan's protected least significant difference test ( $P \leq 0.05$ ). A data normalization test was performed.

### 4. Results

Variance analysis showed a significant difference ( $P \leq 0.05$ ) between extracts with different concentrations (0.005, 0.01, 0.02, and 0.04 µL of extract/ml of metha-

nol) in total phenol, total flavonoid, and total anthocyanin content of peppermint (Table 1). The amounts of the above mentioned phytochemicals were enhanced by the increase of the extract concentration. Antimicrobial activity analysis indicated that there was also a significant difference between extracts with different concentrations in terms of their antibacterial and antifungal activities against all tested strains ( $P \leq 0.05$ ). In addition, the antimicrobial activity of the methanolic extract depended largely upon the concentration of the extract (Table 2).

In the case of *Bacillus subtilis*, the highest concentration of methanolic extract of peppermint (0.04  $\mu\text{L/mL}$ ) showed a growth inhibition zone of 1.39 mm around the disc. The lowest concentration of methanolic extract (0.005  $\mu\text{L/mL}$ ) exhibited a growth inhibition zone of 1.09 mm. The diameter of the microbial inhibition zone for

*Staphylococcus aureus* was found to be 1.29 mm for the highest concentration of methanolic extract. The lowest concentration of methanolic extract demonstrated 0.99 mm of growth inhibition for *Staphylococcus aureus*. For *Escherichia coli* and *Pseudomonas aeruginosa*, the zones of growth inhibition were 1.19 and 1.09 mm for the highest concentration of methanolic extract, respectively, and 0.99 and 0.89 mm for the lowest concentration of methanolic extract, respectively (Table 2).

In the case of antifungal activity, the highest concentration of methanolic extract showed 1.48, 1.49, and 1.39 mm of growth inhibition around the disc on *Trichophyton mentagrophytes*, *Trichophyton verrucosum*, and *Candida albicans*, respectively. The diameters of the growth inhibition zones for the three above mentioned fungi were 1.18, 1.29, and 0.99 mm for the lowest concentration of methanolic extract, respectively.

**Table 1.** Total Phenol, Flavonoid, and Anthocyanin Content of *M. piperita* Methanolic Extract (Based on mg/g Dry Weight)

Phytochemical Factors	Methanolic Extract Concentration, $\mu\text{L/mL}$			
	0.005	0.01	0.02	0.04
Total phenol	0.11 <sup>a</sup>	0.22 <sup>a</sup>	0.43	0.88
Flavonoid	12.26	24.71	49.82	98.47
Anthocyanin	0.0016 <sup>a</sup>	0.0035 <sup>a</sup>	0.0066	0.0134

<sup>a</sup>statistical insignificance ( $P \leq 0.05$ ).

**Table 2.** Zone of Growth Inhibition of the *M. piperita* Methanolic Extract against Bacterial and Fungi Strains (based on mm)

Strain	Disc	Methanolic Extract Concentration $\mu\text{L/mL}$				
		0	0.005	0.01	0.02	0.04
<b>Bacterial strain</b>	<b>Gentamicin Disc</b>					
<i>Bacillus subtilis</i>	2.09	0.89	1.09	1.19	1.29	1.39
<i>Staphylococcus aureus</i>	1.99	0.79	0.99	1.19 <sup>a</sup>	1.21 <sup>a</sup>	1.29
<i>Escherichia coli</i>	1.69	0.89	0.99	1.09 <sup>a</sup>	1.11 <sup>a</sup>	1.20
<i>Pseudomonas aeruginosa</i>	1.49	0.79	0.89	0.99 <sup>a</sup>	1.01 <sup>a</sup>	1.10
<b>Fungi strain</b>	<b>Nystatin Disc</b>					
<i>Trichophyton mentagrophytes</i>	1.98	0.89	1.18	1.29	1.38	1.48
<i>Trichophyton verrucosum</i>	2.08	1.08	1.29	1.38 <sup>a</sup>	1.39 <sup>a</sup>	1.49
<i>Candida albicans</i>	1.79	0.89	0.99	1.08	1.28	1.39

<sup>a</sup>statistical insignificance ( $P \leq 0.05$ ).

## 5. Discussion

In the present study, the inhibition activity of peppermint extract was lower than that of Gentamicin and Nystatin for all tested microorganisms. The highest antibacterial activity was detected against *Staphylococcus aureus* and the highest antifungal activity was obtained against *Trichophyton mentagrophytes* (Table 1). In addition, the highest concentration of extract exhibited the highest growth inhibition of all of the listed bacterial and fungal strains. It is considerable that the lowest concentration of peppermint extract, when compared with the negative control, showed growth inhibition in both bacterial and fungal strains. The varying degrees of sensitivity of the bacterial and fungal strains may be due to the intrinsic tolerance of the bacterial and fungal strains and to the combinations of phyto-compounds present in the extract.

When compared with another extract, methanolic extract expressed higher activity against microorganisms. Chen and Blumberg (2008) reported larger extraction efficiencies of phenolic compounds in methanolic extracts than in aqueous ones, indicating that organic solvents are more efficient in extracting phenolic compounds than aqueous media (16). In a previous study, it was reported that among the alcoholic extracts (methanol, ethanol, and methanol/ethanol) of peppermint, the methanol extract had maximum phenol content (10). The phenolic compounds are known to be synthesized by plants in response to microbial infection. It is therefore possible that they can act as effective antimicrobial substances against a wide array of microorganisms (17). The biologically active constituents of some plant extracts are considered to be antimicrobial agents because of their ability to bind to bacterial adhesions and thus disturb the availability of receptors on the surface (18). Several studies have demonstrated a correlation between antimicrobial activity and chemical compounds in medicinal plants such as alkaloids, anthocyanins, flavonoids, and phenols (15).

The phenolic constituents of the *M. piperita* extract are reported for their antimicrobial and antiviral activities as well as their strong antioxidant and antitumor action (4, 19). The bactericidal properties of menthol and its effective inhibitory potential against gram positive bacteria has also previously been reported (8). In a previous study, Sujana et al. (2013) evaluated the antibacterial potential of six extracts from the leaves, stems, and roots of *M. piperita* against pathogenic bacteria such as *Bacillus subtilis*, *Streptococcus pneumonia*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, and *Klebsiella pneumonia* by the agar well diffusion method (20). They reported that the antimicrobial activity of the leaf extract was more powerful against *Bacillus subtilis*, *Staphylococcus aureus*, and *Proteus vulgaris* than against *Escherichia coli*, *Streptococcus pneumonia*, and *Klebsiella pneumonia*. Their report is consistent with our results. In the present study, all concentrations of the extracts from peppermint were shown

to possess significant antibacterial activity, but growth inhibition was the most effective against *Bacillus subtilis*.

Overall, this study demonstrated the moderate antimicrobial activity of *M. piperita* extract against some human bacterial and fungal pathogens. However, additional clinical trials are necessary to confirm the above results for medical purposes. Due to the strong interest in natural drugs obtained from medicinal and aromatic plants, applications of the *M. piperita* extracts against these pathogens are recommended. We hope that in future, peppermint can be used widely as an antimicrobial agent.

## Acknowledgments

The authors are thankful to the Bu-Ali Sina University for providing the necessary facilities to carry out the work and to Ms. Nazeri, the manager of the medical diagnostic laboratory at Sina Hospital in Hamadan, Iran.

## Footnotes

**Authors' Contribution:** Leila Farzaei: administrative, technical, and material support, drafting of the manuscript; Ali Azizi: study supervision, study concept and design; Razieh Rostami: acquisition of data and statistical analysis; Isa Arji: interpretation of data and critical revision of the manuscript.

**Funding/Support:** This report is a part of the M.Sc. thesis of the first author, which was supported by the ACECR Institute of Higher Education in Kermanshah, Iran.

## References

- Hemaiswarya S, Kruthiventi AK, Doble M. Synergism between natural products and antibiotics against infectious diseases. *Phyto-medicine*. 2008;**15**(8):639-52. doi: 10.1016/j.phymed.2008.06.008. [PubMed: 18599280]
- Nascimento GGF, Locatelli J, Freitas PC, Silva GL. Antibacterial activity of plant extracts and phytochemicals on antibiotic-resistant bacteria. *Brazil J Microbiol*. 2000;**31**(4). doi: 10.1590/s1517-83822000000400003.
- Cowan MM. Plant products as antimicrobial agents. *Clin Microbiol Rev*. 1999;**12**(4):564-82. [PubMed: 10515903]
- McKay DL, Blumberg JB. A review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.). *Phytother Res*. 2006;**20**(8):619-33. doi: 10.1002/ptr.1936. [PubMed: 16767798]
- Cappello G, Spezzaferro M, Grossi L, Manzoli L, Marzio L. Peppermint oil (Mintoil) in the treatment of irritable bowel syndrome: a prospective double blind placebo-controlled randomized trial. *Dig Liver Dis*. 2007;**39**(6):530-6. doi: 10.1016/j.dld.2007.02.006. [PubMed: 17420159]
- Lv J, Huang H, Yu L, Whent M, Niu Y, Shi H, et al. Phenolic composition and nutraceutical properties of organic and conventional cinnamon and peppermint. *Food Chem*. 2012;**132**(3):1442-50. doi: 10.1016/j.foodchem.2011.11.135.
- Patel T, Ishiuiji Y, Yosipovitch G. Menthol: A refreshing look at this ancient compound. *J Am Acad Dermatol*. 2007;**57**(5):873-8. doi: 10.1016/j.jaad.2007.04.008. [PubMed: 17498839]
- Iscan G, Kirimer N, Kurkuoglu M, Baser KH, Demirci F. Antimicrobial screening of *Mentha piperita* essential oils. *J Agric Food Chem*. 2002;**50**(14):3943-6. [PubMed: 12083863]
- Sokovic MD, Vukojevic J, Marin PD, Brkic DD, Vajs V, van Griensven LJ. Chemical composition of essential oils of Thymus and Mentha species and their antifungal activities. *Molecules*.

- 2009;**14**(1):238–49. doi: 10.3390/molecules14010238. [PubMed: 19136911]
10. Farnad N, Heidari R, Aslanipour B. Phenolic composition and comparison of antioxidant activity of alcoholic extracts of Peppermint (*Mentha piperita*). *J Food Measur Characteriz*. 2014;**8**(2):113–21. doi:10.1007/s11694-014-9171-x.
  11. McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. *Food Chem*. 2001;**73**(1):73–84. doi:10.1016/s0308-8146(00)00288-0.
  12. Sultana B, Anwar F, Ashraf M. Effect of extraction solvent/ technique on the antioxidant activity of selected medicinal plant extracts. *Molecules*. 2009;**14**(6):2167–80. doi: 10.3390/molecules14062167. [PubMed: 19553890]
  13. Rapisarda P, Fanella F, Maccarone E. Reliability of analytical methods for determining anthocyanins in blood orange juices. *J Agric Food Chem*. 2000;**48**(6):2249–52. [PubMed: 10888531]
  14. Duraipandiyan V, Ignacimuthu S. Antibacterial and antifungal activity of Flindersine isolated from the traditional medicinal plant, *Toddalia asiatica* (L.) Lam. *J Ethnopharmacol*. 2009;**123**(3):494–8. doi:10.1016/j.jep.2009.02.020. [PubMed: 19481384]
  15. Rios JL, Recio MC. Medicinal plants and antimicrobial activity. *J Ethnopharmacol*. 2005;**100**(1-2):80–4. doi:10.1016/j.jep.2005.04.025. [PubMed: 15964727]
  16. Chen CY, Blumberg JB. In vitro activity of almond skin polyphenols for scavenging free radicals and inducing quinone reductase. *J Agric Food Chem*. 2008;**56**(12):4427–34. doi: 10.1021/jf800061z. [PubMed: 18512942]
  17. Narasimhan MK, Pavithra SK, Krishnan V, Chandrasekaran M. In vitro Analysis of Antioxidant, Antimicrobial and Antiproliferative Activity of *Enteromorpha antenna*, *Enteromorpha linza* and *Gracilaria corticata* Extracts. *Jundishapur J Nat Pharm Prod*. 2013;**8**(4):151–9. [PubMed: 24624206]
  18. Brantner A, Males Z, Pepeljnjak S, Antolic A. Antimicrobial activity of *Paliurus spina-christi* Mill. (Christ's thorn). *J Ethnopharmacol*. 1996;**52**(2):119–22. [PubMed: 8735457]
  19. Sandasi M, Leonard CM, Van Vuuren SF, Viljoen AM. Peppermint (*Mentha piperita*) inhibits microbial biofilms in vitro. *South Afr J Botany*. 2011;**77**(1):80–5. doi: 10.1016/j.sajb.2010.05.011.
  20. Sujana P, Sridhar TM, Josthna P, Naidu CV. antibacterial activity and phytochemical analysis of *mentha piperita* L. (Peppermint) an important multipurpose medicinal plant. *Am J Plant Sci*. 2013;**04**(01):77–83. doi: 10.4236/ajps.2013.41012.