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International Journal of Clinical Biochemistry and Research

Journal homepage: https://www.ijcbr.in/

**Original Research Article**

# Antifungal efficacy of *Mentha species* (*Mentha arvensis, Mentha longifolia, Mentha spicata & Mentha viridis*)

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## Abstract

**Introduction:** Fungal diseases currently devastate a minimum of 125 million tons of the five principal food crops—rice, wheat, maize, potato, and soybean—annually, which could otherwise nourish those suffering from food insecurity. These crops constitute the primary source of calories ingested by individuals. Rice blast, corn smut in maize, stem rust in wheat, soybean rust, and late blight in potatoes are diseases that impair agricultural yield. This has forced researchers to seek novel antifungal chemicals from natural sources, such as medicinal plants, which are more efficacious and less hazardous to human health and the environment.

**Aim & Objective:** The study aims to evaluate antifungal potential of methanolic extract of whole plant of *Mentha species* (*Mentha arvensis, Mentha longifolia, Mentha spicata, Mentha viridis)* against *Alternaria solani and Bipolaris oryzae.*

**Materials and Methods:** The antifungal activity of the test samples (*Mentha species*) was determined by Poisoned Food Technique (a type of agar dilution method) against two pathogenic fungal strains.

**Results:** The results revealed that *Mentha spicata* showed maximum antifungal activity against *Alternaria solani* fungal strain with $IC_{50}$ values of 270.4±1.2 µg/mL.

**Conclusion:** Most frequently fungicides are used to control the diseases caused by plant pathogens. However, there is a serious problem in the effective use of these chemicals due to the development of resistance by the fungi. *Mentha spicata* can be used for the formulation of antifungal agents especially against *Alternaria solani*.

**Keywords:** Mentha arvensis, Mentha longifolia, Mentha spicata, Mentha viridis, Antifungal

**Received:** 24-04-2025; **Accepted:** 17-06-2025; **Available Online**: 02-08-2025



## 1. Introduction

Mentha serves as an effective expectorant, and gas chromatography–mass spectrometry (GC-MS) examination revealed the presence of menthol, menthone, limonene, isomenthone, menthyl acetate, carvone, pinene, 1,8-cineole, pulegone, piperitone oxide, and micene. Every species possesses a distinctive predominant compound. Research conducted on Mentha species has demonstrated antibacterial activity associated with several species within this genus. The plant is predominantly recognized for its antiviral, antibacterial, antifungal, potent antioxidant, and cytotoxic effects.[1] *Mentha arvensis* (field mint) is an erect and branched aromatic herb, up to 60 cm in height, a native of Japan and is cultivated extensively in the temperate regions of Europe, Western and Central Asia, East to the Himalaya, Eastern Siberia and also grows in Western Himalayas at an altitude of 1500-3000 m.[2] The entire plant is utilized for a wide array of ailments, including anorexia, abdominal pain, vomiting, cough, loss of appetite, menstrual abnormalities, joint pain, and diseases of the liver, spleen, and asthma. The leaves are primarily utilized in salads and for medical purposes related to gastrointestinal issues and allergies.[3] The wild mint i.e., *Mentha longifolia* (horse mint), proliferates significantly in Mediterranean regions, Europe, Australia, and North Africa. It possesses a creeping rhizome with erect to creeping stems of 40-120 cm in height. The leaves are oblong-elliptical to lanceolate, thinly to densely tomentose,

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https://doi.org/10.18231/j.ijcbr.v.12.i.2.6

green to greyish-green on the upper surface and white on the underside. The flowers measure 3-5 mm in length, exhibiting lilac, purplish, or white colors, and are borne in dense clusters on elongated, branching, and tapering spikes.[4] *M. longifolia* is utilized in the medicinal, tobacco, and food industries, with specific application in cosmetology. Various plant components, including leaves, flowers, stem, bark, and seeds, have been extensively utilized in traditional folk medicine for their antibacterial, carminative, stimulant, and antispasmodic properties, as well as for treating ailments such as headaches and digestive disorders.[5] *Mentha spicata*, commonly known as spearmint, is a significant aromatic herb cultivated globally. Spearmint is native to Northern England and is cultivated in regions with climates that vary from tropical to temperate, including America, Europe, China, South Africa, and Brazil.[6] Spearmint is regarded as a natural remedy in traditional medicine for the treatment of colds, influenza, respiratory tract issues, gastralgia, hemorrhoids, and stomachache. Moreover, spearmint is regarded as carminative, antispasmodic, and diuretic.[7] *Mentha viridis*, generally referred to as garden or green mint, is indigenous to the Mediterranean region and extensively distributed in Eurasia, Australia, and South Africa, thriving in moist or wet environments.[8] The extract of boiled leaves exhibits anti-infectious, antiflatulent, and anti-inflammatory properties, particularly concerning the digestive system. It has been recommended for viral hepatitis, colitis, gastric acidity, and aerophagia, as well as to enhance digestion; additionally, it possesses invigorating and stimulating attributes.[9]

## 2. Materials and Methods

### *2.1. Authentication and collection of Mentha species*

The above mentioned *Mentha species* were authenticated at site by Dr. L.M. Gupta, Professor, Division of Agroforestry, FoA, SKUAST-Jammu and enough quantity of fresh species were collected.

### *2.2. Chopping and drying of Mentha species*

Freshly collected *Mentha species* were chopped, shade dried, crushed and the coarse plant material was then extracted with methanol at room temperature (35$^0$C) for bioevaluation.

### *2.3. Extraction of Mentha species*

Powdered dried plant material of the above mentioned *Mentha species* was placed in a percolator of appropriate size. The plant material was then submerged in 99% (v/v) methanol depending on the need. Standard protocol[10] was followed for the extraction of single species, which can easily be employed for each *Mentha species.* Dried plant material (100 g) was placed in a conical glass percolator. Sufficient quantity of solvent was added so as to submerge the plant material. After standing for about 16 h (overnight), the percolate was collected and filtered if required. The process was repeated four times, which was generally sufficient for exhaustive extraction of the plant material. The methanolic extract (collected in four attempts) was evaporated to dryness under reduced pressure at 60 $^0$C using rotavapor and round bottom flask (RBF). The final drying was done in a vacuum desiccator. The dried extract was scrapped off from the RBF and transferred to a tared wide mouth glass container of appropriate size. The container was weighed to calculate the quantity of the extract obtained. This formed the “stock extract” of the *Mentha species*. Generally, 8 to 10 g crude extract was obtained from 100 g of the dried plant material. The extracts obtained, were stored at –20 $^0$C under desiccation in deep freezer for further testing.

### *2.4. Determination of antifungal activity*

The antifungal efficacy of the test samples (Mentha species) was assessed using the Poisoned Food Technique (a variant of the agar dilution method) against two pathogenic fungal strains, *Alternaria solani* and *Bipolaris oryzae* (obtained from the Division of Plant Pathology, SKUAST-Jammu). Various quantities of the test component were formulated in sterilized potato dextrose agar and dispensed into 9 cm petri dishes. Subsequently, a 5 mm segment of test fungus was inoculated at the center of the agar plate (mycelial surface of the bit was placed upside down) and the petri plates were incubated at 26 °C. The extension diameter (mm) of hyphae from the center to the dish was recorded at 24-hour intervals until the fungal growth in the control plate reached the edges. The experiment was conducted three times, and the results were presented as the average of the three repetitions. The diameter of fungal growth on each plate with varying amounts of the test component was measured to compute the percentage of growth inhibition. Amphotericin B served as the positive control in the experiment.[11]

### *2.5. Calculations*

The percent inhibition of the fungal growth in presence of test material was calculated using the formula:

Inhibition% = [Radial growth in control (mm) – Radial growth in treatment (mm) / Radial growth in control (mm)] x 100.

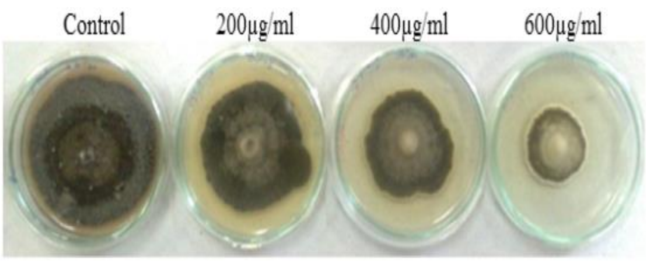


**Figure 1:** Antifungal activity of methanolic extract of *Mentha spicata* against *Alternaria solani*

**Table 1:** Growth inhibitory effect of *Mentha arvensis & Mentha longifolia* on fungal strains

| Extract | Conc. (µg/mL) | Phytopathogenic fungi | |
|---|---|---|---|
| | | ***Alternaria solani*** | ***Bipolaris oryzae*** |
| | | **Growth Inhibition (%)** | |
| Methanolic | 200 | 25 | 25 |
| | 400 | 55 | 49 |
| | 600 | 67.5 | 67.5 |
| | $IC_{50}$ | **427±6.4 µg/mL** | **487±2.6 µg/mL** |
| | 200 | 28 | 22 |
| | 400 | 45 | 43 |
| | 600 | 70 | 67 |
| | $IC_{50}$ | **422.22±1.3 µg/mL** | **435.55±0.2 µg/mL** |
| Amphotericin B (positive control) | **Conc. (µg/mL)** | **Growth Inhibition (%)** | |
| | 10 | 48.5 | 50.5 |
| | 20 | 65.00 | 71 |
| | 40 | 83.60 | 85.5 |
| | $IC_{50}$ | **11.66±0.1 µg/mL** | **9.14±0.2 µg/mL** |

**Table 2:** Growth inhibitory effect of *Mentha spicata &Mentha viridis* on fungal strains

| Extract | Conc. (µg/mL) | Phytopathogenic fungi | |
|---|---|---|---|
| | | ***Alternaria solani*** | ***Bipolaris oryzae*** |
| | | **Growth Inhibition (%)** | |
| Methanolic | 200 | 43.5 | 27 |
| | 400 | 62.5 | 45 |
| | 600 | 83 | 72 |
| | $IC_{50}$ | **270.4±1.2 µg/mL** | **419.64±2.1 µg/mL** |
| | 200 | 25 | 26 |
| | 400 | 52.5 | 38 |
| | 600 | 75 | 60 |
| | $IC_{50}$ | **393.33±0.2µg/mL** | **501.9±3.1 µg/mL** |
| Amphotericin B (positive control) | **Conc. (µg/mL)** | **Growth Inhibition (%)** | |
| | 10 | 48.5 | 50.75 |
| | 20 | 65.00 | 71.50 |
| | 40 | 83.60 | 85.69 |
| | $IC_{50}$ | **11.66±0.1 µg/mL** | **9.14±0.2 µg/mL** |

## 3. Results and Discussion

The methanolic extract of whole plant of *Mentha arvensis* showed antifungal activity against both the fungal strains: *Alternaria solani* with $IC_{50}$ value of 427±6.4 µg/mL and *Bipolaris oryzae* with $IC_{50}$ value of 487±2.6 µg/mL. The methanolic extract of whole plant of *Mentha longifolia* was tested with fungal strains and inhibitory effect was observed - *Alternaria solani* with an $IC_{50}$ value of 422.22±1.3 µg/mL and *Bipolaris oryzae* with an $IC_{50}$ value of 435.55±0.2 µg/mL (**Table 1**). Further, the methanolic extract of *Mentha spicata* exhibited potential inhibitory effect against *Alternaria solani* as shown in **Figure 1** with an $IC_{50}$ value of 270.4±1.2 µg/mL and a moderate effect against *Bipolaris oryzae* with an $IC_{50}$ value of 419.64±2.1 µg/mL. The methanolic extract of whole plant of *Mentha viridis* during its testing against fungal strains showed that the extract exhibited some moderate activity against *Alternaria solani* with an $IC_{50}$ value of 393.33±0.2 µg/mL. However, activity was also observed against *Bipolaris oryzae* with an $IC_{50}$ values of 501.9±3.1 µg/mL. mL (**Table 2**). The essential oil derived from the leaves of *Mentha arvensis* shown fungicidal properties against human pathogens, viz., *Candida albicans*, *Cryptococcus neoformans*, *Sporothrix schenckii*, Microsporum gypsum, and *Trichophyton rubrum*.[12,13] The essential oil of *Mentha longifolia* exhibits notable antimicrobial activity against *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Aspergillus flavus*, *Botrytis cinerea*, *Fusarium oxysporum*, *Pseudomonas aeruginosa*, *Aspergillus niger*, *Trichophyton longifusus*, *Microsporum canis*, and *Mucor ramamnianus*. The essential oil of the plant exhibited fungistatic and fungicidal properties that were markedly superior to those of the more expensive fungicide bifonazole. Menthol has demonstrated efficacy as an antibacterial and antifungal agent against ringworm and various other fungal infections.[14] *Mentha spicata* is recognized for its antibacterial, antimutagenic, and anti-inflammatory qualities attributed to the presence of coffeic acid, rosmarinic acid, and α-tocopherol, as well as its anti-histaminic activity due to compounds such as 5-O-desmethylnobiletin, cirsilineol, thymosin, thymonin, and sideritoflavone.[15] A study evaluated the essential oil of Mentha viridis leaves against four standard bacterial species: two gram-positive bacteria, Bacillus subtilis and Staphylococcus aureus, two gram-negative strains, *Escherichia coli* and *Pseudomonas aeruginosa*, and the fungal strain *Candida albicans*. It demonstrated significant efficacy against gram-negative bacteria (*E. coli* and *P. aeruginosa*). It also showed efficacy against gram-positive bacteria (*B. subtilis* and *S. aureus*) and a fungal strain (*C. albicans*). This study showed that the essential oil derived from *M. viridis* leaves exhibits significant antibacterial activity in vitro.[16]

In the present research work, an attempt was made to elucidate the *in vitro* antifungal potential of some *Mentha* species. The results obtained from our investigation confirmed the therapeutic potency of these species. In addition, these results form a good basis for selection of these *Mentha* species for further phytochemical and pharmacological analysis. The results produced in the research support the folkloric usage of the studied plants and showed that plant extracts possess certain cytotoxic constituents that can be used for developing antifungal agents. *Mentha spicata* can be used for the formulation of antifungal agents especially against *Alternaria solani*. Further studies are required for the isolation of active ingredients from these *Mentha* species for developing antifungal agents.

## 4. Conclusion

A total of four *Mentha* species, *viz*., *Mentha arvensis, Mentha longifolia, Mentha Mentha spicata* and *Mentha viridis* were selected from herbal garden of SKUAST-Jammu. Two pathogenic fungal strains namely *Alternaria solani and Bipolaris oryzae* were used for the purpose of antifungal assay. Methanolic extracts of all the above mentioned *Mentha* species were prepared and used as test material. Poisoned Food Technique was employed for antifungal analysis and amphotericin B was used as positive control. The present study disclosed the presence of phytochemicals in the *Mentha* species that implies that particularly the methanolic extract of *Mentha spicata* may indeed be effective in the management of diseases caused by fungal pathogens and this species is cost effective to treat many infectious diseases of livestock, poultry and human. There is a need for further investigation of this species in order to identify and isolate its antifungal agent.

## 5. Conflict of Interest

None.

## 6. Source of Funding

None.

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**Cite this article:** Rassol I, Sharma V, Sudan K. Antifungal efficacy of *Mentha species* (*Mentha arvensis, Mentha longifolia, Mentha spicata & Mentha viridis*). *Int J Clin Biochem Res*. 2025;12(2):94-97.