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Original Article

# Phytochemical Screening and Antimicrobial Activity of Mentha Arvensis L. [Pudina]: A Medicinal Plant

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## ABSTRACT

*Mentha arvensis* is an essential aromatic, energizer restorative, and medicinal plant in the mint family Lamiaceae. *Mentha arvensis* is found in rugged areas or cold climates of India. Herein, we studied the presence of different dynamic metabolites like- Flavonoids, Saponins, Tannins, terpenoids, steroids, Carb, anthraquinones, Heart glycosides, and alkaloid. In the given study, the phytochemical and antimicrobial action of leaves concentrates on pudina (*Mentha arvensis L.*). The broth dilution method has been used to check the antimicrobial activity of Mentha arvensis. In vitro antimicrobial movement was studied against pathogenic microbes such as *Escherichia coli, Staphylococcus aureus, Streptococcus Pyogenes, Pseudomonas aeruginosa, Candida albicans, Aspergillus Niger, Aspergillus clavatus* by agar well dispersion method. When used on bacterial colonies and fungal colonies, the separated extract showed the maximum zone of inhibition against *Staphylococcus aureus, Escherichia coli, Streptococcus Pyogenes, Pseudomonas aeruginosa, Candida albicans, Aspergillus Niger, Aspergillus clavatus* over the control. The maximum zone of inhibition was found in Methanolic extracts against Pseudomonas aeruginosa and Aspergillus clavatus over the control. Thus, the present approach can be useful to find new bioactive segments to improve new drugs. Our findings showed that the Mentha arvensis plant gives 25- 100 MIC (ug/ml) to inhibit the growth of the mentioned microorganisms. Thus, it can be used as a strong antimicrobial agent against pathogens, mainly *Aspergillus Clavatus*.

## Introduction

*Mentha arvensis L.* belongs to the family Lamiaceae and is typically known as *Pudina, menthol mint, corn mint*. In recent times, the identification and search for compounds with antimicrobial activity have gained more importance because the rate of infection by antibiotic-resistant microorganisms increases exponentially [1]. The leaves of *pudina* are one of the significant wellsprings of palatable sweet-smelling compounds. The *pudina* Exceptionally significant unstable oils, tars, tannins, and coumarins, alkaloids. So, in the current examination, we studied phytochemical and antimicrobial screening of pudina leaves against some pathogenic microbes. *Mentha arvensis L.* is a largely utilized spice in the kitchen as a feature of food. It is additionally utilized as a home solution for a stomach issue and mouth disinfection. It is an upstanding fragrant spice with suckers, round and hollow stem, straightforward and inverse leaves. *Mint* has a vital spot in Ayurveda and Unani arrangement of medication. Its juice, glue, powder, separate distillate can be utilized exclusively or used to treat - dyspepsia, acid reflux, and so on [2]. It is customarily used in hypertension and patients with ischemic coronary disease. Juice of leaves is used for the treatment of diarrhea. It is likewise utilized to treat liver infection, spleen infection, asthma, and jaundice. Regarding these properties, they are immensely used for a medicinal purpose [3]. The infusion of these leaves is also utilized to treat heartburn, rheumatic agonies, joint pain, and is used as a solution for kindled joints. Menthol derived from mint's fundamental oil is utilized in drug, perfumery, and food enterprises. Menthol is a germicide, carminative, refrigerant, energizer, and diuretic in properties and is used against skin diseases [4]. Studies have shown that some commonly utilized restorative plants and their photochemical have radio-defensive impacts [5]. Recently, many developed and developing countries used traditional medicine for primary health care [6]. The present study aimed to assess the anti-bacterial and anti-fungal activity of Mentha arvensis L. Figure 1 shows the photograph of a mint plant. Figure 2 presents the application of a mint plant.

*Figure 1. Photograph of a mint plant.*
Source: UBC Botanical Garden [7].

https://botanyphoto.botanicalgarden.ubc.ca/2015/04/mentha
Scientific name: *Mentha arvensis*, Higher classification: *mint*; Family: Lamiaceae; Kingdom: Plantae; Order: Lamiales


*Figure 2. Application of mint plant.*
Source: Benefits of Mint and Its Side Effects [8].

## Materials and Methods

Fresh leaves of *Mentha arvensis L.* were collected from the Parul university garden. The crushed powder was recognized and validated in the college of Agriculture of Parul College. The fresh leaves were washed altogether with faucet water, followed by the use of two-fold refined water. At long last, the washed leaves were air-dried and homogenized to give a fine powder. This fine powder was set in an impermeable container for additional investigations. The dry homogenized powder of *Mentha arvensis L.* leaves was dissolved in methanol and purified by using the soxhlet apparatus. The extract of selected plant material was prepared in Methanol. The 50 Gms of dried powder was extracted with 500 ml solvent using Soxhlet apparatus for 24 hrs. The aqueous methanol extracts were lyophilized and stored at 4° C. The resulting extract was sent to the NABL Accredited Microcare lab (Surat) to test the antimicrobial activity of *Mentha arvensis.* Figure 3 demonstrates different applications of *Mentha arvensis L.*


*Figure 3. Different Applications of Mentha arvensis L.*
Source: Robert et al. [9].

## Phytochemical Screening Test

The phytochemical tests of the different extracts of pudina were studied by different researchers recently [10, 11, 12, and 13].

*Test for Flavonoids*
In 1 ml extracts of different solvents, add 1 ml 10 % lead acetate solution. The formation of yellow precipitation indicates a positive test for flavonoids.

*Test for Saponins*
In 2 ml leaves extracts, add a small amount of 2 N HCl, shake well and decant the aqueous layer. Finally, add two drops of Mayer's reagents. The formation of intense color foaming lather was taken as a positive test for saponin.

*Test for Alkaloids*
Heat 2 ml leaves extracts of pudina by adding 10 % NaOH solution in a test tube. The white precipitate was taken as a positive test for alkaloids.

*Test for Tannins*
For tannins, 2 ml leaves extract of pudina were heated by adding concentrated HNO3 with excess ammonia. The development of white precipitation shows the presence of tannins.

*Test for Carbohydrates (Molisch's Test)*
The leaf concentrates of pudina were treated with Molisch reagent and concentrated Sulphuric Acid Corrosion. The reddish violet ring shows the presence of carbohydrates.

*Test for Terpenoid*
Mixed, 5 ml of leaves extract with 2 ml of chloroform, and then 3 ml of concentrated sulphuric acid was carefully added to form a layer. A reddish-brown coloration at the interface demonstrated the presence of the terpenoids.

*Test for Cardiac Glycosides*
0.5 gm leaf extracts diluted by 5 ml refined water, add 2 ml of glacial acetic acid containing one drop ferric chloride solution. At last, cautiously add 1 ml concentrated sulphuric acid. A brown ring at the interphase showed the presence of cardiac glycosides.

**Effect of Powdered Extract on Microorganisms to Study Antimicrobial Activity**
The antimicrobial activity of Mentha arvensis was tested by sending samples to Microcare lab, Surat. The four bacterial *species* (i.e., *Staphylococcus aureus, Escherichia coli, Streptococcus Pyogenes, Pseudomonas aeruginosa*), and fungal species (i.e., *Candida albicans, Aspergillus niger, Aspergillus clavatus*) were used. The antibacterial movement was evaluated by estimating the distance across the zone of a hindrance.

**Evaluation Techniques**
The following techniques were used as agar diffusion method: Agar Cup method, Agar Ditch method, and Paper Disc method. In addition, we have used the Broth Dilution Method to evaluate the antibacterial activity. It was one of the non-automated in vitro bacterial susceptibility tests. This classic method yields a quantitative result for the number of antimicrobial agents needed to inhibit specific microorganisms' growth. It is carried out in tubes.

- Macro dilution method in tubes.
- Micro dilution format using plastic trays

1 All the synthesized drugs were used for antibacterial test procedures

2 All necessary controls like:

- Drug Control
- Vehicle Control
- Agar Control
- Organism Control
- Known antibacterial drugs control
- All MTCC cultures were tested against above mentioned known and unknown drugs.
- Mueller Hinton broth was used as a nutrient medium to grow and dilute the drug suspension for the test bacteria.
- Inoculum size for test strain was adjusted to 108 cfu [colony forming unit] per milliliter by comparing the turbidity.
- The strains were procured from the Institute of Microbial Technology, Chandigarh.
- DMSO was used as diluents/vehicle to get the desired concentration of drugs to test upon Standard bacterial strains.
- To determine the median inhibitory concentration (IC50) value.
- The percentage (%) of the bacterial growth inhibition was determined as [(Ac–At)/Ac] 100, where Ac is an average of six replicates of light absorption values at wavelength --- nm of the negative controls, and was an average of six replicates of light absorption values at wavelength --- of the samples. The IC50 value was calculated using the linear relation between the inhibitory probability and concentration logarithm according to the method of Sakuma. The IC50 value is expressed as the mean ± standard deviation of three independent experiments.

**Minimal Inhibition Concentration (MIC)**

The main advantage of the 'Broth Dilution Method' for MIC determination lies in the fact that it can readily be converted to determine the MIC.

1. Serial dilutions were prepared in primary and secondary screening.
2. The control tube containing no antibiotic was immediately subcultured (before inoculation) by spreading a loopful evenly over a quarter of plate of medium suitable for the growth of the test organism and put for incubation at 37 overnight). The tubes are then incubated overnight.
3. The MIC of the control organism is read to check the accuracy of the drug concentrations.
4. The Minimum inhibitory concentrations (MICs) were recorded.

The amount of growth from the control tube before incubation (which represents the original inoculum) is compared.

**Methods Used for Primary and Secondary Screening**

Each synthesized drug was diluted, obtaining 2000 microgram /ml concentration, as a stock solution.

*Primary screen:* In preliminary screening, 1000 microgram/ml, 500 microgram/ml, and 250 microgram/ml concentrations of the synthesized drugs were taken. The active synthesized drugs found in this primary screening were further tested in the second set of dilution against all microorganisms.

*Secondary screen:* The drugs found active in primary screening were similarly diluted to obtain 200 microgram/ml, 100 microgram/ml, 50 microgram/ml, 25 microgram/ml, 12.5 microgram/ml, and 6.250 microgram/concentrations.

## Results

As presented in Table 1, the result of the preliminary phytochemical screening was carried out on the Methanolic crude leaves extracts of *Mentha arvensis L*. The phytochemical results uncovered the presence of different bioactive auxiliary metabolites in the methanolic dissolvable concentrates of *pudina* leaves. The most extreme auxiliary metabolites were seen in Methanolic extricates.

Table1

*Phytochemical Analysis of Leaves Extracts of Mentha Arvensis*

| Sr. no. | Phytochemical analysis | Methanol |
|---|---|---|
| 1 | Flavonoids | + |
| 2 | Saponins | _ |
| 3 | Alkaloids | _ |
| 4 | Tannins | + |
| 5 | Carbohydrates | _ |
| 6 | Terpenoid | + |
| 7 | Cardiac Glycosides | _ |

*[+] = Present; [-] = Absent*

Antimicrobial activity analysis of *M. arvensis* was done using broth dilution assay. The antimicrobial action of leaf removes was analyzed against pathogenic microscopic organisms by estimating the zone of a hindrance. The results revealed that the high antimicrobial activity of leaf extracts of pudina was observed for methanolic solvents against *Staphylococcus aureus, Escherichia coli, Streptococcus pyogenes, Pseudomonas aeruginosa*, *Candida albicans, Aspergillus Niger*, and *Aspergillus clavatus* (Table 2). The maximum zone of inhibition was found in methanolic extracts against *Pseudomonas aeruginosa*, and *Aspergillus clavatus* over the control.

Table 2

*Antibacterial Activity and Antifungal activity Analysis of M. Arvensis*

| CODE NO. | *Bacteria* | | | | *Fungi* | | |
|---|---|---|---|---|---|---|---|
| | *E. COLI* | *P. AERUGINOSA* | *S. AUREUS* | *S. PYOGENUS* | *C. ALBICANS* | *A. NIGER* | *A. CLAVATUS* |
| | MTCC 443 | MTCC1688 | MTCC 96 | MTCC 442 | MTCC227 | MTCC282 | MTCC1323 |
| Extraction of *Mentha Arvensis* | 100 µg/ml | 125 µg/ml | 100 µg/ml | 0.625 µg/ml | >1000 µg/ml | 500 µg/ml | 1000 µg/ml |

*NOTE*. Minimal inhibition concentration [µg /ml], (MTCC-Microbial type culture collection).

## Observations

Table 3 presents the Minimal bactericidal concentration and Minimal fungicidal concentration of standard drugs on specific microorganisms:

1. Gentamycin showing zone of inhibition at 0.05 ug/ml on *E.coli.*
2. Ampicillin showed zone of inhibition at 100 ug/ml on *E.coli*.
3. Chloramphenicol showed zone of inhibition at 50 ug/ml on *E.coli, Staphylococcus aureus, Streptococcus pyogenes, and Pseudomonas aeruginosa*.
4. Ciprofloxacin showed a zone of inhibition at 25 ug/ml on *E.coli* and *Pseudomonas aeruginosa.*
5. Norfloxacin exhibited zone of inhibition at 10 ug/ml on *E.coli, Staphylococcus aureus, Streptococcus pyogenes, and Pseudomonas aeruginosa*.
6. Nystatin revealed zone of inhibition at 100 ug/ml on *Candida albicans, Aspergillus Niger*, and *Aspergillus clavatus*.
7. Griseofulvin showed zone of inhibition at 100 ug/ml on *Aspergillus Niger*, and *Aspergillus clavatus*.

Table3

*Minimal Bactericidal Concentration and Minimal Fungicidal Concentration of Standard Drugs on Specific Microorganisms*

| DRUG | *E. COLI* | *P. AERUGINOSA* | *S. AUREUS* | *S. PYOGENUS* | *C. ALBICNS* | *A. NIGER* | *A. CLAVATUS* |
|---|---|---|---|---|---|---|---|
| | MTCC443 | MTCC1688 | MTCC96 | MTCC442 | MTCC227 | MTCC282 | MTCC1323 |
| | (µg/ml) | | | | | | |
| Gentamycin | 0.05 | 1 | 0.25 | 0.5 | - | - | - |
| Ampicillin | 100 | 100 | 250 | 100 | - | - | - |
| Chloramphenicol | 50 | 50 | 50 | 50 | - | - | - |
| Ciprofloxacin | 25 | 25 | 50 | 50 | - | - | - |
| Norfloxacin | 10 | 10 | 10 | 10 | - | - | - |
| Nystatin | - | - | - | - | 100 | 100 | 100 |
| Greseofulvin | - | - | - | - | 500 | 100 | 100 |

*Pudina* also showed the same inhibition act at 0.05 concentration on *E. coli*. Means function of *pudina* extract might be the same as ampicillin. Further cytotoxicity studies are needed to confirm the same plant against *Staphylococcus aureus, Escherichia coli, Streptococcus Pyogenes, Pseudomonas aeruginosa, Candida albicans, Aspergillus Niger, Aspergillus clavatus*. We have simultaneously studied the effect of antibiotics on such microorganisms. A concentration of antibiotics up to 100 ug/ml (more than 100 ug/ml) is the best drug or better medicine against certain species of microorganisms. *Mentha arvensis* exhibited MICs against *Staphylococcus aureus, Escherichia coli, Streptococcus Pyogenes, Pseudomonas aeruginosa*, when compared with antibiotics like Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin. *Mentha arvensis* showed MICs to inhibit the growth of *Candida albicans, Aspergillus Niger, Aspergillus clavatus* when compared with antibiotics like nystatin and *Griseofulvin*.

## Discussion

The subjective phytochemical examination (Table 1) of *pudina* leaves was reported in the present investigation. The antimicrobial action of leaf extricates inspected against pathogenic microbes by

estimating the zone of a hindrance as observed in our experimental studies. The phytochemical results showed the presence of different bioactive optional metabolites in the dissolvable concentrates of pudina leaves. The flavonoid, Tannins, and Terpenoid were present in the dissolvable concentrates. As presented in Table 2, the most extreme zone of hindrance was recorded in methanolic separates against Pseudomonas aeruginosa and Aspergillus clavatus over the control. The antimicrobial movement of the leaf concentrates of pudina was more successful than other contemplated microorganisms. As shown in Table 3, *Mentha arvensis* extract showed its activity similar to the Gentamycin, Ampicillin, Chloramphenicol, Ciprofloxacin, Norfloxacin, nystatin, and griseofulvin. If the concentration of any drug and extract acts on the same organisms, they behave in the same manner. A study by Kailash Sontakke [11] conducted preliminary phytochemical investigation of leaves *Mentha piperita L*. by using diverse dissolvable concentrates. The leaves of *Mentha piperita L*. showed the presence of alkaloids, flavonoids, tannins, phenols, saponins, steroids, terpenoids, proteins, and carbs. Alkaloids were found to be absent in chloro-form extracts, while acetone and aqueous extracts showed the highest presence of alkaloids. Similarly, flavonoids, terpenoids, and proteins were also found to be absent in the chloroform extracts. Most of all, phytochemicals showed their presence in ethanol, acetone, and aqueous solvent extracts [11]. As reported by Elhadi Sulieman [12], the chemical composition of the *spearmint* leaves was determined as follows: moisture (76.01 ± 0.033%), fiber (2.1 ± 0.03%), ash (3.48 ± 0.001%), protein (1.75 ± 01%.), fat (3.20 ± 0.003%), and carbohydrates (14.46 ± 0.15%), In addition, the acid value, peroxide value, iodine value, free fatty acids, and refractive index at 27C$^{o}$ and density at room temperature were 0.0614, 1.0, 0.564, 0.0305, 1.4572 and 0.8395, respectively [12]. Shahzad [13] investigated the qualitative phytochemical compounds of various extracts of leaves, stem, and root of *M. arvensis*. Findings showed the presence of different phytochemical compounds, such as alkaloids and flavonoids polyphenols, tannins, cardiac glycosides, indicating their distribution in the whole plant. Test for saponins showed their absence from leaves, stem, and root extracts. The results also demonstrated that diterpenes are available in leaves and stem extracts but are absent in root extricates. The data for the quantitative determination of secondary phytochemicals from leaves, stem, and roots of *M. arvensis*. This showed that the phytoconstituents level is high in leaves when compared with stem and roots. Among all phytochemicals, flavonoid content is high in leaves, stems, and roots (22.86>19.9>19.09%), respectively. A considerable amount of total phenol (Polyphenols;3.51%) has been reported to be present in leaves. Alkaloid content was found to be more in stem extracts when compared with leaves and roots. *Mentha* root was reported to have high tannins (16.84) and cardiac glycosides (5.61%) content compared to leaves and stem extracts. The findings exhibited that all plant parts (leaves, stem, and root) were a rich source of various important phytoconstituents [13]. Zaidi and Dahiya [14] investigated the antibacterial activity of *Mentha spicata* and *Mentha piperita* essential oils, where they were capable of inhibiting the growth of bacterial and fungal clinical isolates *in varying differing* ways. Both the essential oils were assessed using the agar well diffusion method by measuring the diameter of inhibition zones. In both the oils tested, gram-positive organisms *(Staphylococcus aureus)* were more susceptible than Gram-negative organisms (*E. coli, P. aeruginosa, Salmonella* spp.). Among the Gram-positive microbes, both the essential oils revealed maximum activity against *S. aureus* 1, creating the maximum zone of inhibition (21±0.09 mm) in *Mentha spicata* and *Mentha piperita* (19.2±0.07 mm). Among Gram-negative organisms tested, *Acinetobacter* spp. Was more sensitive to Mentha spicata

essential oil (18±0.11 mm zone of inhibition) than E. coli (14±0.05 mm) *Klebsiella* spp. (12.7±0.07 mm). No inhibition was also found for *S. Typhi, S. paratyphi,* and *P. aeruginosa*. Regarding the fungal clinical isolates tested, *C. Albicans* exhibited a remarkable inhibition zone (11.7±0.12 mm) compared to *Rhizopus nigricans*, possessing an inhibition zone of 8.3±0.05 mm in *Mentha piperita* essential oil. A considerable antifungal activity against *Aspergillus Niger* (inhibition zone of 15.7±0.09 mm) was found by *Mentha spicata* essential oil. The oil additionally has antifungal activity against *Aspergillus* spp. (13±0.13 mm) and *Candida albicans* (11.8±0.10 mm) [14]. B. Sugandhi and Bai [15] investigated the antibacterial activity of the ethanol extract of *Mentha arvensis* L. leaves studied against *E.coli, Pseudomonas aeruginosa, Shigella flexneri, Klebsiella pneumoniae,* and *S.aureus*. at various concentrations of ethanolic extract. The ethanolic extract of ethanol extract of *Mentha arvensis* L exhibited significant antibacterial activity against the aforementioned organisms. The antibacterial activity of *S.aureus* was found to be higher than the other bacteria (20 mm zonation at 10`% concentration and 7mm at 0.3% concentration). *P. aeruginosa* showed the lowest inhibition zone, ranging from 7mm to 12mm at 0.6% to 10% of concentration. The inhibition zone of *E. Coli, K. pneumoniae*and*S. Flexneri* was found to be between 7mm to 14mm at 0.3% to 10% of concentration. The plant extract also showed moderate antibacterial activity against these three bacteria. The aforementioned study reported that *M. arvensis* L was categorized as "very active antibacterial activity" for *S. aureus*, "active" for *E. coli, K. pneumonia,* and *Shigella flexneri*, and "partially active" for *P. aeruginosa* [15]. Pramila et al. [16] assessed the antimicrobial potential of the methanolic leaf extract by antimicrobial susceptibility test, where antimicrobial activity of the plant extract against *E.coli,* Acinetobacter, *S. aureus,* and two fungi (i.e., *Candida albicans,* and *Candida glabrata*). Among these bacterial pathogens, *E. coli* was found to be more sensitive as compared with *S. aureus* and Acenatobacter. For the fungal pathogens, *C.* Albicans revealed a remarkable inhibition zone in comparison with *C. glabrata*. The minimum inhibitory concentration and minimum bactericidal concentrations of the plant extract against the tested organisms were found to be 3.125 and 6.25 µg/ml, respectively [16]. Al-Sum et al. [17] evaluated antimicrobial activity of the aqueous extract of Mentha species .against *Bacillus fastidious, S. aureus, Proteus mirabilis, Proteus Vulgaris, Salmonella choleraesuis, E. coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*, and *Serratia odorifera*. Mint concentrate at various concentrations was capable of inhibiting the growth of all tested bacteria except for *S.aureus,* and the highest inhibitory activity was reported for *S. mutans* by applying the well diffusion method. Antibacterial activity of aqueous extracts of Mint was also showed against multidrug-resistant bacteria [17]. Overall, our observation and literature review revealed that *M. arvensis*has effective antibacterial and antifungal activity. This is a preliminary study that should be done at the next level by using instrumental *chromatographic* studies, including HPTLC, resulting in confirmation of active ingredients of these plants for revealing their medicinal or therapeutic potential.

## Conclusion

The present study showed that *Pudina* (*Mentha arvensis*) has a wide range of valuable auxiliary metabolites and may be used to treat various bacterial and fungal diseases. *Mentha arvensis* plant had inhibitory effects (25 - 100 MIC ([ug/ml]) against 5 pathogens including. *aureus, E. coli, S.pyogenes, P. aeruginosa, C. albicans, A. Niger,* and *A. clavatus*.

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## Authors' Contribution

Dr. Vijay Upadhye Guided the Students in the preparation of the manuscript and design of the study.

## Conflict of Interest

The author declares that there is no conflict of interest.

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